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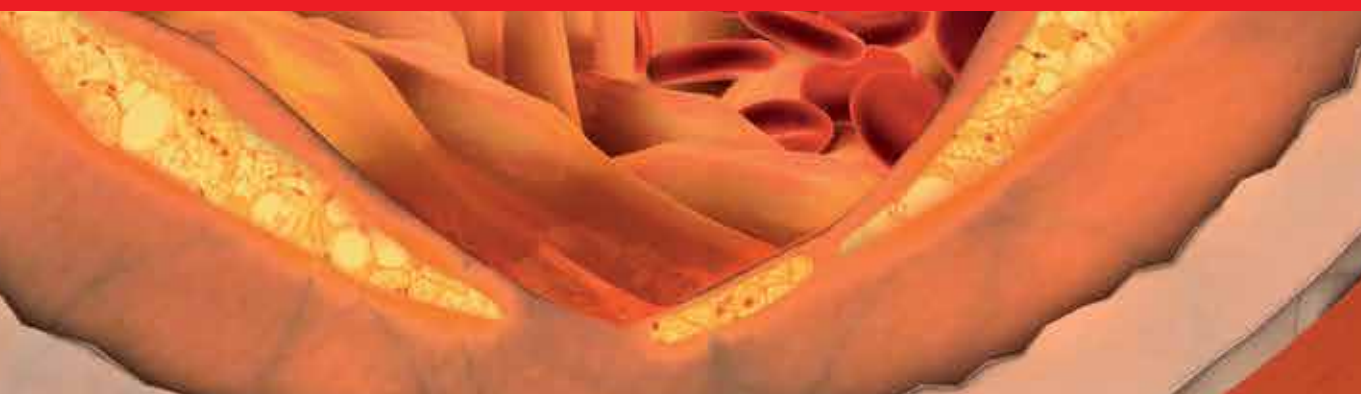
Coronary Angiography

Advances in Noninvasive Imaging Approach for Evaluation of Coronary Artery Disease

Edited by Branislav Baskot



CORONARY ANGIOGRAPHY – ADVANCES IN NONINVASIVE IMAGING APPROACH FOR EVALUATION OF CORONARY ARTERY DISEASE

Edited by **Branislav Baškot**

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Notice

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Meet the editor

Branislav Baskot MD PhD Ass Prof; was born 1958. in Ruma, Serbia. He finished Medicine Faculty in Sarajevo, Bosnia and Hercegovina, and specialization of Nuclear Medicine on Military Medical Academy in Belgrade, Serbia in 1994. The main occupation in the field of Nuclear medicine was nuclear cardiology. He obtained his Doctoral degree in 2005.; "Determination of culprit lesion before and after percutaneous coronary intervention by myocardial perfusion imaging" and Master's Degree; "Myocardial perfusion imaging with 99mTc-Tetrofosmin in the diagnosis ischemic heart disease" in 2003. He is an Associate professor of medicine, on Institute of Nuclear Medicine Medical Military Academy Belgrade, Serbia; Associate Professor on US Medical School, the first private medical school in Belgrade, Serbia; Associate Professor on European University Belgrade, Serbia; and Director and owner Department of Nuclear Medicine in Belgrade, Serbia.

Contents

Preface

This book brings together contributions from around the world, investigators who are clinical versus imaging science in their orientation, and representatives from academic medical centers and the imaging industry. Each article is written to be accessible to those with a basic knowledge of coronary imaging but also to be stimulating and educational to those who are experts and investigators in medical imaging.

This book covers where advances have been dramatic in the past two decades and shows the major contributions of the imaging scientists and engineers from both academia and industry. Patients with know or suspected coronary artery disease who are asymptomatic or who have stable symptoms are often evaluated noninvasive. Functional test, such as stress electrocardiography, stress echocardiography, and stress nuclear perfusion imaging, detect and quantity the presence of ischemia based on electrical, mechanical, or perfusion abnormalities, indirectly, but nuclear perfusion imaging directly, establishing the burden of coronary artery disease. Multidetector CT (MDCT) has emerged as a tool to evaluate noninvasive the coronary anatomy. MDCT has overcome many of its original limitations and now provides ECG-gated acquisition with short acquisition time, sub millimeter spatial resolution, allowing excellent visualization of the coronary arteries. Over the last 15 years, the rate of technologic advancements leading to improved coronary angiography with MDCT has rapidly exceeded those of other cardiac imaging modalities. Image quality is undergoing constant refinement, and the number of uninterpretable coronary studies has gradually decreased from 20%-40% using for detector, to 15% - 25% with 16-detector, and is now as low as 3% to 10% with 64-detector systems.

But this section is also devoted to the current state of myocardial perfusion imaging (MPI). MPI is well establishment imaging techniques and is already integral part of the management of coronary artery disease (CAD), and is included in a number of professional guidelines. Coronary angiography, considered the "gold standard" for the diagnosis of CAD, often does not provide information about the functional significance of coronary stenosis, especially in borderline lesions. Andres Gruentzig said; *when coronary angiography founded coronary narrowing, I would like to have some kind of diagnostic procedure who gives me functional significance that lesion.* MPI is very important diagnostic tool for the diagnosis culprit lesions, and indicating who patients have for cardiovascular intervention (PCI or ACBP). The predominant theme is that

MPI finding can serve as the gatekeeper for more costly and more risky invasive strategies in the evaluation and treatment of patients with coronary artery disease.

The book Coronary Angiography – Advances in Noninvasive Imaging Approach for Evaluation of Coronary Artery Disease includes a series of articles that provide a state-of-the-art summary of the current clinical applications of cardiac CT, reviews data that support the accuracy and the prognostic use of CT coronary angiography and reports of the newest technological advances and promising future applications of these imaging modalities. Its also provide other diagnostic approach like functional test, which finding helps to make decision about invasive strategies with best benefit for patients.

Finally, the next decades should see even greater advances in the field, and such breakthroughs will be instrumental in further enhancing the information that can be derived from functional testing for the assessment of myocardial blood flow, cardiac function, and myocardial viability.

Readers of *Coronary Angiography* will enjoy in this book and will find the information and expert opinions very useful to their clinical practice.

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1

Utilization of Functional Tests Prior to and Adherence to Guidelines on Coronary Angiography

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1. Introduction

Coronary angiography is one of the most commonly performed investigations in clinical cardiology and remains the "gold standard" in the anatomical diagnosis of coronary artery disease. It is often required to establish the diagnosis of coronary disease and to provide a map of a patient's coronary artery anatomy prior to percutaneous coronary intervention or coronary artery bypass surgery.

Despite its importance, invasive coronary angiography should not be performed in all patients suspected to have coronary artery disease. Functional tests, such as stress ECG, echo or nuclear perfusion imaging, are often recommended as initial tests for many of these patients. These functional tests are widely available and practised. In addition to their diagnostic value, functional tests provide independent and additional prognostic information (Marwick et al., 1997). Furthermore, functional tests are often required to guide management of patients with intermediate lesions on invasive coronary angiography. According to the Bayesian theorem, the impact of a screening test is most significant in patients with intermediate pre-test probability of disease. Furthermore, cost-effectiveness analyses often reveal that the use of screening tests in these patients is the most favourable approach. Despite the established roles of functional tests and their extensive incorporation in best practice guidelines, there is little data on the extent of their use and on how the results of such tests are utilised prior to referral to coronary angiography in patients with low to intermediate pre-test probabilities of coronary disease.

Guidelines have proliferated in cardiology in recent years. Major professional bodies like the American College of Cardiology, American Heart Association and the European Society of Cardiology have published guidelines on a wide range of cardiovascular disorders and cardiovascular investigations. These guidelines incorporate the latest evidence base and provide recommendations, which are intended to improve the quality of patient care and clinical outcomes whilst minimising costs. These recommendations are based on the most effective and evidence-based strategies. The American College of Cardiology and American Heart Association have published comprehensive guidelines on the use of coronary angiography (Scanlon et al., 1999). The guidelines were initially published in 1987 and were revised in May of 1999. These guidelines provide recommendations for coronary

angiography in clinical scenarios such as patients with known or suspected coronary artery disease, stable or unstable angina pectoris, acute coronary syndromes, recurrence of symptoms after revascularization, congestive heart failure or other conditions.

There has been considerable interest in evaluating compliance with guidelines in clinical practice. This is particularly pertinent as improved compliance with treatment guidelines is associated with better clinical outcomes in patients with acute coronary syndromes (Schiele et al., 2005). Despite the widespread dissemination of the guidelines on coronary angiography, the compliance rate with these guidelines in clinical practice and the relationship between compliance and results of angiography has not been prospectively evaluated.

As discussed in other chapters, computed tomography (CT) coronary angiography is now increasingly being used to evaluate patients with suspected coronary artery disease. The American College of Cardiology, together with other professional bodies, has published criteria for the appropriate use of CT coronary angiography (Hendel et al., 2006). CT coronary angiography will increasingly be incorporated into clinical practice as an important imaging modality for the evaluation of patients suspected to be suffering from coronary artery disease. An important consideration for clinicians and administrators will be the diagnostic value of imaging tests and their cost effectiveness in these patients. More data has recently become available regarding the incremental value of functional testing and other imaging modalities like CT coronary angiography in patients with suspected coronary artery disease. In particular, the incremental value of non-invasive testing in risk stratification and the prediction of adverse events in these patients will be of interest in guiding practice and, more importantly, health care policy.

2. Utilisation of functional tests prior to coronary angiography

As discussed earlier, despite their well-documented clinical usefulness, there is little information on the pattern of use of functional tests in patients prior to undergoing invasive coronary angiography. In particular, for patients who are subsequently found to have no significant coronary artery disease on coronary angiography, it will be interesting to examine how and why they ended up having invasive coronary angiography. It may be argued that, for these patients, a "failure" of the investigative algorithms led them to undergo an invasive test, which may not have been indicated and should not have been performed. These tests should have been avoided as they exposed the patient to needless risks and might have been unnecessary monetary, resource and manpower wastes. By examining where the process has "failed", one will hopefully be able to learn how to minimise future such "failures". The purpose of our study was to analyse the patterns of use and the results of functional tests in patients found to have normal coronary arteries on invasive coronary angiography.

2.1 The study - methods

Over a 7 and a half-year period, a total of 6,409 patients underwent 8,069 coronary procedures at our hospital. Our hospital is the only tertiary referral centre serving a population of about 800,000 people. Only patients referred for coronary angiography for evaluation of coronary artery disease were included in the analysis. Angiographic studies on patients referred for valvular or haemodynamic indications were excluded from the

analysis. Patients with documented coronary artery disease referred for coronary angioplasty or other percutaneous intervention were also excluded. Therefore, the study included 6,053 patients who underwent a total of 6,830 coronary angiographic procedures.
Of the 6,830 procedures, 4,610 were for male patients and 2,220 were for female patients. The mean age of the patients was 60.9 ± 11 years. Clinical information, including age and gender, referrer details, indications for angiography, type of the study and subsequent results, was prospectively collected and entered into a computerised database.
Coronary angiography was performed according to standard techniques via either the femoral, brachial or radial approaches. Patients with no angiographically detectable disease or irregularities in any of the epicardial coronary arteries were considered to have normal coronary arteries on angiography. Patients who had previously undergone coronary artery bypass surgery and who were found to have patent bypass grafts on angiography were not considered to have normal coronary arteries.
Patients who were subsequently found to have normal coronary arteries on angiography were identified. The clinical records of these patients were then reviewed. Their clinical characteristics and presenting symptoms, including risk factors for coronary artery disease, were analysed. Chest pain as the main presenting symptom was characterized on retrospective chart review as typical, atypical or non-anginal/non-specific pain. Five risk factors were considered: diabetes mellitus, cigarette smoking, hypertension, hypercholesterolemia and family history of coronary artery disease. Patients' pre-test probabilities of coronary artery disease were estimated from age, gender and presenting symptoms (Diamond & Forrester, 1979).
The types and results of functional tests, if performed for these patients, were recorded and analysed. Results of functional tests including exercise ECG, exercise or pharmacologic stress echocardiogram or nuclear myocardial perfusion studies were sought. None of the patients had CT coronary angiography as it was not available at the time of the study. Functional tests were considered negative if no evidence of inducible ischaemia was detected on testing and if the level of the stress was considered adequate. Functional tests were considered inconclusive if there was equivocal evidence of inducible ischaemia or if there was no inducible ischaemia at inadequate levels of stress.
Information on the physicians who referred these patients (referrers) for coronary angiography was also recorded. Referrers were classified into cardiologists or other physicians according to the field of specialisation. In particular, for cardiologist referrers, those who performed coronary angiography were considered proceduralists whilst cardiologists who do not perform angiography were considered non-proceduralists.

2.2 Results

2.2.1 Patients

Seven hundred and fifty six patients undergoing 762 procedures were found to have normal epicardial coronary arteries on angiography. This means that 11.2% of the coronary angiograms performed were for patients with normal coronary arteries. The mean age of these patients was 54.9 ± 11.5 years with female patients comprising 54.9%. Clinical information was obtainable in all but 4 patients (99.5%). The mean number of coronary risk factors was 1.5 ± 1. The mean pre-test probability of coronary artery disease was 41.7 ± 30% (median 46.1%, inter-quartile range 14.1 - 58.9%). Three hundred and thirteen patients underwent coronary angiography as hospital inpatients while 445 patients underwent coronary angiography as a day-only procedure on an outpatient basis. There were no

significant differences in gender distribution, number of coronary risk factors and pre-test probability of coronary artery disease between patients who underwent coronary angiography as inpatients and those who underwent the procedure as day-only patients. However, patients who underwent coronary angiography as inpatients were significantly younger and more likely to have presented with non-anginal chest pain (Table 1).

Parameters	Inpatient procedure (n=313)	Day-only procedure (n=445)	p
Men/Women	149/164	199/246	0.43
Age (years)	53.6 ± 12.5	55.8 ± 10.8	0.01
Number of risk factors	1.48 ± 1.1	1.49 ± 1	0.73
Presenting symptom n (%)			<0.001
Typical angina	62 (19.8%)	99 (22.2%)	
Atypical angina	110 (35.1%)	172 (38.7%)	
Non anginal chest pain	85 (27.2%)	71 (15.9%)	
Dyspnea	23 (7.3%)	48 (10.8%)	
Others	26 (8.3%)	34 (7.6%)	
Asymptomatic	2 (0.6%)	16 (3.6%)	
Pre-test probability of coronary disease (%)	39.6 ± 30	43.2 ± 29.8	0.11

Table 1. Clinical characteristics of patients who were subsequently found to have angiographically normal coronary arteries divided according to whether they were hospital inpatients or not at the time of angiography. (Reproduced with permission from: Leung DY, Lo ST, Liew CT, Wong A, Hopkins AP, Juergens CJ. Utilization of functional tests prior to coronary angiography in patients with angiographically normal coronary arteries. International Journal of Cardiology 2005; 104(3):326 – 331. Elsevier Limited)

2.2.2 Utilization of functional tests

Only 483 of the 758 patients (63.7%) had undergone functional tests as part of the diagnostic workup prior to coronary angiography. Two hundred and fifty three patients (33.4%) underwent exercise electrocardiography, 140 underwent stress nuclear perfusion imaging (18.5%), 9 underwent stress echocardiography (1.2%) and 81 underwent more than one functional test (10.7%). There were no significant differences in gender distribution, age, number of coronary risk factors and pre-test probability of coronary disease between patients who had undergone functional tests and those who had not. However, inpatients were significantly less likely to have undergone functional tests prior to angiography compared to day-only patients (Table 2).

Multiple logistic regression analysis identified inpatient status as the only independent predictor of undergoing functional tests prior to coronary angiography in these patients (OR 5.9, 95% confidence interval 5.0 - 7.0, p <0.001).

Of the 483 patients who underwent functional tests prior to coronary angiography, inducible ischaemia was detectable in only 241 patients (49.6%). Therefore less than half of the performed tests were positive. Functional tests were reported negative for inducible

Parameters	Functional tests (n = 483)	No functional tests (n=275)	p
Men/Women	218/265	130/145	0.57
Age (years)	55.3 ± 10.7	54.2 ± 12.8	0.2
Number of coronary risk factors	1.5 ± 1.1	1.45 ± 1.1	0.52
Pre-test probability of coronary disease (%)	42.5 ± 29	40.2 ± 31	0.32
Inpatient/day-only patient (n)	126/357	187/88	<0.001

Table 2. Clinical characteristics of the patients when subdivided according to whether they had undergone functional testing prior to coronary angiography. (Reproduced with permission from: Leung DY, Lo ST, Liew CT, Wong A, Hopkins AP, Juergens CJ. Utilization of functional tests prior to coronary angiography in patients with angiographically normal coronary arteries. International Journal of Cardiology 2005; 104(3):326 – 331. Elsevier Limited)

ischaemia for 245 patients. Of these, 151 patients achieved adequate stress levels whilst in 91 cases the results were inconclusive. Of the 151 patients with negative functional tests, 114 (75.5%) underwent coronary angiography as outpatients and 37 as inpatients. Only 22 patients (14.5%) had history of previous admission to hospital with chest pain prior to angiography.

Patients were further divided into 3 subgroups according to their pre-test probability of coronary disease: low risk (pre-test probability <20%), intermediate risk (pre-test probability 20% to <80%) and high risk (pre-test probability ≥ 80%). There were no significant differences in the utilisation and the results of functional tests across the clinical risk subgroups for day-only patients (Figure 1, p = 0.2) or for inpatients (Figure 2, p = 0.76). However, a higher proportion of day-only patients underwent functional tests prior to angiography irrespective of pre-test probability of disease compared to inpatients.

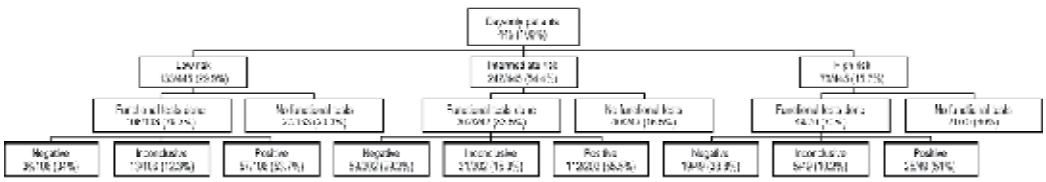

Fig. 1. Use and results of functional tests in patients undergoing coronary angiography as a day-only procedure. (Reproduced with permission from: Leung DY, Lo ST, Liew CT, Wong A, Hopkins AP, Juergens CJ. Utilization of functional tests prior to coronary angiography in patients with angiographically normal coronary arteries. International Journal of Cardiology 2005; 104(3):326 – 331. Elsevier Limited)

A total of 33 physicians referred these patients to our institution for coronary angiography. Twenty (60.6%) were cardiologists and 13 (39.4%) were other physicians. Patients referred by cardiologists were significantly more likely to have undergone functional tests compared

Fig. 2. Use and results of functional tests in patients undergoing coronary angiography as inpatients. (Reproduced with permission from: Leung DY, Lo ST, Liew CT, Wong A, Hopkins AP, Juergens CJ. Utilization of functional tests prior to coronary angiography in patients with angiographically normal coronary arteries. International Journal of Cardiology 2005; 104(3):326 – 331. Elsevier Limited)

with those referred by other physicians (66% vs 50.8%, p = 0.001). Of the cardiologists, 9 were proceduralists and 11 were non-proceduralists. There was no significant difference between procedural cardiologists and non-procedural cardiologists in the use of functional tests in patients prior to coronary angiography (68% vs 64%, p = 0.28).

2.3 Discussion

This study examined the use and results of functional tests in a large consecutive series of patients who were subsequently found to have angiographically normal coronary arteries at our institution over the study period. The overall utilisation of functional tests in our patients was only modest and a significant proportion of patients either had no functional tests done prior to angiography or proceeded directly to coronary angiography despite negative functional tests. Referrer characteristics and patient status, rather than pre-test probability of coronary artery disease, appeared to have a greater impact on the use of functional tests prior to angiography.

Functional tests have established and pivotal roles in the investigation and management of patients with suspected or confirmed coronary artery disease. They have a high sensitivity and specificity in the non-invasive diagnosis of coronary artery disease in patients with suggestive symptoms. Information like blood pressure, exercise capacity and heart rate response to exercise are of further prognostic value. The addition of cardiac imaging during functional tests provides incremental prognostic information (Marwick et al., 1999). Therefore, functional tests not only allow diagnosis of coronary artery disease but also allow clinicians to risk-stratify patients. Higher risk patients should be referred onto coronary angiography whilst lower risk patients can safely be managed without expensive and invasive investigations. In the American College of Cardiology/American Heart Association guidelines for coronary angiography, functional tests play a central role (Scanlon et al., 1999).

It has been suggested that the diagnostic as well as the follow up costs were lower for those who had undergone functional tests prior to coronary angiography, irrespective of the pre-test probability of coronary disease, without significant differences in the medium term outcome (Shaw et al., 1999a). The cost differences between the two strategies may reflect a decreased need for coronary angiography in patients with normal perfusion scan results (Shaw et al., 1999b). Functional tests, therefore, have the potential to accrue cost savings by acting as a "gate-keeper" for coronary angiography by excluding those with normal results who have no significant coronary disease and excellent short to medium term outcome.

According to the Bayesian theorem, the post-test probability of coronary artery disease is dependent on the prevalence of the disease in the population being tested. Impact of screening tests is highest in patients with intermediate pre-test probability. Proceeding to coronary angiography without functional tests may be justifiable in patients with high pre-test probability of coronary artery disease. In patients with multiple coronary risk factors and typical angina, coronary angiography without functional tests may be more appropriate as the results of functional tests are less likely to obviate the need for angiography in these patients. Cost-effectiveness analyses by Patterson et al suggested that proceeding straight to coronary angiography in patients with high pre-test probability of disease (>80%) may be more cost-effective (Patterson et al., 1995). One would expect that the use of functional tests would be higher in patients with low to intermediate pre-test probability of coronary disease. However, the results of the present study showed that this is not the case; the use of functional tests in these patients was low (Figures 1 and 2). The use of functional tests was particularly low for hospital inpatients. Inpatients were less likely to have undergone functional tests prior to angiography compared with day-only patients across all three groups of pre-test probability of coronary disease. For intermediate risk patients, 83.5% of day-only patients compared with only 42.1% of inpatients had undergone functional tests prior to angiography. For low risk patients, 79.7% of day-only patients compared with only 36.3% of inpatients had undergone functional tests. This cannot be explained by any differences in risk factor profiles or pre-test probability of disease.

There could be a number of possible explanations for these findings. Hospital inpatients may be more likely to be perceived as unstable, which may have made the treating physicians reluctant to subject them to functional testing. Some of our regional referring hospitals do not have stress testing facilities and direct referral to our institution for coronary angiography might have been an easier solution. The delay in obtaining stress echocardiography or stress perfusion nuclear imaging contrasted with the overriding pressure to discharge patients from hospital may make such functional testing less attractive to treating physicians.

Functional tests are neither 100% sensitive nor 100% specific. In our study, a high proportion of patients with negative functional tests with adequate stress still proceeded to angiography. This may have resulted from the suspicion of a false negative functional test, frustration on the part of the physicians about the lack of a definitive diagnosis and the desire to answer the question "once and for all". However, despite the lack of 100% sensitivity, patients with normal stress echocardiograms or stress nuclear perfusion imaging had very low cardiac event rates on follow up, in the range of <0.5%/year (Metz et al., 2007). Functional tests may also be falsely positive. However, patient and test characteristics associated with false positive functional tests are well described. Exercise ECG is well known to be non-specific in young or middle aged women. The basal inferior wall changes on stress echocardiogram and diaphragmatic and breast attenuation artefacts on stress nuclear perfusion imaging are well-described sources of false positive findings. The recent advent of CT coronary angiography may help in ruling out coronary artery disease and its use in equivocal or un-interpretable functional tests is considered appropriate.

The increased use of coronary angiography, regardless of whether it is indicated or not, may have more than just an economic impact. In addition to the increased risks of procedural complications, increased diagnostic testing has been shown to result in an increased therapeutic intervention rate downstream (VerrilliWelch, 1996; Wennberg et al., 1996). While the concern of increased downstream therapeutic intervention is minimal in patients

with angiographically normal coronary arteries, the risks of procedural complications and the increased overall costs of investigations cannot justify indiscriminate use of angiography in patients with low to intermediate pre-test probability of disease.

There are considerable variations in the use of coronary angiography (Pilote et al., 1995), which was closely related to the availability of cardiac catheterization facilities (Every et al., 1993). With the use of angiography closely related to its availability, there have been concerns over the appropriateness of coronary angiography (Bernstein et al., 1999; Chassin et al., 1987b). A significant proportion of coronary angiography was found to be "inappropriate" (Gray et al., 1990), with "inappropriate" use of coronary angiography higher in high-use sites (Chassin et al., 1987a). In the study by Chassin et al (Chassin et al., 1987b), patients without angina or with atypical angina and who had not undergone exercise testing constituted the most common subgroup of inappropriate angiography. In a random audit of 320 patients referred for coronary angiography (Gray et al., 1990), only 53% of the patients had undergone functional tests prior to angiography. In a consecutive series of 3631 patients referred for coronary angiography, 5% were performed for inappropriate indications and another 33% for uncertain indications (Hemingway et al., 2001). Similarly, asymptomatic patients and patients with atypical angina or mild angina who had not undergone exercise testing comprised the majority of the inappropriate and uncertain indications. Furthermore, the appropriateness ratings for angiography predicted both the angiographic findings of coronary disease, subsequent rates of revascularisation and mortality rates after a mean follow-up time of 2.5 years (Hemingway et al., 2001).

There has been considerable interest in examining the difference in treatment (Borowsky et al., 1995), procedural use (Nash et al., 1997), and patient outcome between cardiologists and non-cardiologists in patients with coronary artery diseases. It has been reported that cardiologists were more likely than non-cardiologists to recommend "clinically necessary" coronary angiography (Borowsky et al., 1995). Some reports suggested that cardiologists were more likely to prescribe medical therapies of proven efficacy in the care of patients with myocardial infarction (Ayanian et al., 1994), while others suggested that myocardial infarct patients in the care of cardiologists had a lower risk-adjusted mortality rate (Nash et al., 1997). The results of our study suggested that cardiologists were more likely to have referred patients with suspected coronary artery disease for functional tests prior to angiography and procedural cardiologists were as likely as their non-procedural counterparts to utilise functional tests prior to referral for angiography.

Our study is only a single centre experience and may not reflect experience of other centres. We selected only patients with angiographically normal coronary arteries as these patients represent a lower clinical risk subgroup where functional tests should have been more widely used and clinically relevant. As a result of the selection, we did not include patients who had undergone functional tests but were not referred for angiography. Furthermore, our aim was to document the use of functional tests and not to judge whether referral of low to intermediate risk patients without functional tests was "appropriate" or not.

In conclusion, use of functional tests prior to coronary angiography was only modest and was particularly low for hospital inpatients in our large consecutive series of patients with angiographically normal coronary arteries. A significant proportion of patients proceeded to coronary angiography despite negative functional tests. Referrer characteristics and hospital inpatient status, rather than pre-test probability of coronary artery disease, appeared to have greater impact on utilization of functional tests.

3. Adherence to guidelines on coronary angiography

The American College of Cardiology and The American Heart Association initially published their guidelines on the use of invasive coronary angiography in 1987. An update was issued in 1999 (Scanlon et al., 1999). The guidelines examined the indications for coronary angiography in a wide range of commonly encountered clinical situations. These included patients with known or suspected coronary artery disease with minimal or stable symptoms, patients with unstable coronary symptoms, patients after myocardial infarction, patients after revascularisation, patients with heart failure or with haemodynamic instability, patients undergoing non cardiac surgery and patients with valvular or congenital heart disease. These guidelines incorporate the latest available evidence and provide guidance to clinicians to the best evidence based practice. Despite the wide dissemination of these guidelines, there is little information on how they are incorporated into daily clinical practice and how closely they are adhered to. It would be interesting to find out how compliant clinicians are with these guidelines and how compliance is translated into the clinical results. Furthermore, it will be helpful to identify areas where compliance is low so that further efforts may be spent on these problem areas to improve compliance.

3.1 The study - methods

A total of 802 consecutive patients referred to our cardiac catheterisation laboratory for coronary angiography were prospectively enrolled and evaluated over a 5-month period in 2002. Clinical history including coronary risk factors, presenting symptoms, electrocardiograms and laboratory test results were recorded prospectively. Chest pain as a presenting symptom was assessed and classified as typical angina, atypical angina or non-anginal chest pain. Five coronary risk factors were considered: diabetes mellitus, cigarette smoking, hypertension, hypercholesterolemia and family history of coronary artery disease. Electrocardiographic changes were considered ischaemic if there was horizontal ST segment depression or elevation of ≥ 1 mm or if there was symmetrical T wave inversion of ≥ 3 mm in ≥ 2 contiguous leads.

The physicians who referred these patients were classified as cardiologists or general physicians according to their primary field of specialisation. Cardiologists were further sub-classified into non-invasive or invasive cardiologists according to whether they performed coronary angiography. The type and results of functional tests, if performed, were recorded and information on left ventricular function, if available, was also collected. Functional tests were considered positive it there was evidence of inducible ischaemia on testing (ST segment deviation of ≥ 1mm on exercise electrocardiography, inducible new segmental wall dysfunction on stress echocardiography or reversible perfusion defects on nuclear perfusion imaging). They were considered negative if there was no evidence of inducible ischaemia on testing and if the level of the stress was considered adequate for exercise stress (peak heart rate ≥ 85% age predicted maximum). Functional tests were considered inconclusive if there was equivocal evidence of inducible ischaemia or if there was no inducible ischaemia at inadequate levels of stress for exercise stress.

Patients with no irregularities detected in any of the epicardial coronary artery on angiography were considered to have normal coronary arteries on angiography. Patients with less than 50% diameter stenosis in any of the epicardial coronary arteries or its major branches were considered to have minor coronary artery disease. Angiograms on patients

who had previously undergone coronary artery bypass surgery and were found to have all bypass grafts patent (<50% diameter stenosis) and no ungrafted but stenotic native vessels on angiography were not considered to have significant flow limiting stenosis. Complications of coronary angiography, if any, were also recorded.

Compliance with guidelines on coronary angiography was assessed for these 802 patients by 2 independent assessors, blinded to the results of angiography, according to the American College of Cardiology/American Heart Association guidelines on coronary angiography (Scanlon et al., 1999). Referrals were considered compliant with guidelines if they fulfilled either the Class I or Class II indications. Referrals were considered non-compliant (outside guidelines) if they fulfilled Class III indications or none of the Class I or II indications. Disagreements between the 2 assessors were reconciled with arbitration by an independent third assessor. Intra-observer agreement was assessed with the same assessor evaluating compliance on the same 802 patients, 12 months after the initial assessment. Both assessments were done with the assessor blinded to the results of the initial assessment and coronary angiography.

3.2 Results

3.2.1 Patients characteristics

We evaluated a total of 802 patients (Table 3). The indications for coronary angiography were; assessment of chest pain with known or suspected coronary artery disease in 491 patients (61.1%), non ST segment elevation myocardial infarction in 127 patients (15.8%), ST segment elevation myocardial infarction in 72 patients (9%), congestive heart failure or dyspnoea in 70 patients (8.7%), valvular heart disease in 20 patients (2.5%), prior to non cardiac surgery in 11 patients (1.5%), arrhythmias in 5 patients and miscellaneous in 6 patients (0.8%). Two hundred and thirty six patients (29.4%) had had coronary angiography previously.

One hundred and nineteen patients (14.8%) were referred by general physicians (internists), 341 (42.5%) by non-invasive cardiologists and 342 (42.6%) by invasive cardiologists. Cardiac Troponin T levels were measured in 354 patients and a significantly higher proportion of patients referred by general physicians had raised cardiac Troponin T levels compared to those referred by cardiologists (56 of the 69 patients versus 141 of 285 patients, $p<0.001$)

3.2.2 Investigations prior to angiography

All patients had 12-lead electrocardiography before coronary angiography as part of the initial diagnostic work-up. Twelve-lead electrocardiography was normal in 261 patients (32.5%), showed non-specific changes in 174 patients (21.7%), was un-interpretable in 30 patients (3.7%) and showed ischemic changes in 337 patients (42%).

Left ventricular function was assessed as part of the diagnostic work up before coronary angiography with echocardiography in 232 (28.9%) patients and with radionuclide ventriculography in another 123 patients (15.3%). Left ventricular function was assessed in a further 37 patients (3.6%) from previous contrast ventriculography. Left ventricular function was not assessed prior to coronary angiography in 410 patients (51.1%). Inpatients were significantly less likely to have their left ventricular function evaluated prior to coronary angiography compared with day-only patients (36.9% vs 62.9%, $p<0.001$). Of the 392 patients who had left ventricular function assessment, 149 (38%) had an ejection fraction of < 50% and 243 patients had normal function (ejection fraction ≥ 50%).

Age	62 ± 11 years
Male / Female (n)	522 / 280
Average number of coronary risk factors	2.7 ± 1.3
Medications, n (%)	
Aspirin	642 (80%)
Beta blockers	542 (67.6%)
Calcium antagonists	177 (22.1%)
Nitrates	289 (36%)
Angiotensin converting enzyme inhibitors	375 (46.8%)
Statins	547 (68.2%)
Clopidogrel	111 (13.8%)
Unfractionated heparin or enoxaparin	220 (27.5%)
Angiotension receptor blockers	103 (12.8%)
Inpatient / day only procedure (n)	433 / 369
Previous coronary artery bypass surgery, n(%)	89 (11.1%)
Previous percutaneous coronary intervention, n(%)	88 (11%)
History of myocardial infarction, n(%)	141 (17.6%)
Acute coronary syndrome during index hospital admission (for inpatients), n(%)	199 (24.8%)
Renal impairment, n(%)	71 (8.9%)

Table 3. The baseline clinical characteristics of the 802 patients. (Reproduced with permission from: Leung DY, Hallani H, Lo ST, Hopkins AP, Juergens CP. How compliant are we with guidelines for coronary angiography in clinical practice? Internal Medicine Journal 2007, Oct;37(10):699-704. John Wiley and Sons)

Serum cardiac Troponin T levels were measured in 347 of the 433 inpatients (80.1%) and were elevated to ≥ 0.03 ng/ml in one or more blood samples in 194 patients (44.8%). Cardiac Troponin T levels were measured in only 7 of the 369 day-only patients and were elevated in 3 patients (0.8%).

3.2.3 Functional test results

Functional tests were performed in 262 of the 369 (71%) day-only patients and in only 75 of the 433 inpatients (17.3%, $p<0.001$). Even after the 197 patients with raised cardiac Troponin T levels were excluded from analysis, inpatients were still significantly less likely to have

had functional tests prior to coronary angiography compared with day-only patients (28.5% vs 71.6%, p<0.001). Patients with history of percutaneous coronary intervention, coronary artery bypass surgery or documented myocardial infarction were significantly less likely to have functional tests prior to coronary angiography (79/224, 35.3%) than patients with no such history (258/578, 44.6%, p=0.016). Only 288 of the 491 patients (58.6%) referred for assessment of chest pain not associated with either non-ST elevation or ST elevation myocardial infarction had functional tests prior to angiography. One hundred and nine patients (13.6%) underwent exercise electrocardiography, 41 patients (5.1%) underwent exercise echocardiography, 57 (7.1%) underwent exercise nuclear perfusion scan and 130 patients (16.2%) underwent vasodilator stress nuclear perfusion scan. Table 4 shows the results of functional tests in these 337 patients.

	Positive functional test (n)	*Negative functional test (n)*	*Total (n)*
Adequate stress level (For exercise stress, n)*	81 (74)	23 (20)	104 (94)
Inadequate stress level* (For exercise stress, n)	65 (58)	38 (33)	103 (91)
Vasodilator stress (n)	108 (83)	22 (20)	130 (103)
Total (n)	254 (215)	83 (73)	337 (288)

Table 4. Results for functional tests in the 337 patients (The numbers in parentheses are the number of patients with assessment of chest pain as indications for coronary angiography in each category). (Reproduced with permission from: Leung DY, Hallani H, Lo ST, Hopkins AP, Juergens CP. How compliant are we with guidelines for coronary angiography in clinical practice? Internal Medicine Journal 2007, Oct;37(10):699-704. John Wiley and Sons)

3.2.4 Coronary angiography results

The coronary arteries were angiographically normal in 152 patients (19%) and showed only minor disease in another 111 patients (13.8%). One hundred and sixty six patients (20.7%) had single vessel disease, 145 (18.1%) had double vessel disease and 228 (28.4%) had triple vessel disease. Of the 89 patients who had previous coronary artery bypass surgery, 40 patients (45%) had no significant graft disease. The overall rate of angiography showing either normal or minor diseases was 37.7%. The overall complication rate of coronary angiography was low. There were 51 cases of access site haematoma (6.4%), one case for each of pseudo-aneurysm, arterio-venous fistula, neurologic deficit, significant arrhythmia and contrast allergy. No deaths as a result of the angiography were recorded.

3.2.5 Compliance with guidelines

Referrals for coronary angiography were considered outside the guidelines for coronary angiography in 34.3% and 36.2% as evaluated by the 2 independent assessors. The concordance rate between the 2 independent assessors was 88.2% (kappa 0.74, p<0.001). The concordance rate between the 2 independent evaluations by the same assessor was 97.5% (kappa 0.945, p<0.001).

Coronary angiography showed normal coronary arteries or only minor coronary disease in a significantly higher proportion of patients when the referrals were outside published guidelines compared with referrals within the guidelines (181 of the 264 referrals, 68.4% versus 121 of the 538 referrals, 22.6%, $p<0.001$).

There were no significant differences in complications of coronary angiography between the group where referrals were within guidelines (6.7%) and the group where referrals were outside guidelines (7.2%, $p = 0.79$).

Table 5 shows the compliance rate for each of the indications of coronary angiography. The compliance rates were high with indications of non-ST elevation and ST elevation myocardial infarction, valvular heart disease and arrhythmias. However, the compliance rates were lower with indications of assessment of dyspnoea or heart failure and prior to non-cardiac surgery and were particularly low with assessment of chest pain (n = 491, mean age 61.3 ± 11 years, 300 men). Two hundred and ninety five of these 491 patients (60%) were day-only patients. Only 288 of these 491 (58.7%) had functional tests and only 254 (51.7%) had assessment of left ventricular function prior to coronary angiography.

Indication	Non-compliant with guidelines n(%)	Compliant with guidelines n(%)	Total (n)
Assessment of Chest pain	230 (46.8%)	261 (53.2%)	491
Non-ST elevation myocardial infarction	1 (0.8%)	126 (99.2%)	127
ST elevation myocardial infarction	3 (4.2%)	69 (95.8%)	72
Dyspnea/heart failure	18 (25.7%)	52 (74.3%)	70
Valvular disease	4 (20%)	16 (80%)	20
Prior to non cardiac surgery	3 (27.3%)	8 (72.7%)	11
Arrhythmia	1 (20%)	4 (80%)	5
Others	4 (66.7%)	2 (33.3%)	6
Total	264 (32.9%)	538 (67.1%)	802

Table 5. The compliance rates for coronary angiography to the American College of Cardiology/American Heart Association guidelines according to the indications for angiography. (Reproduced with permission from: Leung DY, Hallani H, Lo ST, Hopkins AP, Juergens CP. How compliant are we with guidelines for coronary angiography in clinical practice? Internal Medicine Journal 2007, Oct;37(10):699-704. John Wiley and Sons)

Concentrating on referrals from cardiologists (n=683), referrals from non-invasive cardiologists were significantly more likely to be outside published guidelines compared

with referrals from invasive cardiologists (141 of the 341 referrals, 41.3% versus 103 of the 342 referrals, 30.1%, p = 0.002). Multivariate logistic regression analysis identified younger age (OR 1.04 for every year younger, 95% CI 1.029 – 1.048, p<0.001), female gender (OR 2.67, 95% CI 2.24 – 3.19, p<0.001), day-only procedure (OR 2.27, 95% CI 1.91 – 2.69, p<0.001) and non-invasive cardiologist referrer (OR 1.41, 95% CI 1.19 – 1.67, p = 0.046) to be independent predictors of non-compliance with published guidelines.

When patients with raised cardiac Troponin T (n = 197) were excluded from the multivariate analysis, day-only procedure was no longer a significant independent predictor of non-compliance. Younger age (OR 1.04 for every year younger, 95% CI 1.03 – 1.05, p<0.001), female gender (OR 2.24, 95% CI 1.87 – 2.69, p<0.001) and non-invasive cardiologist referrer (OR 1.47, 95% CI 1.29 – 1.67, p = 0.004) remained independent predictors of non-compliance with guidelines.

3.3 Discussion

In our large consecutive series of patients referred for coronary angiography, we found that more than a third of the referrals were outside the American College of Cardiology/American Heart Association guidelines for coronary angiography. The inter-observer and intra-observer agreement in assessing compliance were high. The rate of coronary angiography showing either normal coronary arteries or only minor diseases was significantly higher when the referrals were outside guidelines. The compliance rate was particularly low with indications of assessment of chest pain. Younger age, female gender, day-only procedure and non-invasive cardiologist referrals were independent predictors of non-compliance with the guidelines.

Practice guidelines have proliferated in clinical medicine in the past 2 decades in all major fields. The compilation and publication of these practice guidelines represent efforts by professional bodies to incorporate an ever-expanding evidence based medicine into best clinical practice. A systemic review suggested significant improvement of care after introduction of clinical guidelines although the size of the improvement varied considerably (GrimshawRussell, 1993). The dissemination and implementation of these guidelines have emerged as major challenges. Furthermore, awareness of these guidelines does not necessarily equate to compliance. Therefore, the full potential for these guidelines to improve health care delivery and clinical outcomes has yet to be completely realized.

A number of studies have found significant gaps between clinical practice and guidelines in a number of areas (Brand et al., 1995; Leape et al., 2003; Vikman et al., 2003). In a study of antithrombotic therapy in atrial fibrillation, a report found that only 47% of the eligible patients received warfarin according to published guidelines and 4 patients had a stroke during a 12-month follow-up period (Nair et al., 2005). These 4 patients were not on warfarin despite recommendations by the guidelines. In a random audit of Medicare data in 5 US states showed that 30% of percutaneous coronary angioplasties was rated as Class III indications according to the 1988 American College of Cardiology/American Heart Association guidelines whereas 24% were class III by use of the 1993 guidelines (Leape et al., 2003). Similar gaps were found between clinical practice and the European Society of Cardiology guidelines for the management of non-ST elevation myocardial infarction (Vikman et al., 2003) and only about 50% of patients post myocardial infarction received beta blockers as recommended by the guidelines (Brand et al., 1995).

Adherence to guidelines has been suggested to lead to an improved clinical outcome. A clear relationship was found between extent of guideline implementation and one-year mortality in patients with acute myocardial infarction (Schiele et al., 2005). Compliance remained an independent predictor of survival even after adjustment for clinical risk. Similarly, compliance with the guidelines significantly improved prognosis in acute coronary syndrome regardless of risk score (Gulati et al., 2004) and resulted in an improved outcome in high risk patients with non ST elevation myocardial infarction (Vikman et al., 2004). In evaluating the impact of compliance with guidelines for coronary angiography, patient outcome such as survival may not be appropriate. Nevertheless, we were able to demonstrate that the rate of coronary angiography showing either normal coronary arteries or only minor diseases was significantly lower when the referrals were within the guidelines. Rates of normal coronary angiography may be a reasonable surrogate for measuring the impact of compliance as a high negative rate has significant implications due to inappropriate costs, superfluous resource utilisation and unnecessary risks for the patient.

Little is known about the barriers to compliance with guidelines by physicians. Potential barriers may include awareness, familiarity, disagreement with the guidelines, resistance to change, and absence of disincentives or penalties for not adhering to recommendations on the part of the physicians (Cabana et al., 1999). In addition to physician factors, our study also identified clinical parameters and scenarios that were predictive of non-compliance with the guidelines. Younger age and female gender were found to be predictors. One may postulate that physicians may be more aggressive in recommending coronary angiography outside guidelines in younger patients as they do not want to "miss" significant coronary disease in such patients. The compulsion for a "definitive" diagnosis and fear of litigation in an increasing medico-legal environment may also have contributed. Day-only procedure as a predictor of non-compliance may be explained by the fact that patients with acute coronary syndrome with raised cardiac Troponin T levels almost always underwent angiography as inpatients. This is supported by the fact that day-only procedure was not an independent predictor when patients with elevated Troponin levels were excluded from the multivariate analysis. In our study, non-invasive cardiologists were more likely to refer outside the guidelines for coronary angiography. This may be because non-invasive cardiologists are potentially less familiar with the guidelines. Furthermore, in the present study, clinical scenarios of assessment of chest pain in patients with suspected or known coronary artery disease appeared to be areas where the non-compliance rate was particularly high. These may be areas in which physicians have the most difficulties adhering to the recommendations of the guidelines.

Only a small proportion of patients, especially for inpatients, had functional tests prior to coronary angiography. Functional tests, which play an important role in the risk stratification of patients with suspected or confirmed coronary artery disease as depicted in the published guidelines, were performed in a relatively small proportion of the patients in our study. Our previous study on a different patient population also found a low rate of utilisation of functional tests. Referrer characteristics and inpatient status, rather than pre-test probability of coronary disease, appeared to have the greatest impact on utilisation of functional tests (Leung et al., 2005). Non-utilisation of functional tests

prior to coronary angiography was a common reason for "inappropriate" referral for coronary angiography, as established by previous studies (Chassin et al., 1987b; Gray et al., 1990).

Our study represents the experiences of a single tertiary referral hospital, and hence may not be representative of other centres around the world. However, we feel that our study population was representative as our institution is the only cardiac catheterisation laboratory in a public hospital tertiary referral centre serving a population of about 800,000. The aim of the present study was to evaluate compliance with the American College of Cardiology and American Heart Association guidelines for coronary angiography and not to judge whether the referrals were appropriate. Referral outside guidelines may be entirely appropriate depending on the patient's specific clinical situation. These guidelines for coronary angiography were published in 1999. As the evidence base has been evolving and improving, these guidelines may not reflect contemporary practice. Although the rate of angiography showing either normal coronary arteries or minor disease was significantly higher when the referrals were outside published guidelines, which can have significant cost-effective implications, our results do not allow us to perform cost-effectiveness analysis.

4. Conclusions

Coronary angiography is one of the most commonly performed cardiac procedures. It continues to play an important role in the management of cardiac patients and is indispensible in patients considered for coronary revascularisation. It is an invasive procedure not without significant risks and, together with its inherent costs, should not be carried out or repeated without sufficient justification. Functional tests, including stress ECG, stress echo, nuclear perfusion imaging and magnetic resonance imaging are all accepted functional tests that provide important diagnostic and prognostic information and should form part of the body of investigations in patients suspected to be suffering from coronary artery disease. More recently, CT coronary angiography is being used as a "ruling out" test and is considered an appropriate indication in certain subsets of patients with chest pain. Improvement in the use of these functional tests may lead to better risk stratification so that low risk patients may be spared the risks and costs of invasive coronary angiography and higher risk patients can have their angiography expedited. We identified that the use of functional tests, especially in low to intermediate risk patients, was suboptimal and have identified certain problematic areas where their use was very low. The ACC and AHA have published guidelines on coronary angiography. Although the guidelines were published in 1999, it is still a useful document applicable to clinical practice today. There is ample evidence to suggest, in multiple areas of cardiology, that adherence to guidelines was associated with improved patient outcomes. In our study, we found that the adherence to the guidelines on coronary angiography was only modest in certain indications and non-adherence led to a higher rate of normal coronary angiography. Attention should be paid to these problem areas to identify the underlying reasons for non-compliance so that efforts can be made by both individual physicians and professional bodies to improve the rate of compliance so that the full potential of these guidelines can be realised.

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2

Transthoracic Echocardiography in the Assessment of Coronary Arteries

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1. Introduction

Quantitative coronary angiography remains the reference standard for assessing coronary anatomy, measuring anatomic severity of the stenotic lesion and assisting in the process of intracoronary interventions. Thus, treatment of coronary artery disease (CAD) is performed primarily on the basis of anatomic measurements of stenosis severity, although the disease severity correlates better with physiologic disturbances which can be revealed by the analysis of coronary artery flow and coronary flow reserve (CFR). Direct invasive measurements of coronary flow signal using Doppler flow wires and catheters provide a lot of information on the pathophysiology of coronary flow dynamics (Chamuleau et al, 2001; Bax et al, 2006; Braden, 2006; Werner et al., 2006; Kaul & Jayaweera, 2008; Courtis et al., 2009). But in clinical practice, these invasive techniques are rarely applied because of the time and expense required. Alternative methodology in detecting coronary flow and CFR is positron emission tomography which is feasible but expensive and scarcely available (West & Kramer, 2009). In fact, a large-scale assessment of such important functional parameters is hampered by the lack of a reliable, low-cost, noninvasive method that might be used for this purpose (Pellikka, 2004). Some years ago transesophageal echocardiography was proposed for evaluation of coronary flow and CFR in man. However, this method demonstrates some important limitations: it is semiinvasive, and has optimal feasibility in visualizing the flow in only very proximal part of the left anterior descending coronary artery (LAD) (ranging from 69% to 97%) and right coronary artery (RCA) (ranging from 66% to 83%) which allowes the assessment of transstenotic or prestenotic CFR but not poststenotic CFR (Vrublevsky et al., 2001, 2004). Until recently, transthoracic echocardiography (TTE) evaluation of the CAD was aimed at the assessment of regional and global left ventricular function (Youn & Foster, 2004). Direct transthoracic visualization of the coronary arteries was attempted in children and occasionally in adults with coronary artery anomalies, arteriovenous fistulas, and aneurysms (Harada et al., 1999; Hiraishi et al, 2000; P.C. Frommelt & M.A. Frommelt, 2004). However, with the advent of harmonic imaging, contrast agents and high-frequency transducers, direct transthoracic Doppler visualization of non-dilated arteries and measurement of coronary artery flow is now relevant in the majority of patients. The aims of this review are to outline the technical aspects of coronary artery visualization and flow measurements both at rest and with pharmacological stress, to demonstrate pathologic coronary artery flow patterns by TTE and to discuss clinical implications of TTE for patients with suspected or confirmed CAD.

2. How coronary arteries should be visualized by TTE

2.1 New technical considerations for transthoracic echocardiography evaluation of coronary arteries: high-frequency transducers, multi-frequency transducers, harmonic imaging and contrast agents

One of the first reports on TTE visualization of distal LAD as a vessel with a most superficial location (3-7 cm from skin) was presented by Fusejima et al., 1987. However, clinical application of the method was limited by a low success rate of the LAD assessment because adequate signals were available in only 35% of control subjects and 50% of patients with cardiac disease. The introduction of high-frequency (7.5-MHz) transducers allowed a more frequent visualization of distal LAD (Ross et al., 1990; Kenny & Shapiro, 1992). But a major limitation of Doppler evaluation of blood flow in distal LAD associated with a low-intensity Doppler signal caused by ultrasound attenuation was overcome with echocontrast agents alone. Several reports on echocardiographic contrast enhancement of coronary artery images have been published and shown considerable improvement of visualization rate of distal LAD. Caiati et al., 1999a, 1999b, during TTE gave a peripheral injection of Levovist, a contrast agent consisting of a suspension of galactose microbubbles, and demonstrated enhanced coronary color flow Doppler signals. Lambertz et al., 1999, reported the increase of visualization rate of mid-distal LAD during high-frequency TTE from 40% without a contrast agent to 88% with Levovist. In addition, contrast administration facilitated the positioning of the pulsed sample Doppler volume. Okayama et al, 2002, demonstrated a success rate of CFR assessment in the LAD in 70% of cases without an echocontrast agent and in 97% after a Levovist injection with particular improvement of systolic pattern quality. With the routine use of contrast, coronary flow and CFR measurements in the distal LAD become feasible in the majority of patients. Several reports gave little information on transthoracic visualization of the posterior descending coronary artery (PDA) (Voci et al., 2002; Tokai et al., 2003). Voci et al., 2002, could estimate coronary blood flow in the PDA in 76% of patients at rest, but CFR assessment was performed in 54% of patients only because of hyperventilation caused by adenosine. Tokai et al., 2003, demonstrated beneficial effect of Levovist on coronary blood flow signals in the PDA, with increase of visualization rate from 30% without a contrast agent to 86% with it.

High frequency transducers can be most successfully used for scanning the apical area, that is for examination of distal LAD or PDA. Unlike distal LAD and PDA, non-distal LAD, circumflex artery (Cx) and right coronary artery (RCA) are located too deep for TTE, and high-frequency transducers preferred by many investigators can not be used (Boshchenko et al., 2008). Standard low-frequency transducers provide a good penetration and seem to be the optimal choice for scanning the RCA, Cx and proximal and mid LAD. Unfortunately, as standard cardiac low-frequency transducers have lower resolution and lower sensitivity of Doppler exam, low success rate of visualization of non-distal coronary arteries was reported in earlier studies (Voci & Pizzuto, 2001). In recent years, the advantage of using a multifrequency (1.7- to 3.5-MHz) transducer with second tissue harmonics lies in the fact that coronary flow imaging and standard TTE can be readily alternated (Pellikka, 2004), and it is true for estimating both distal LAD and PDA, and proximal-mid segments of coronary vessels. Wideband of low ultrasound frequencies of multifrequency transducers at the exit (1.7-3.5 MHz) provides a good scanning depth which is particularly important for visualizing non-distal LAD and PDA, and second tissue harmonics enables to receive reflected ultrasonic waves of high-frequency range (3,5-7 МГц) at the entrance improving

the image quality for both near and far setting zones. According to our data the feasibility of LAD and PDA visualization with second harmonics has progressively improved, and harmonic imaging facilitated the examination considerably (Table 1).

Authors	N of patients	Transducers, contrast agents	Success rate, %
distal LAD			
Fusejima et al., 1987		high-frequency transducer	35%healthy volunteers 50% CAD patients
Hozumi et al.,1998a	23	high-frequency transducer	78
Lambertz et al., 1999	45	high-frequency transducer, contrast agent	80
Caiati et al., 1999a	56	harmonic, contrast agent	88
Lethen et al., 2003	33	high-frequency transducer	91
Boshchenko et al., 2008	150	harmonic	93
Sicari et al., 2008	1779	harmonic, contrast agent	96
PDA			
Krzanowski et al., 2000	50	harmonic	33
Tokai et al., 2003	50	harmonic, contrast agent	86
Takeuchi et al., 2004	151	harmonic, contrast agent	83
Otsuka et al., 2005	129	harmonic, contrast agent	97
Boshchenko et al., 2008	150	harmonic	91
distal Cx			
Krzanowski et al., 2000	50	harmonic	0
Boshchenko et al., 2008	150	harmonic	33

Table 1. Success rate in visualizing distal coronary arteries by TTE

2.2 Main ultrasonic windows, positions, views by which main coronary arteries can be visualized, and hints concerning optimization of ultrasound images of coronary arteries such as setting depth, Nyquist limit, Doppler angle, size of the sample volume

Modern, high quality ultrasound systems are required for success scanning. In most cases TTE is performed with the help of Acuson Sequoia 512 (Acuson) or HDI 5000, HDI 5000 SonoCT and iE33 (Philips). We used Vivid 7 (GE HealthCare) with 1.7-3.5 MHz narrow-band transducers with second harmonic mode (Boshchenko et al., 2008, 2009).

After obtaining optimal quality B-mode image, the search for coronary arteries is started with color Doppler mapping with or without the use of harmonics. To achieve the best image quality the sample size of color Doppler should be kept at minimum. As the Doppler velocities of coronary blood flow are low, the velocity range should be set with a low Nyquist limit (15-20 cm/s), and filters should be decreased. Too low Nyquist limit can be a cause of more color Doppler artifacts obscuring the images (<10-13 cm/s), too high Nyquist limit is not able to detect low blood flow velocity within coronary arteries (Pellikka, 2004; Korsarz & Stein, 2004; Boshchenko et al., 2008, 2009). Some ultrasound systems offer special color maps for coronary artery examination including second or third harmonics of B-mode and color Doppler mapping, low Nyquist limit, special gain for B-mode and Doppler mode, etc.

2.2.1 Technique of examination for each segment of main coronary arteries

There are several windows by which coronary arteries can be visualized with the patients in the supine or left decubitus positions (Krzanowski et al., 2000, 2003; Saraste et al., 2005; Boshchenko et al., 2008). Standard parasternal short- and long-axis views from second- or third intercostal space or low parasternal short- or long-axis views from fourth- or fifth intercostal space should be used. Alternatively, a modified apical 2, 3 or 5-chamber view or subcostal scanning can be performed. TTE has a very high time resolution, while spatial resolution is low due to a small size of scanning windows. So, coronary arteries appear as linear intramyocardial color fragmental structures of approximately 0.5-2.5 cm in length and 2 to 4 mm in diameter. Initially, a short part of arteries can be visualized. Then, by step-by-step movement of the transducer according to the course of the vessel, a longer fragment of arteries can be assessed.

The scanning depth for the search of proximal and mid coronary artery segments should be set at 10-15 cm. The transducer should be placed at the left parasternal position from second or third intercostal space and a modified short-axis B-view of great vessels should be obtained. The search of the left main coronary artery (LMCA) and proximal LAD can be started in B-mode (figure 1A, B) by consecutive clockwise and cranial rotation of the transducer, but color Doppler mapping which makes images clearer can also be recommended for initial search. The LMCA has approximately 1-3 cm in length, and the vessel should be visualized along its entire extension.

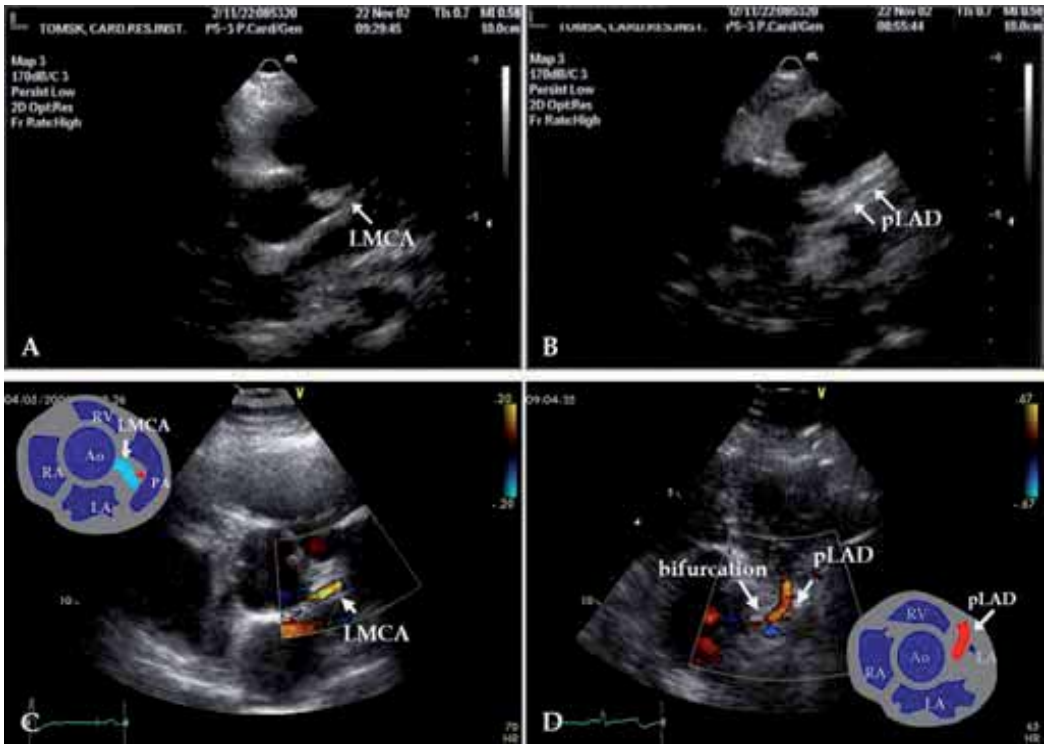

Fig. 1. Images of the LMCA and proximal LAD (pLAD). The left parasternal position, a modified parasternal short axis view of great vessels, Nyquist limit is 20-67 cm/s. A – LMCA, B-mode; B – pLAD, B-mode; C – LMCA, Color Doppler mapping; D - bifurcation of the LMCA; Color Doppler mapping.

The normal anterograde blood flow in the LMCA is identified on color Doppler map as a linear structure dawning from left coronary sinus of Valsalva and has a red or blue color depending on the placement of the transducer, anatomic features of chest and LMCA origin (figure 1C). Bifurcation of the vessel into the LAD and Cx is a marker of LMCA distal board (figure 1D). If the origin of the Cx can not be found, the LMCA and proximal LAD should be assessed as a common segment. Proximal LAD should be assessed after the LMCA by a slight change of the imaging plane in a parasternal or low parasternal short-axis B-view or by change of the position in a modified parasternal long-axis view. The origin of the first diagonal branch can be used as a distal mark of proximal LAD (fig. 2A). Mid LAD should be searched in the low left parasternal position from third- to fifth intercostal spaces and a modified short- or long-axis view of the left ventricle in the anterior interventricular groove (fig 2B, C). Distal LAD should be assessed from the low left parasternal position to a modified apical five-chamber position at varying levels using different short- and long-axis views in the anterior interventricular groove before, at or after the apex of the left ventricle (fig 2D,E). The setting depth should be reduced approximately to 6-10 cm, and the transducer may be substituted for a high-frequency one, because the location of distal LAD is superficial. The LAD is the best vessel for TTE assessment as there are clear anatomic marks for its search and segmentation, such as: anterior interventricular groove, origin of the first diagonal branch and papillary muscles. The segment of the LAD from the origin of diagonal branch to papillary muscles in the short-axis view should be assessed as its mid segment, and the segment of the LAD apical to papillary muscles should be marked as distal LAD. Apical window is usually the best one to obtain low Doppler angles for velocity measurements in the LAD. The normal anterograde blood flow in the entire LAD before the apex is identified as a red linear color signal on color Doppler map, which reflects the direction of the flow from base to apex of the left ventricle.

Unlike the LAD, the scanning depth for the RCA and Cx should be set at 12-15 cm. Proximal Cx should be visualized in the left parasternal position and a modified short-axis B-view of great vessels with the slight caudal tip of the transducer. The normal anterograde blood flow in proximal Cx is identified as a blue linear color signal on color Doppler map, reflecting the direction of the flow from the transducer (fig. 3A). There are no clear anatomic marks for the distal border of proximal Cx. Visualization of mid and distal segments of the Cx is very difficult and possible in a few patients only. Mid and distal Cx should be examined with the same position of the transducer as proximal Cx. As the mid and distal Cx are located in the coronary sulcus at the border between the left atrium and left ventricle, the angulation from heart basis to apex at the level of papillary muscles is usually required (fig 3B). The posterior papillary muscle can be used as a symbolic border between mid and distal Cx. The first or second obtuse marginal branches (OMB) presenting distal parts of the Cx can be assessed from fourth- and fifth intercostal spaces in the apical long-axis position in a modified four- or five-chamber view at either lateral or inferior wall of the left ventricle (fig. 3C).

Proximal RCA should be examined in the left parasternal position from second- or third intercostal spaces in modified short- or long-axis B-views as a structure dawning from right coronary sinus of Valsalva and lying along the anterior wall of the aorta (fig. 4A, B). It may be relatively easy to visualize proximal RCA in B-mode and Color Doppler mapping but pulse-wave Doppler examination is rarely correct because the angle between the direction of coronary blood flow and Doppler beam exceeds 60 degrees. The mid and distal RCA should be searched using Color Doppler mapping only. The subcostal position, a short-axis B-view or a modified apical two-chamber position with cranial angulation of the transducer should be used for the search of mid RCA (fig. 4C). Good quality images of the PDA – usually distal

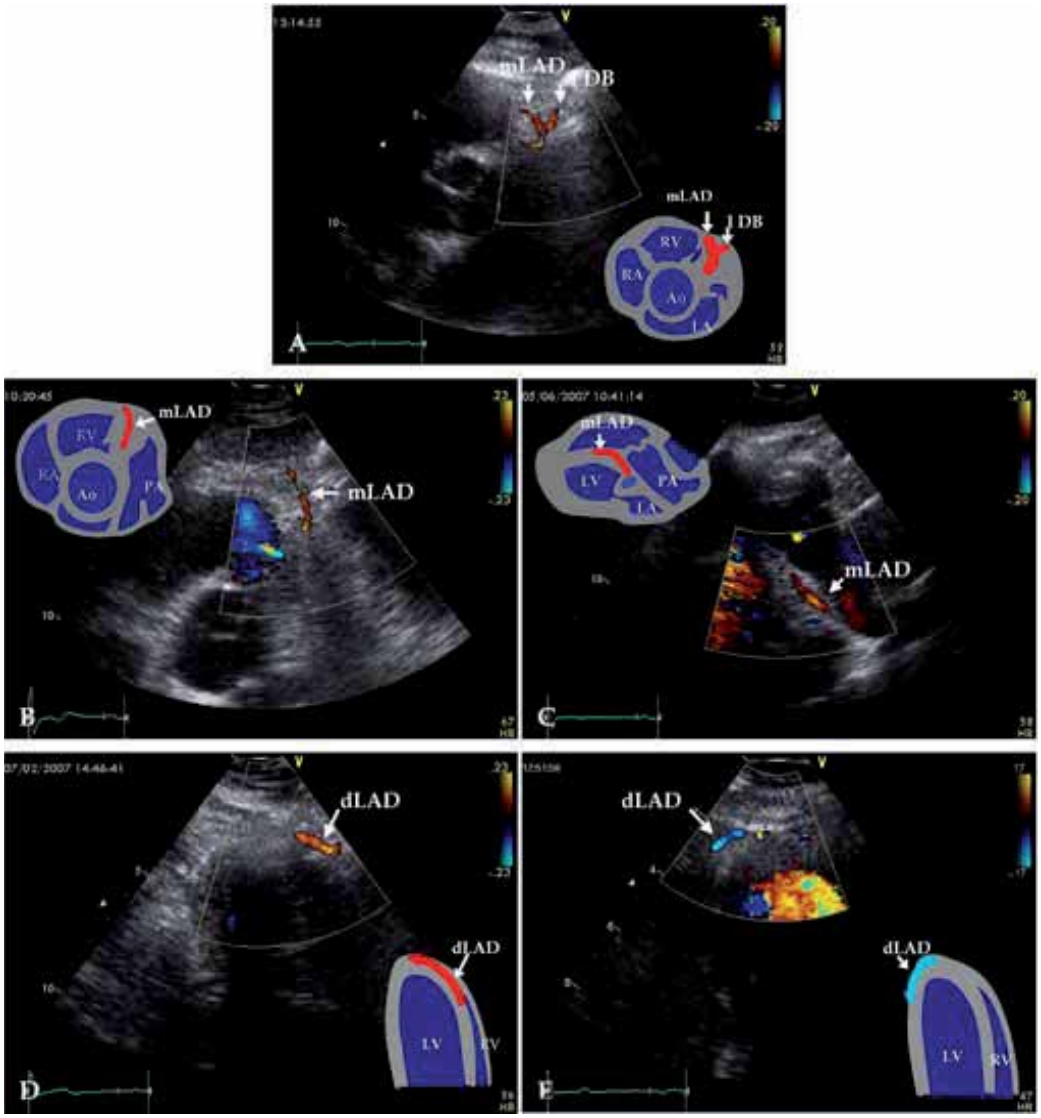

Fig. 2. Doppler Color images of the LAD. A – distal board of proximal LAD with origin of the first diagonal branch (I DB); B – mid LAD (mLAD), the left parasternal position, a modified parasternal short axis view of great vessels; C – mLAD, the left parasternal position, a modified parasternal long axis view; D – distal LAD (dLAD) before circumflexing the cardiac apex, a modified apical five chamber position; E - dLAD after circumflexing the apex of the left ventricle.

RCA – can be obtained from fourth- and fifth intercostal spaces in the apical long-axis position in a modified two- or three-chamber view with caudal tip of the transducer in the posterior interventricular groove (fig. 4D). There are no anatomic marks for segmentation of the entire RCA. The direction of the normal mid RCA and PDA flow is the same as that of the normal LAD flow.

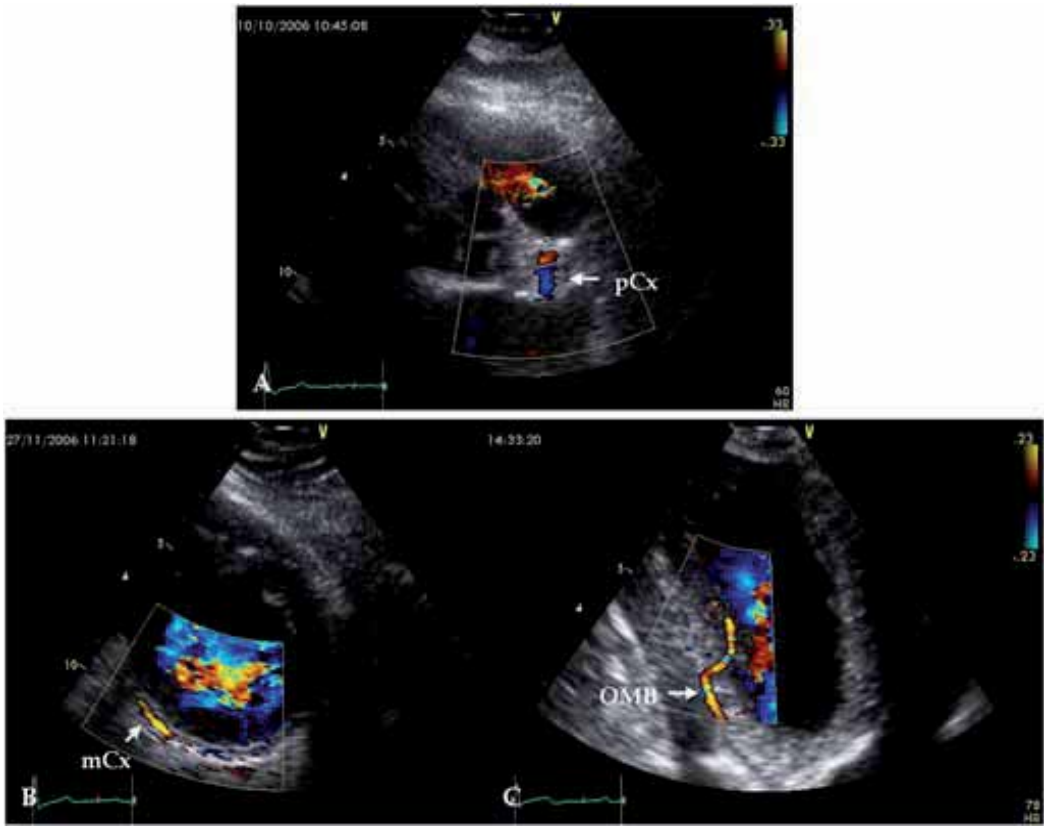

Fig. 3. Color Doppler images of the Cx, Nyquist limit is 20-33 cm/s. A – proximal Cx (pCx); the left parasternal position, a modified parasternal short-axis view of great vessels; B - mid Cx (mCx); the left parasternal position, a modified parasternal short-axis view at the level of papillary muscles; C – the first obtuse marginal branches (OMB) presenting distal Cx, the apical long-axis position, a modified five-chamber view.

2.2.2 Main pitfalls at visualizing coronary arteries by TTE

Main pitfalls of assessment of coronary arteries by TTE arise due to partial visualization of the vessels and anatomic features of coronary bed (Boshchenko et al., 2008). Certain large branches of coronary arteries – the intermediate coronary artery, diagonal and marginal branches can occasionally be visualized and confused with main coronary arteries, most frequently with the LAD (fig. 5A, B). It is more typical for the occluded main artery in which case the branches enlarge and take over its function (Krzanowski et al., 2003). To avoid this mistake, the LAD should be searched in the anterior interventricular groove along its entire extension with the end points in the LMCA and distal LAD. By moving step-by-step towards the aorta or downwards to the left ventricle apex without loosing the LAD sight, the LAD can be correctly identified. The diagonal and marginal branches have no connections with either the LMCA or distal LAD or both.

The distinction between various vessels seen at the lateral and inferior walls of the ventricle can be challenging (Krzanowski et al., 2003). As first and second OMB is lying parallel and close to the PDA, they may be taken for the PDA. If the artery is circumflexing the posterior

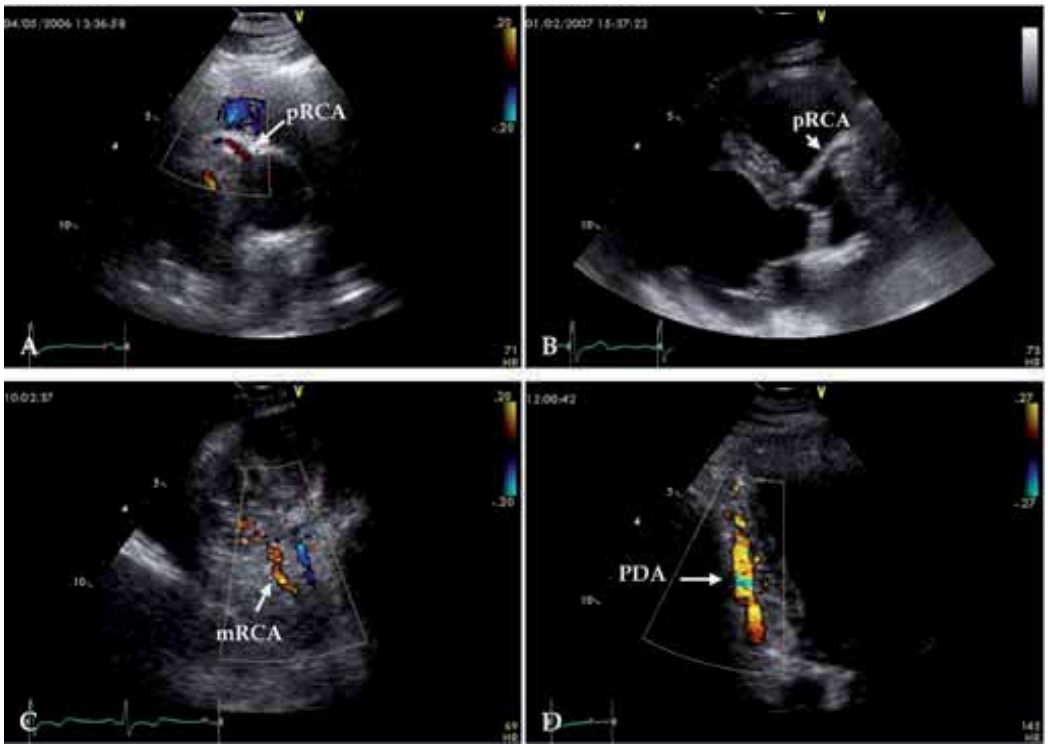

Fig. 4. Color Doppler images of the RCA. Nyquist limit is 17-20 cm/s. A – proximal RCA (pRCA); the left parasternal position, a modified parasternal short-axis view of great vessels; B – pRCA; the left parasternal position, a modified parasternal long-axis view. C – mid RCA (mRCA); a modified apical two-chamber position with cranial angulation of the transducer; D - the PDA as distal RCA, the apical long-axis position, a modified two-chamber view.

surface of the apex of the left ventricle, this vessel is detected by coronary angiography as the PDA in the majority of cases. On the other hand, if the artery is sending a branch to the posterior papillary muscle or lying next to the lateral wall of the left ventricle, this vessel is identified by coronary angiography as the OMB. The PDA which is usually a distal RCA may arise from the Cx if the left coronary artery is strongly dominant, and this fact may result in diagnostic mistakes too.

In scanning mid or distal LAD, certain extracardiac arteries visualized on color Doppler mapping may be confused with the LAD, for example, the left internal thoracic artery (LIMA). However, the pulse-wave Doppler assessment allows an accurate distinction between them: unlike coronary arterial flow which is biphasic systole-diastolic with predominant diastolic phase, the extracardiac arteries show a typical peripheral arterial flow with high velocity predominantly systolic flow and very low anterograde or retrograde diastolic flow.

As there are no clear anatomic marks for segmentation of the Cx and RCA, the pitfalls in assessing the place of coronary stenoses can be observed.

Frequently, in coronary artery occlusions and retrograde filling distal to occlusions in particular, the mistakes in distinction between coronary artery and concomitant vein may be made, i.e. mid cardiac vein may be assessed as the LAD or posterior cardiac vein – as the

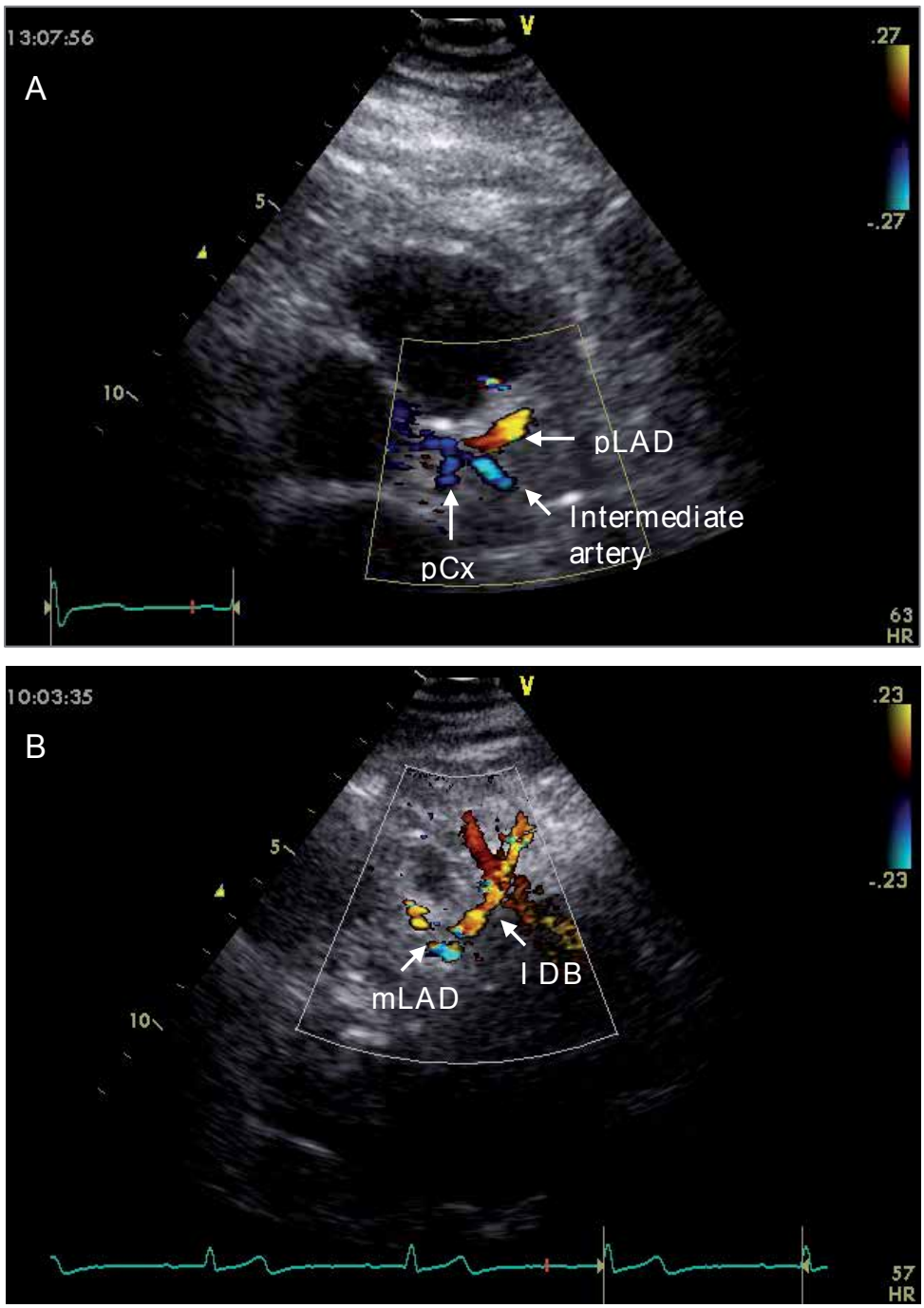

Fig. 5. Large branches of coronary arteries confused with main coronary arteries: A - the intermediate coronary artery, the diameter of which is equal to those of the LAD and Cx; pLAD – proximal LAD, pCx – proximal Cx; B – the first diagonal branch (I DB), which is larger, than mother LAD; mLAD – mid LAD.

PDA (fig 6). As veins are larger, located closer to the right ventricle than arteries and demonstrate a three-phase predominantly systolic flow with very high respiratory variations, it is not very difficult to differentiate between the vessels. Krzanowski et al., 2003 and Youn & Foster, 2004, observed that a strong signal confusing coronary artery flow may

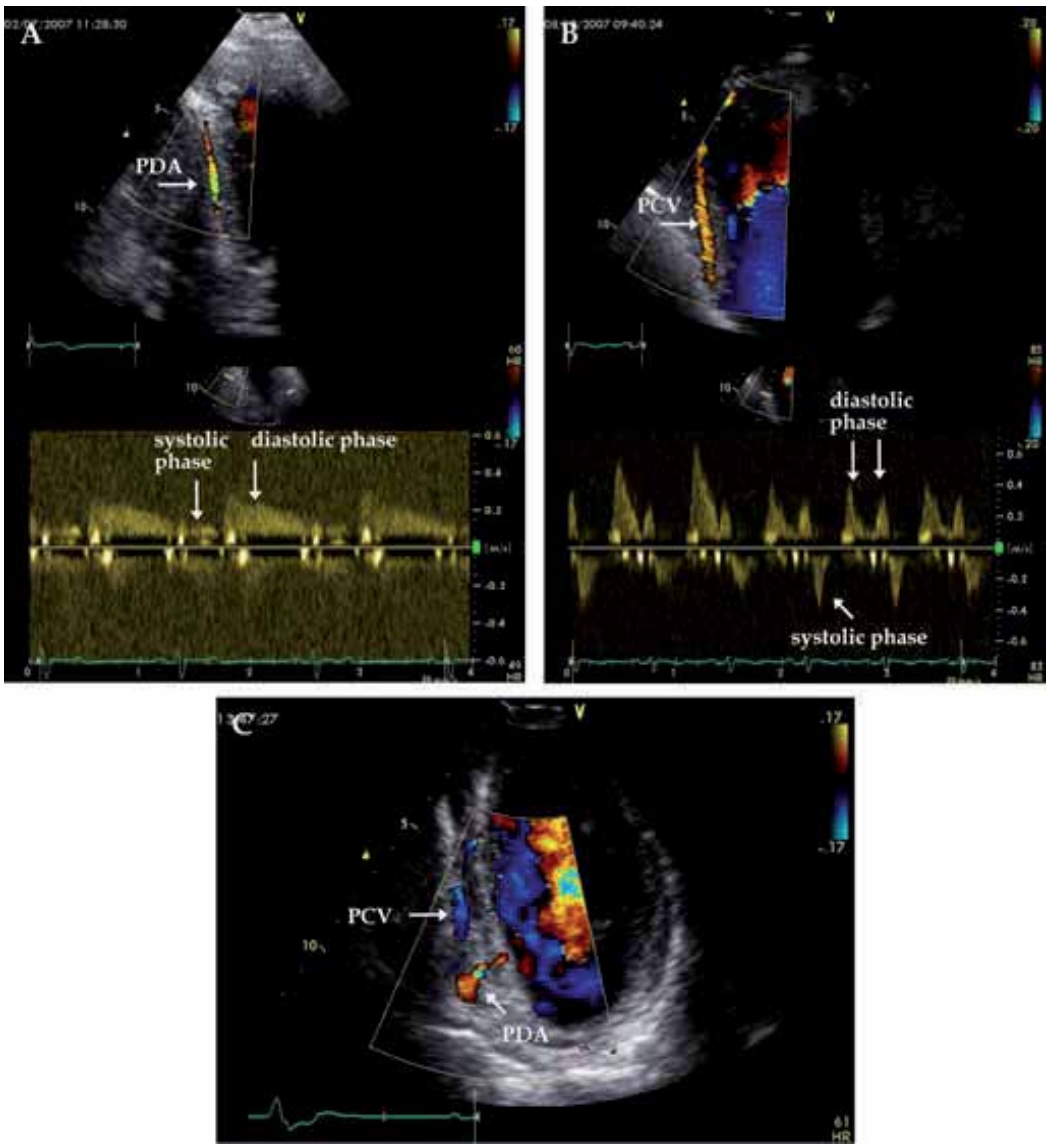

Fig. 6. Distinction between the posterior descending coronary artery (PDA) and concomitant posterior cardiac vein (PCV): A – the PDA is slighter, located closer to the left ventricle and demonstrates a biphasic predominantly diastolic flow without respiratory variations; B – the PCV is larger, located closer to the right ventricle and demonstrates a three-phase predominantly systolic flow with high respiratory variations; C- simultaneous image of the PDA and PCV.

be generated by pericardial fluid, but the flow within the pericardial sac is most pronounced in systole while the coronary artery flow is predominantly diastolic. We did not have similar difficulties in our practice.

2.3 Feasibility of transthoracic echocardiography in visualizing coronary arteries

Feasibility of TTE in visualizing coronary arteries with the addition of harmonics, newer transducers, and contrast agents has been reported to be as high as 100% for distal LAD and 33-97% for the PDA (table 1). Imaging of the entire Cx and proximal and mid RCA has been possible with a low rate (table 1, Kenny & Shapiro, 1992; Krzanowski et al., 2000, Watanabe et al., 2001; Saraste et al, 2006, etc.). Our data (Boshchenko et al., 2008) of TTE feasibility in the assessment of main coronary arteries with both success Color Doppler mapping and good quality of pulse-wave Doppler recording presented in tables 2 and 3 agree with the data of other authors. TTE demonstrated adequate success rate of color Doppler mapping of the LMCA, all LAD segments and PDA, and poor feasibility in detecting other segments of main coronary arteries.

Vessel	proximal segment	mid segment	distal segment
LMCA	70		
LAD	82	83	93
Cx	35	5	31
RCA	25	35	95

Table 2. Success rate of the detection of main coronary arteries by TTE (%) (Boshchenko et al, 2008)

Vessel	one segment (proximal or mid or distal)	two segments (proximal and mid or proximal and distal or mid and distal)	three segments (proximal and mid and distal)
LAD	93	92	68
Cx	35	0	0
RCA	95	51	6

Table 3. Success rate of the detection of one and more segments of each coronary artery by TTE (%) (Boshchenko et al, 2008)

Thus, although this technique requires experience and practice, and only skilled operators can be expected to achieve a 90% success rate in visualizing the coronary arteries, it is possible to use current technology in a clinical setting.

2.4 Normal Doppler systole-diastolic coronary flow pattern and coronary blood flow velocity at rest

2.4.1 Pulse-wave Doppler recording of coronary blood flow velocity by TTE

With pulse-wave Doppler assessment efforts should be directed at maintaining the optimal Doppler angle, and size of the sample volume (Hozumi et al., 1998a, 1998b; Caiati et al, 1999a, 1999b). The angle of incidence between the Doppler beam and flow direction should

be minimized, less than 30 degrees being optimal for coronary artery. The sample volume or gate should be reduced (1.5-3.0 mm) and positioned within the coronary artery (Pellikka, 2004). We usually use a 2-3 mm gate for pulse-wave Doppler, a 3 mm gate for proximal segments and a smaller gate (2 mm) for distal segments.

2.4.2 Normal Doppler systole-diastolic coronary flow pattern in coronary arteries

According to coronary physiology, most coronary flow occurs during diastole with a smaller systolic component (fig. 7) (Gould et al, 1974; Bax, 2006). So, coronary blood flow on pulse-wave Doppler image is presented as continuous, biphasic systole-diastolic flow with predominant diastolic phase and low velocities.

2.4.3 Normal Doppler coronary flow velocities at rest

Transthoracic measurements of coronary flow velocity are proved to be highly reproducible and correlate with invasive measurements and measurements with positron emission tomography (Hozumi et al., 1998a; Caiati et al, 1999a, 1999b, Lethen et al., 2003; Ueno et al., 2002b). The measurements can include assessment of peak velocity, time velocity integral, and mean velocity in systole and diastole (fig. 8). But, in evaluating coronary flow, most investigators measure the diastolic component, and peak diastolic blood flow velocity is assessed most frequently as an easily and quickly estimated characteristic. The duration of diastolic and systolic flow is another potential measure (Crowley & Shapiro, 1998; Hozumi et al., 2000; Daimon et al., 2005; Youn et al., 2005).

Fig. 7. Doppler examination in the PDA: A – laminar flow in the PDA is presented by red linear structure on color Doppler map; B - velocity pattern registered by pulse-wave Doppler; s – systolic phase, d – diastolic phase.

In previous works, control subjects with normal coronary angiograms and normal left ventricular systolic function showed that the peak diastolic velocity in distal LAD was 21.2±7.9 cm/s and the duration of diastolic coronary artery flow was 58.5±6.4% of the R-R interval at rest within the range of physiologic heart rates (60-100 b/m) (Youn et al, 2002). In studies including the participants without significant LAD stenosis, the peak diastolic velocity in distal LAD was ranging from 21±8 cm/s to 28±9 cm/s (Hozumi et al, 1998a, 1998b; Youn et al, 2002; Pizzuto et al, 2004). In our study (Boshchenko et al., 2008) with

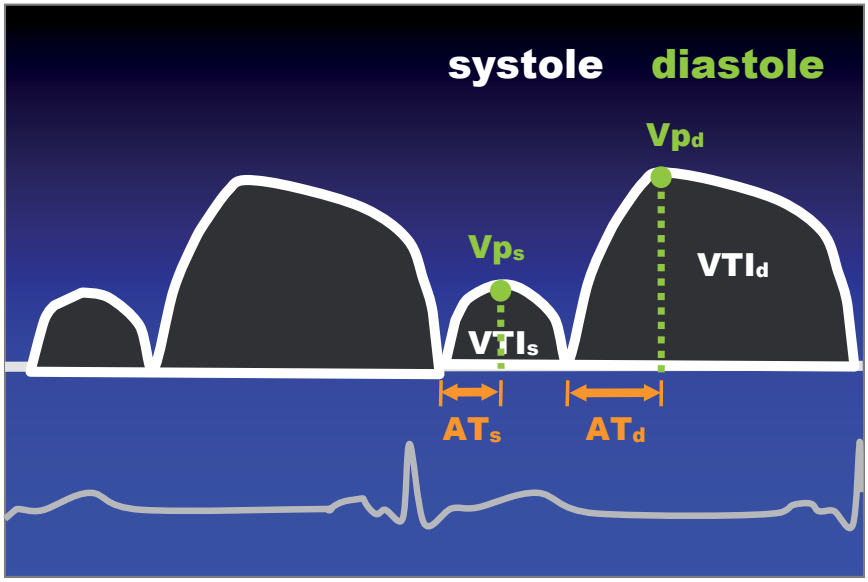

Fig. 8. Scheme of normal coronary blood flow pattern; Vp_s and Vp_d – systolic and diastolic peak coronary flow velocities, VTI_s and VTI_d – systolic and diastolic time velocity integrals, AT_s and AT_d – acceleration time in systole and diastole.

healthy volunteers the coronary flow characteristics were comparable in the conforming segments of the LAD, Cx and RCA, and coronary flow velocities showed a non-significant decrease from proximal segments to distal segments of coronary arteries (tab. 4). But the limits of normal distal LAD flow at rest have not yet been largely settled due to the multitude of variables affecting the baseline coronary flow velocity.

Characteristics	proximal LAD (n=17)	mid LAD (n=16)	distal LAD (n=17)
Vp_s , cm/s	17 (15-21)	14 (12-16)	14 (13-15)
Vm_s , cm/s	13 (12-16)	11 (9-12)	11 (9-12)
VTI_s , cm	3.7 (3.2-4.9)	3.3 (2.7-3.7)	2.8 (2.2-3.6)
AT_s, ms	118 (107-126)	111 (89-118)	111 (96-111)
Vp_d , cm/s	28 (22-35)	25 (22-30)	25 (21-27)
Vm_d , cm/s	22 (16-27)	19 (16-22)	19 (16-20)
VTI_d , cm	10.9 (7.9-13.8)	10.6 (8.8-13.5)	10.4 (8.6-11.9)
AT_d, ms	204 (126-244)	200 (155-222)	156 (133-171)

Table 4. Coronary blood flow in the LAD (mediana (range)). Vp_s and Vp_d – systolic and diastolic peak coronary flow velocity, Vm_s and Vm_d – systolic and diastolic mean coronary flow velocity, VTI_s and VTI_d – systolic and diastolic time velocity integrals, AT_s and AT_d – acceleration time in systole and diastole.

3. Detection of coronary artery stenosis and occlusion by TTE at rest

In adults, TTE is successfully attempted for detection of coronary artery anomalies, arteriovenous fistulas, and coronary aneurysms (Harada et al., 1999; Hiraishi et al., 2000; P.C. Frommelt & M.A. Frommelt, 2004). Thus, the overall sensitivity and specificity of TTE for accurate identification of coronary aneurysms are 95% and 99%, respectively (Hiraishi et al, 2000). Unlike aneurysms, direct visualization of atherosclerotic plaques in non-dilated or mild-dilated coronary arteries with the help of TTE is rather an exception. Accordingly, TTE can not assess correctly the structure and length of atherosclerotic plaques. Unlike computed tomography, TTE as well as coronary angiography detects coronary stenoses basing not on visualizing atherosclerotic plaque *per se* but revealing stenosis of coronary artery lumen in the site of the plaque. But coronary angiography examines anatomy of the entire coronary tree by assessing multipositional images of coronary artery lumen, while TTE assesses primarily the function of coronary vessels by detecting stenosis as focal zones with acceleration and turbulence of coronary blood flow.

3.1 Direct detection of atherosclerotic plaques and assessment of coronary artery wall thickness

Direct detection of atherosclerotic plaques is rarely possible. It is reliable only in case of a major proximal plaque with acoustic shadow due to calcium (fig. 9). Maxted et al, 1998, tried to search for coronary artery stents in proximal and mid LAD, using increased echogenicity, markedly thickened walls of the artery in the stent site, and a stented lumen

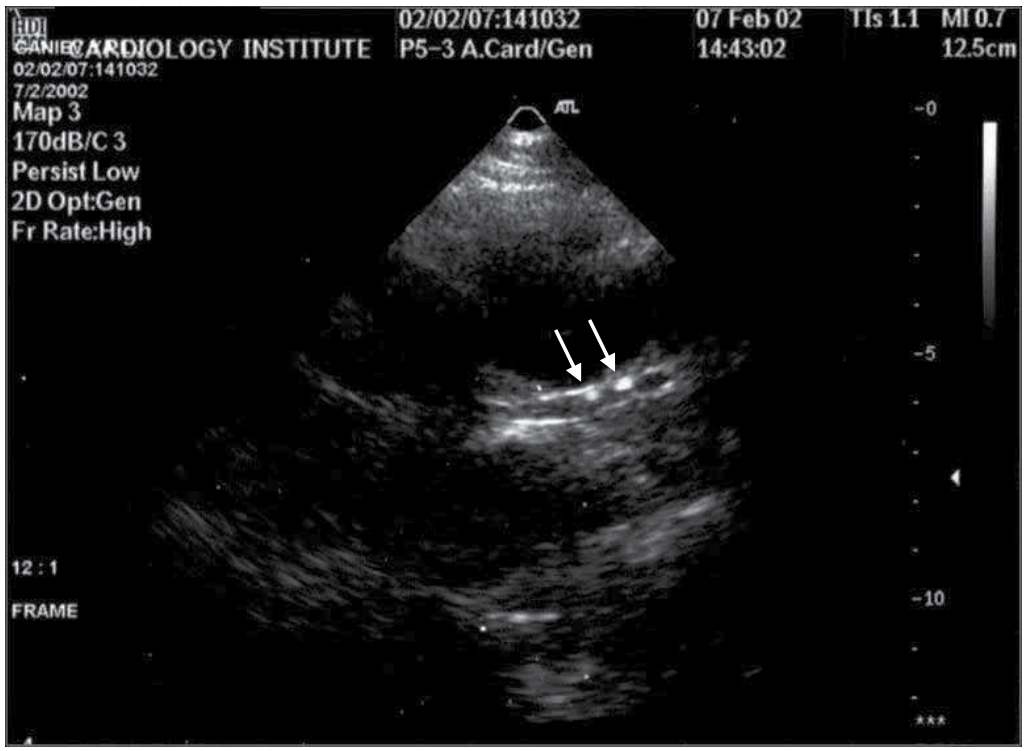

Fig. 9. The cascade proximal plaques with calcium in proximal LAD. B-mode.

with relatively straight edges and a diameter approximately equal to that of the deployed stent. Stents were determined in 10 of 13 cases, but examinations were time consuming, each taking approximately 60 minutes to obtain, process, and review the images. Despite our optimism with this technology, we realize that this method for direct assessment of the plaques and stents *per se* has several limitations.

On the other hand, intravascular ultrasound and epicardial echocardiography studies have demonstrated that coronary atherosclerosis is a diffuse pathological process and before CAD is clinical evident, >90% of the coronary artery tree is atherosclerotic. So, coronary artery wall structure, artery lumen diameter and their qualitative or quantitative changes can be predictors of coronary artery stenosis and direct markers of coronary vasomotor function (Voci & Pizzuto, 2001). High-frequency TTE using a 7.5 or 10 MHz transducer can be used to correctly and accurately measure the wall thickness and diameter of distal LAD and to detect their changes (Kenny et al., 1990; Perry et al., 208a, 2008b). Takeuchi et al, 2006, demonstrated that wall thickness and external diameter of the LAD enlarged with the increase of the number of CAD risk factors, and wall thickness of the LAD >0.72 mm was a predictor of LAD stenosis with 74% sensitivity and 87% specificity. Perry et al, 2008a, found the wall thicknesses and external diameters of the LAD in patients with CAD to be significantly larger than those in normal volunteers, indicating atherosclerotic buildup. TTE can detect vasodilating effects of nitroglycerin and salbutamol on the LAD, correlating with peripheral vascular reactivity to these vasodilators (Perry et al, 2008b). So, TTE is a probably useful tool for noninvasive assessment of coronary vasoreactivity and provides a chance to be a surrogate marker of coronary stenosis (Perry et al, 2008a, 2008b).

3.2 Main Doppler principles of the detection of coronary stenosis by TTE

The detection of coronary stenosis by TTE should be performed basing on Doppler methods – color Doppler mapping and pulse-wave Doppler recoding.

3.2.1 Focal aliasing and flow acceleration in the site of a significant stenosis

First, Color Doppler mapping should be used for stenoses search. Even at low Nyquist limits (13-18 cm/s), the color Doppler flow pattern in the normal coronary artery is uniformly consistent with laminar flow. The focal flow acceleration and turbulence may be detected as aliasing zone on color Doppler map and can assist in localizing the stenosis site (Krzanowski et al, 2000; Hozumi et al., 2000; Takeuchi et al., 2001; Saraste et al., 2005). Second, Doppler velocity patterns should be registered by pulse-wave Doppler. If Doppler color mapping shows a color laminar flow, Doppler velocity patterns should be obtained in the site with the best signal on color Doppler map. If a local increase of the velocity appears on Doppler colour flow map as a localized area of aliased and disturbed signal, Doppler velocity patterns should be obtained in the site of alising (fig. 10).

Third, coronary blood flow should be measured. Velocities of coronary blood flow in the stenosis site are increased compared with non-stenosis sites (both prestenotic and poststenotic) (Hozumi et al., 2000, Okayama et al., 2008). Some authors have shown redistribution of coronary flow in LAD stenosis with the increase of systolic wave due to stenosis and decrease of diastolic-to-systolic velocity ratio in the poststenotic site (Crowley & Shapiro, 1998; Daimon et al., 2005). There are transthoracic quantitative diagnostic markers of coronary artery stenosis >50%, offered by Krzanowski et al, 2000, and Anjaneyulu et al., 2008, based on the measurement of peak diastolic velocity in the stenosis site. The peak diastolic velocity >1.5 m/s has demonstrated 85% sensitivity and 88%

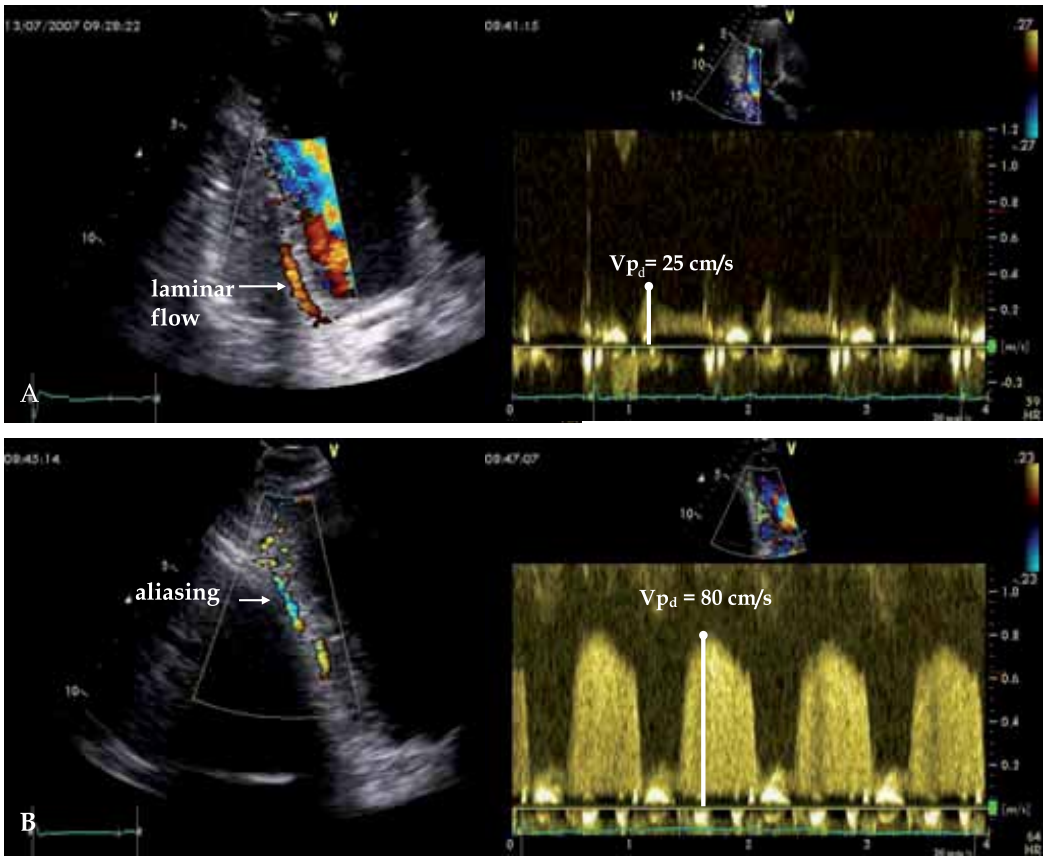

Fig. 10. Examples of Color Doppler flow mapping of distal RCA presented by PDA: upper panel – patient C. without RCA stenosis: artery color flow map is red indicating low velocity with peak diastolic flow velocity (Vp_d) equal to 25 cm/s; lower panel – patient S. with 75% stenosis of mid RCA: Changes in the color flow map for color aliasing in the stenosis site and distal to it with Vp_d acceleration up to 80 cm/s.

specificity diastolic velocity >1.5 m/s has demonstrated 85% sensitivity and 88% specificity in the detection of LMCA stenosis>50% (Anjaneyulu et al., 2008). Krzanowski et al., 2000, and Saraste et al., 2005, showed that a local peak diastolic flow velocity >2.0 m/s could be used as a sign of diameter reduction>50% for all three main coronary arteries. It seems attractive and easy to detect coronary stenoses basing on the search of the aliasing zone and the measurement of peak diastolic flow velocity only, but both methods are semi-quantitative and, unfortunately, inaccurate. As previously reported, laminar peak diastolic velocity in the coronary artery is from 0.21 ± 0.08 m/s (Youn et al., 2002, Hozumi et al., 1998) to 0.28 ± 0.09 m/s (Pizzuto F., 2004), and the velocity will not exceed 1 m/s even in case of its 3-4-fold increase in the stenosis site. So, local peak diastolic flow velocity >2.0 m/s is a highly specific, but low sensitive marker of coronary artery stenosis. On the other hand, some other reasons, such as reologic factors, heart rate, etc. can cause disturbed Doppler signal, hence local aliasing may be a highly sensitive but low specific sign of coronary stenosis. So, Hozumi et al, 2000, have observed localized aliasing by color flow mapping in 100% of patients with LAD restenosis >50% after percutaneous transluminal coronary

angioplasty (PTCA), and 56% of patients without restenosis. Finally, both markers are strongly dependent on different hemodynamic factors (perfusion pressure, heart rate, blood pressure, myocardial mass, etc.) and technical setting (depth of scanning, quality of pulse-wave Doppler pattern, angle of incidence between the Doppler beam and flow direction, etc). So, most authors have supposed that the ratio of stenotic-to-prestenotic diastolic velocity is more correct and allows an exclusion or minimization of hemodynamic influence.

3.2.2 The ratio of stenotic to prestenotic blood flow velocity

To measure the stenotic to prestenotic velocity ratio, the localized color aliasing corresponding to local flow acceleration should be searched first to obtain coronary flow velocity in the stenosis site. When localized aliasing is detected, the sample volume of pulse-wave Doppler should be set at the aliasing and Doppler velocity pattern should be recorded. Then, the sample volume should be slightly moved from the aliasing to the prestenosis site and spectral Doppler recording should be made again (fig. 11).

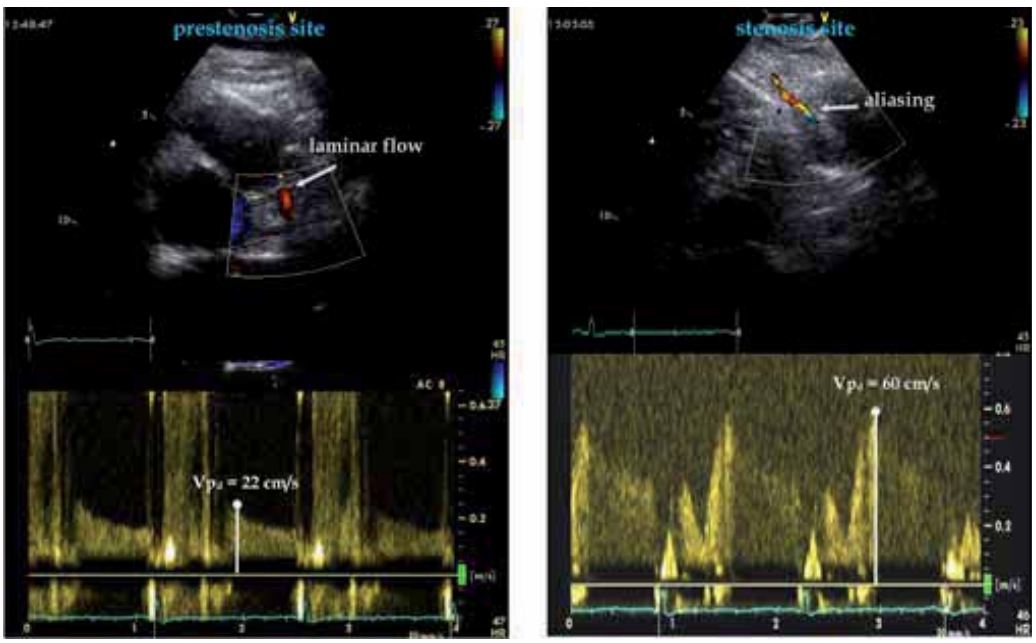

Fig. 11. 70% stenosis in mid LAD. Left panel – prestenosis site; right panel – stenosis site; Vp_d – peak diastolic blood flow velocity. The stenotic to prestenotic flow velocity ratio is 60/22 = 2.72 (>2.0).

Krzanowski et al., 2000, and Saraste et al., 2005, determined the stenotic-to-prestenotic peak blood flow velocity ratio over than 2.0 to be a sign of >50% stenosis with 62-100% sensitivity and 92-100% specificity for the LAD, 63% sensitivity and 96% specificity for the RCA, and 38% sensitivity and 99% specificity for the Cx. The same sensitivity (86%) and specificity (93%) were demonstrated by Hozumi et al., 2000, for the ratio of prestenotic-to-stenotic mean diastolic velocity <0.45 in the detection of LAD restenosis after PTCA. TTE with the use of both a local peak diastolic flow velocity >2.0 m/s and stenotic-to-prestenotic peak blood flow velocity ratio >2.0 permitted to reveal 48% of all LAD stenoses, 30% of all Cx

stenoses and 14% of all RCA stenoses (Krzanowski et al., 2000). Like the assessment of peak diastolic velocity this approach is easy, semi-quantitative and, though to a lesser extent, dependent on hemodynamic factors too. On the other hand, it is known that volume blood flow velocity in the prestenosis site is equal to that in the stenosis one, and, assuming the constant artery diameter and measuring diastolic time velocity integrals in both stenosis and prestenosis sites, the vessel % stenosis can be calculated according to a modified continuity equation. The feasibility of this approach for transesophageal echocardiography was demonstrated by Isaaz et al, 1998 (as cited in Vrublevsky et al., 2001). We use this concept for TTE. Diastolic time velocity integral (VTI_d) is measured in stenosis and prestenosis sites, and the stenosis % area is calculated as: stenosis, % = 100 × (1 - prestenotic VTI_d / stenotic VTI_d) (fig.12). The sensitivity and specificity of >50% stenosis identification by a modified continuity equation in the LAD are 72% and 96%, in the Cx - 40% and 94%, and in the RCA – 50% and 93%, respectively, for the segments which are successfully visualized (Boshchenko et al., 2008). The data on all segments, successfully visualized and unvisualized, are shown in table 5.

Coronary artery	Coronary angiography	Transthoracic echocardiography						
	stenosis	stenosis	false positive	false negative	sensitivity	specificity	positive predictive value	negative predictive value
LMCA (n=100)	1	0	0	1	-	100	-	97
LAD (n=300)	55	39	8	24	56	96	79	89
Cx (n=300)	28	6	4	26	7	94	33	70
RCA (n=300)	47	21	9	35	26	93	57	78

Table 5. Diagnostic accuracy of Doppler TTE in the detection of >50% stenosis in main coronary arteries (Boshchenko et al., 2008).

Thus, TTE is highly specific for identification of coronary stenosis >50%, but, being low sensitive for Cx and RCA stenosis, may be helpful as a screening tool for LMCA and LAD stenosis >50%, limiting the use of groundless catheterization.

3.3 Main Doppler principles of the detection of coronary occlusion by TTE

TTE permits visualizing separate segments of coronary arteries only, and the detection of coronary occlusion as well as stenoses by TTE should be performed basing on Doppler color-coding of the flow velocity and direction. The first offered TTE sign of coronary artery occlusion was the absence of coronary blood flow on color Doppler map, and it demonstrated very low feasibility of the method (Krzanowski et al., 2000) because it was difficult to differentiate between real absence of coronary flow due to occlusion and absence of visualization of coronary artery due to TTE limitations. So, at present, TTE does not seem to be a suitable method for the assessment of acute coronary occlusions. But transthoracic color-Doppler ultrasound can be useful after acute myocardial infarction for the detection of an open LAD, reflecting adequate myocardial reperfusion basing on anterograde flow in the LAD alone or together with perforators (Voci et al, 2002).

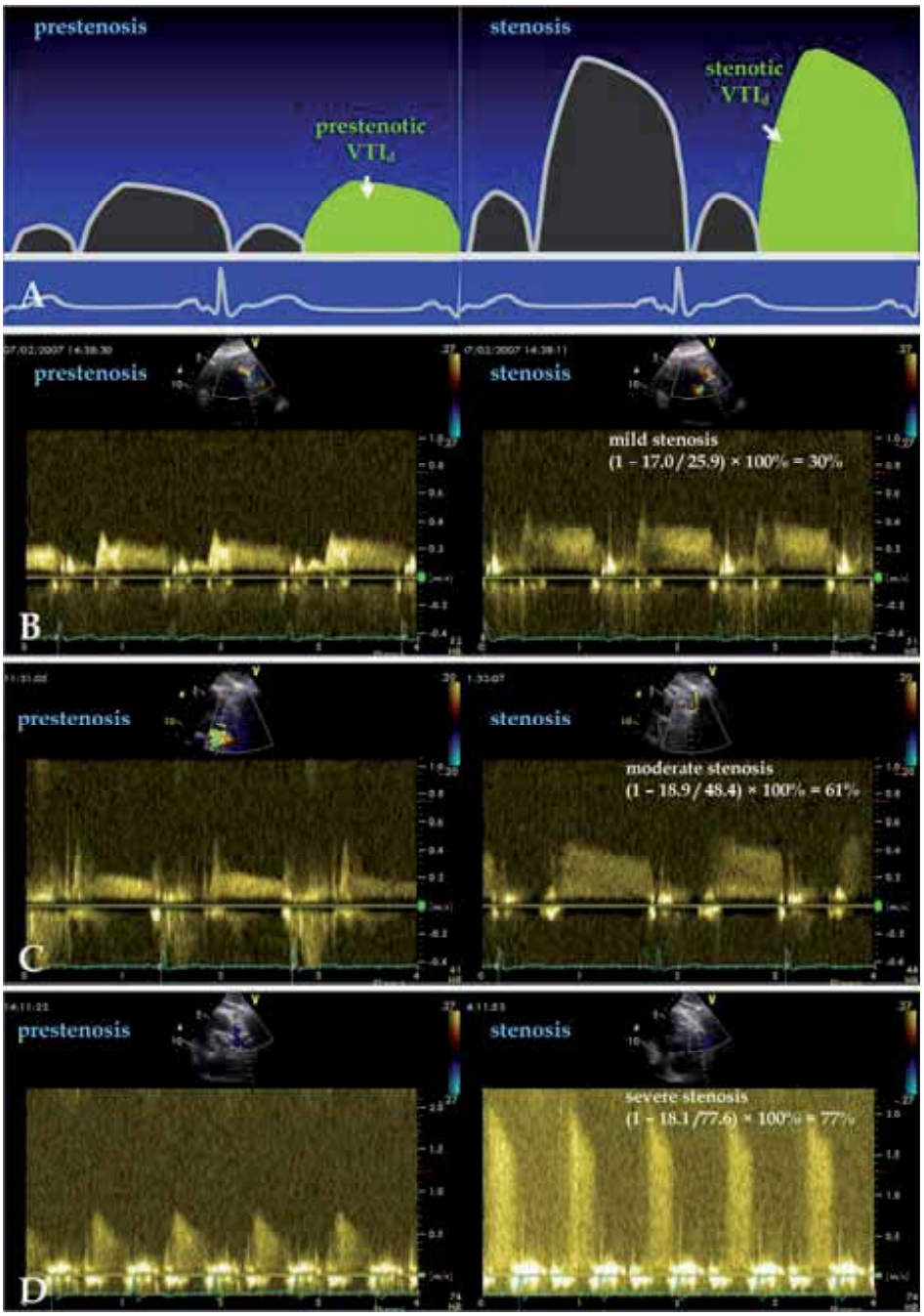

Fig. 12. Calculation of stenosis % area of proximal LAD according a modified continuity equation by TTE. A - scheme of calculation by TTE; B - example of Doppler flow patterns in patient with 35% stenosis according to coronary angiography data; C - Doppler flow patterns in patient with 60% stenosis; D - Doppler flow patterns in patient with 80% stenosis; VTId – diastolic time velocity integral

Unlike acute occlusion, chronic total coronary occlusion (CTO) of over 1-month duration leads to the formation of stable collateral coronary pathways (Werner & Figulla, 2002; Braden, 2006; Werner et al., 2006). Recently, feasibility of TTE for the CTO identification of the LAD and RCA has been established. Watanabe et al., 2001, proposed the inversion of the coronary blood flow in the epicardial collateral vessels on color Doppler map to be a main ultrasound sign of CTO. It has been established that retrograde flow in distal LAD is a good marker of LAD occlusion with 88% sensitivity and 100% specificity (Hirata et al., 2004) and retrograde flow in the PDA is a good marker of RCA occlusion with 67% sensitivity and 100% specificity (Otsuka et al., 2005). But Pizzuto et al., 2006, doubted such a high sensitivity of TTE in the detection of LAD occlusion having revealed a retrograde flow in distal LAD in only 43% of patients with CTO and normal anterograde flow in more than half patients (55%). Angiography data demonstrate that in LAD and RCA occlusions, besides collateral flow distal to the occluded region through the connections on the epicardial surface, 63-86% of patients have intramyocardial collateral channels lying usually in the interventricular septum. According to these findings an additional examination of the retrograde flow in the septal branches of the LAD and RCA has demonstrated the increase of TTE sensitivity in the CTO detection from 88 to 96% for the LAD (Hirata et al., 2004) and from 67 to 80% for the RCA (Otsuka et al., 2005, Saraste et al., 2005).

According to our data (Boshchenko et al., 2009), the sensitivity and specificity of retrograde flow for identification of the occluded LAD by TTE in distal LAD alone were 77% and 97%, and those in both distal LAD and septal branches of the LAD - 85% and 97%, respectively. The sensitivity and specificity of retrograde flow for identification of the occluded RCA by TTE in the PDA alone were 77% and 98%, and those in both the PDA and septal branches of the RCA - 88% and 98%, respectively. Basing on these data the following order of TTE detection of LAD and RCA occlusion could be offered. First, potential epicardial collateral vessels should be examined. The retrograde flow in distal LAD will be a highly specific marker of LAD occlusion, and the retrograde flow in the PDA will be a sign of RCA occlusion, respectively. In this case, the examination can be stopped. Second, if the flow in distal LAD and RCA is anterograde, the septal (intramyocardial) collateral pathways of the LAD and RCA should be examined additionally, because 8% of patients with LAD CTO and 11% of patients with RCA CTO demonstrate an inversion of the flow direction in the septal branches only (Boshchenko et al, 2009) (fig. 13). Unlike the LAD and RCA, low success rate of visualization of the Cx does not allow the use of TTE for CTO detection.

TTE detection of CTO is based on the identification of coronary blood flow direction alone without the assessment of the rest Doppler coronary flow velocities and, thus, it does not depend on the hemodynamic conditions of the patients and does not require discontinuation of the therapy including antianginal, hypotensive, and antiarrhythmic drugs. So, TTE can be used in unstable patients, patients with life-threatening rhythm and conductance disturbances. TTE can be rather an attractive non-invasive method with little time consumption and good specificity in standardized approach, answering the principal question – whether the LAD or RCA is chronically opened or closed. The length and accurate site of CTO can not be assessed by TTE.

4. Noninvasive assessment of coronary flow reserve in main coronary arteries by TTE

Over the last two decades it has been demonstrated that measurements of coronary flow reserve (CFR) are a diagnostic approach providing a lot of additional information on the

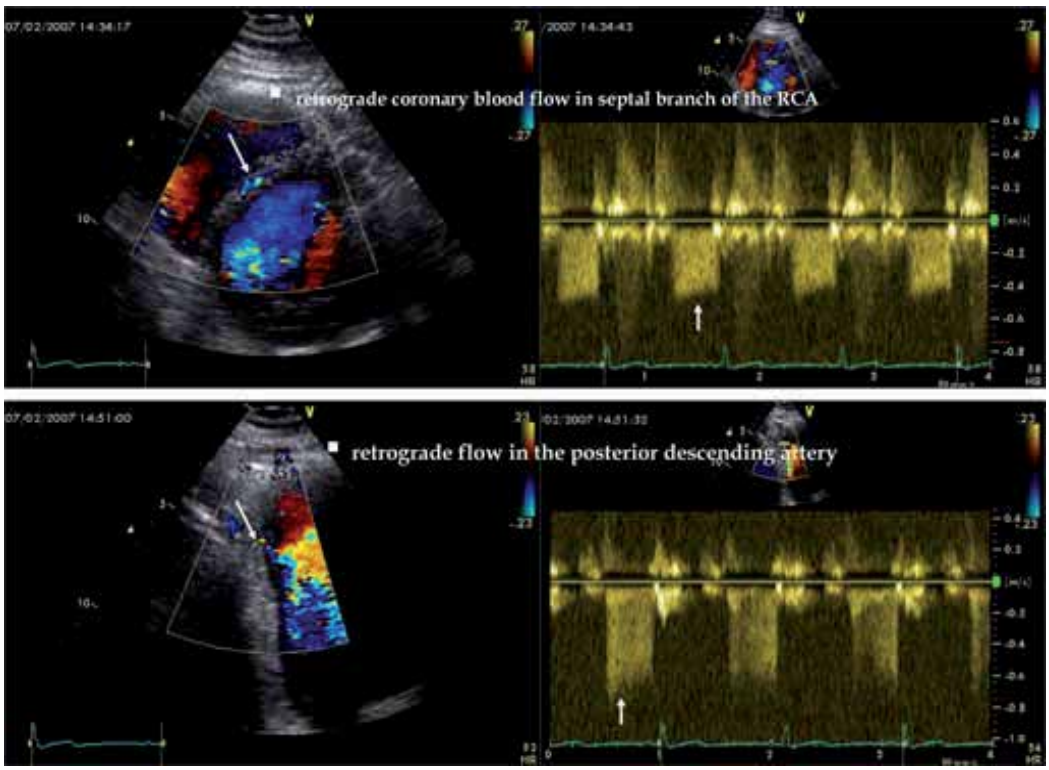

Fig. 13. Patient B. Proximal occlusion of the right coronary artery (RCA). Examples of the retrograde coronary blood flow in septal branch of the RCA and in the PDA (intramyocardial and epicardial collateral flow, respectively).

function of coronary artery and assisting in the decision-making process of cardiac interventions. Reserve of coronary blood flow is defined as the ability of coronary flow volume to increase under maximal coronary hyperemia when compared with coronary flow volume at rest. Coronary flow velocity correlates well with flow volume, and measurements of the ratio of hyperemic-to-rest coronary flow velocity can substitute direct measurements of the ratio of hyperemic-to-rest flow volume. At present, intracoronary Doppler with adenosine or papaverine infusion remains to be the reference standard for the assessment of coronary flow velocity reserve (CFVR) in vivo. In normal coronary bed, microvascular flow at rest is low-optimal for the maintenance of wall perfusion, and it increases at stress due to maximal peripheral vasodilatation according to myocardial demand; normal epicardial coronary artery with a high degree of elasticity under these conditions demonstrates a high CFVR. In agreement with experimental and intracoronary wire studies (Gould et al., 1974; Gould & Lipscomb, 1974), three main mechanisms, alone or in combination, may explain why coronary flow does not increase or even decreases during adenosine infusion in stenosis. First, in mild, moderate or tight severe stenoses, CFVR reflects the state of microcirculation to a greater degree. Epicardial stenoses induce increased resistance to flow, and microvascular resistant vessels already are dilated maximally at rest to maintain the basal flow. Therefore, a hyperemic stimulus results in a smaller increment of poststenotic flow, and the change of flow or the ratio of hyperemic-to-rest flow are less, and CFVR distal

to the stenosis site is reduced. Second, in incompletely calcified severe coronary stenosis, the coronary artery preserves some degree of elasticity and may collapse during adenosine infusion allowing a decrease in intraluminal tense pressure, which is induced by flow acceleration in the stenosis site. This collapse may translate distally into a damped flow producing at times a coronary steal effect. Third, prestenotic collaterals may be opened at stress, causing coronary redistribution from the critically hypoperfused bed to less stenotic regions. So, CFVR should be measured in the most distal part of the epicardial artery for assessing its function along the entire length.

TTE is a good method for detection of distal flow in the LAD and PDA (usually distal RCA), and it has the main advantage among other noninvasive technologies being valid for easy, fast, direct assessment of distal CFVR. That is particularly important in cases of repeated monitoring of stenosis progressive changes. Hozumi et al, 1998a, 1998b, performed the first validation of TTE comparing CFVR in the LAD with simultaneous intracoronary Doppler guide wire assessment. TTE reflected invasive measurement of coronary flow velocity and CFVR accurately, and the agreement between the two methods was 0.97 for averaged diastolic and systolic peak velocities, 0.98 for diastolic peak velocity and 0.97 for CFVR. A very good correlation was observed between TTE and intracoronary Doppler guide wire not only for the LAD (Caiati et al., 1999a, 1998b; Lethen et al., 2003) but for the PDA as well (Ueno et al., 2002b). The correlation ranging from 0.79 to 0.97 has been reported for noninvasive techniques compared with invasive ones in cases of decision-making process (Hozumi et al., 1998a, 1998b; Caiati et al., 1999a, 1998b) and intracoronary intervention (Lethen et al., 2003). Thus, TTE can be used as a possible substitute of the invasive method for correct clinical measurement of CFVR, particularly in the LAD.

4.1 Comparison and choice of optimal stress agents for transthoracic measurement of CFR

A variety of agents can be used as vasodilators to examine CFVR by TTE including adenosine, dipyridamole and dobutamine with or without additional atropine (Pellikka, 2004; Korsarz & Stein, 2004). All drugs have potent endothelium-independent vasodilating properties, but act by different mechanisms. Dobutamine increases metabolic myocardial demand inducing the rise of rate-pressure product, and accordingly contributes to the increase of coronary flow. Both adenosine and dipyridamole induce coronary arteriolar vasodilatation associated with hyperemic coronary flow as a result of stimulation of adenosine A2-receptors on arteriolar smooth-muscle cells causing vasorelaxation.

As dobutamine stress echocardiography is widely available, it is appropriate to consider a combination of CFVR examinations and these tests (Pellikka, 2004). But dobutamine infused with the traditional rate of 5-10-20 mcg/kg/min induces a smaller acceleration of the flow and lower CFVR than adenosine as a reference standard (Meimoun et al., 2006). Similar CFVR values can be obtained with two drugs at peak dobutamine infusion only (with the infusion rate of 40 mcg/kg/min). But it is not always possible in patients with positive dobutamine stress echocardiography and chest pain. Furthermore, CFVR assessment with recording of the LAD or RCA flow velocity at each step is time-consuming and requires an average of 25-30 minutes. A number of technical difficulties during TTE with dobutamine also must be stressed because a high heart rate and consequently a reduced diastolic filling time and increased myocardial contractility can impair good-quality recording of the coronary flow pattern. This is particularly true for very high heart rates at peak stress test.

Like intracoronary Doppler wire assessment of CFVR, adenosine, a direct coronary vasodilator, seems to be a drug of the first choice for TTE. Intravenous adenosine has a short half-life (8-10 seconds) inducing rapid onset of vasodilatation and resulting in short examination periods. CFVR assessment may be performed at bedside within a few minutes. Adenosine should be infused intravenously at the rate of 140 mcg/kg/min for 2 minutes. Coronary flow velocities are measured before and immediately after the cessation of adenosine infusion. But if simultaneous evaluation of regional function is necessary, repeated infusion of adenosine is required, and the effect of ischemic preconditioning can be observed. Adenosine may be a cause of adverse effects, attributed to nonselective stimulation of the A1-, A2B-, and A3-receptors, because of its lack of specificity for the A2-receptors. Among these effects, arterial hypotension, dyspnea and chest discomfort are frequent and tend to impair LAD flow velocity recording during hyperemia.

Dipyridamole is an indirect vasodilator, decreasing the cellular uptake of adenosine and increasing its endogenous level. Compared with adenosine, the duration of the infusion is longer (0.56 mg/kg for 4 minutes or 0.84 mg/kg for 6 minutes) and, depending on the dose administered and infusion rate, its effects can last up to 30 minutes allowing a combined CFVR and wall motion evaluation. Peak vasodilating activity is obtained 2 to 4 minutes after the cessation of dipyridamole infusion. In agreement with the comparative TTE study of Lim et al, 2000, dipyridamole with the infusion rate of 0.56 mg/kg may induce lower CFVR than adenosine, and CFVR value, achieved with adenosine, can be obtained with dipyridamole infusion rate of 0.84 mg/kg only. Adverse effects after dipyridamole infusion appear slowly and have a less intensity than those after adenosine, without marked dispnea and arterial hypotension. Both adenosine and dipyridamole are contraindicated in patients with active asthma or severe chronic obstructive pulmonary disease with wheezing. The vasodilative effect of both drugs is reversed by antagonists, such as methylxanthines, which compete for the A2 receptor, and therefore, foods and drinks containing xantines (chocolate, coffee, tea, colas, etc.) should be avoided for at least 12 hours before testing.

In summary, adenosine should be a drug of choice for TTE, if CFVR assessment is required in one artery only. If combined evaluation of regional function and CFVR assessment in the LAD or both the LAD and PDA are planned, the choice should be made in favor of dipyridamole with the infusion rate of 0.84 mg/kg. Dobutamine could be a good alternative to adenosine and dipyridamole for CFVR assessment in patients with contraindications to both drugs or scheduled dobutamine stress echocardiography. If echocontrast infusion is necessary, a separate intravenous line should be made.

4.2 Cut-off level for normal and pathologic CFVR

4.2.1 TTE calculation of CFVR

For CFVR assessment, the correction of angle incidence between the Doppler beam and flow direction may be used; however, as evaluation is performed on the basis of ratio values, the absolute velocity is of less importance. However, the angle should not change between the baseline and hyperemic phases of the test, so the differences in blood flow velocity can still be compared. After obtaining the baseline and hyperemic coronary flow velocities, CFVR should be expressed as the ratio of coronary flow velocity under maximal vasodilatation to coronary flow velocity at rest (fig.14) (Picano et al., 1999; Korcarz & Stein, 2004).

Various investigators consider that the peak diastolic flow velocity (Vp_d), mean diastolic flow velocity (Vm_d), and average peak diastolic flow velocity (AVp_d) can be used as a reference parameter for CFVR assessment. Vp_d, being a most easily and quickly measured

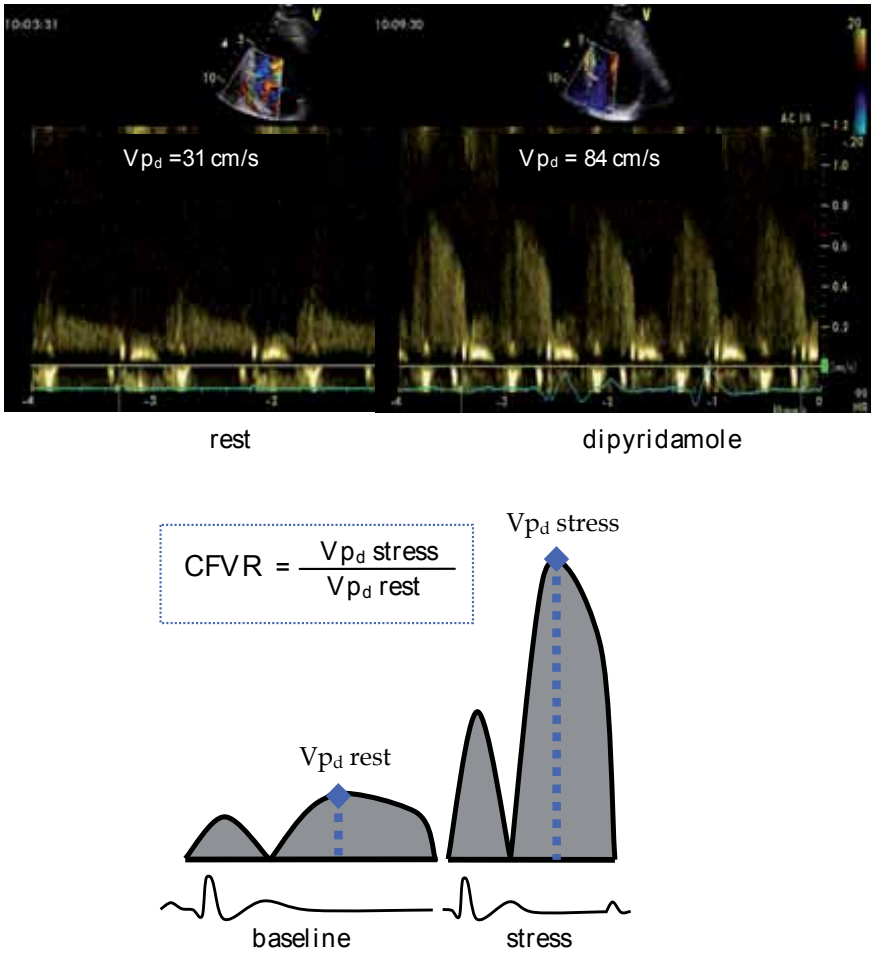

Fig. 14. Example of CFVR evaluation in the PDA. Vp_d – peak diastolic coronary flow velocity.

index, demonstrates a higher inter- and intra-observed variability of assessment than Vp_m and AVp_d. But CFVR evaluation is based on the ratio values of the same parameters, and similar values of CFVR were reported for Vp_d and Vm_d (Hozumi et al., 1998; Caiati et al., 1999b). The time required to complete a CFVR test in one artery is around 5–8 minutes depending on the investigators' experience.

4.2.2 Cut-off level of CFVR for significant stenosis in the LAD and RCA

Pizzuto et al, 2001, 2003 revealed CFVR value for the LAD ranging from 2.90±0.58 to 3.05±0.81 in control group of patients with angiographically normal coronary arteries, and similar values for CFVR were found in <50% LAD in-stent restenosis (Lambertz et al., 1999; Pizzuto et al, 2003). Later, CFVR in angiographically normal LAD patients with remote CAD (presence of previous remote myocardial infarction and wall motion abnormalities) was determined also as preserved (Pizzuto et al, 2004). According to numerous studies using

TTE the cut-off value of CFVR is generally accepted to be 2.0 for predicting significant LAD stenosis in patients under decision-making process, after intracoronary intervention, and with in-stent restenosis (table 6). The sensitivity and specificity of Doppler TTE measurement of CFVR for detecting significant angiographic disease were satisfactory, ranging from 77% to 94% and 65% to 100%, respectively, slightly depending on the study population, dose of stress medications and cut-off value of diameter % stenosis. In one study, CFVR with a cut-off value <2.0 was a significantly better predictor (90%sensitivity, 96%specificity) of LAD stenosis than multidetector computed tomography (80%sensitivity, 93%specificity) and improved the diagnostic accuracy of the latter from 90 to 96% (Pizzuto et al., 2009). A cut-off value <2.0 was appropriate even for the diagnosis of significant stenosis of donor LAD giving collaterals to other arteries (Pizzuto et al., 2004; Iwata et al, 2006).

Several investigators studied the accuracy of Doppler TTE measurement of CFVR in the PDA to detect stenosis in the RCA, and the same cut-off value of CFVR (<2.0), demonstrating sensitivity 84-89% and specificity 83-96%, was accepted for predicting significant RCA stenosis (Voci et al., 2002; Takeuchi et al., 2004).

Authors	Diameter stenosis, %	CFVR, cut-off value	Reference parameter	Stress agent	Sens	Specif	Population
Hozumi et al., 1998b	>70%	<2.0	Vp_d, Vm_d	adenosine	92%	82%	unselected
Caiati et al., 1999a	>70%	<2.0	Vp_d, Vm_d	dipyridamole	86%	90%	unselected
Voci et al., 2002	>70%	<2.0	Vp_d	adenosine	89%	100%	unselected
Voci P et al., 2003	>90%	<1.0	Vp_d	adenosine	92%	98%	unselected
Nohtomi Y. et al., 2003	>50%	<2.0	Vp_d	dipyridamole	94%	65%	unselected
Lethen et al., 2003	>70%	<2.0	S/D mean velocity	adenosine	89%	90%	after PTCA
Takeuchi et al., 2004	>50%	<2.0	AVp_d	adenosine	91%	75%	unselected
Matsumura et al., 2003	>70%	<2.0	Vm_d	adenosine	90%	93%	unselected
Rigo et al., 2003	>50%	<1.9	Vp_d	dipyridamole	81%	84%	unselected
Okayama et al., 2002	>70%	<2.0	AVp_d	adenosine	94%	88%	unselected
Pizzuto et al., 2003	>70%	<2.0	Vp_d, Vm_d	adenosine	91%	95%	after stenting
Lowenstein et al., 2003	>70%	<2.0	Vp_d	dipyridamole	87%	73%	unselected
Hirata et al., 2006	>50%	<2.0	Vm_d	adenosine	77%	100%	after PTCA
Pizzuto et al., 2009	>70%	<2.0	Vp_d	adenosine	90%	96%	unselected

Table 6. Sensitivity and specificity of Doppler TTE measurement of CFVR for detecting significant LAD stenosis. Vp_d – and Vm_d – peak and mean diastolic coronary flow velocities, AVp_d – average peak diastolic velocity of coronary blood flow, S/D mean velocity – systole-diastolic mean velocity of coronary blood flow, Sens – sensitivity, Specif – specificity.

4.2.3 Cut-off value of CFVR for occluded LAD and RCA

CFVR can be a marker of chronic occluded LAD or RCA function. CFVR is always depressed in the collateral dependent vascular area, and the value<2.0 is registered in the LAD or PDA distal to the occlusion in all cases (Takeuchi et al., 2005, Pizzuto et al., 2006). But TTE is able to reveal patients with CFVR <1.0 reflecting coronary steal effect which

develops in 33% to 47% of occluded vessel territories (Takeuchi et al., 2005, Pizzuto et al., 2006). Coronary steal effect occurs when collateral circulation is poor and the donor artery is significant stenotic (Pizzuto et al, 2006; Braden, 2006).

4.3 CFVR for detecting myocardial ischemia and assisting in the decision-making process in patients with chest pain

Transthoracic CFVR measurement may have incremental predictive value in addition to stress echocardiography for detecting CAD in LAD and RCA distribution. In the study by Rigo et al., 2003, CFVR<1.9 in the LAD correlated well with stenosis severity and wall-motion abnormalities. The inverse correlation between CFVR and peak wall motion score index in the overall study population was significant ($r=0.46$: $p<0.001$), and the concordance between the 2 techniques was good (80%) (Rigo et al, 2003). CFVR had excellent sensitivity (81-94%) but moderate or good specificity (65-84%) in detecting significant LAD stenosis whereas wall motion score index showed lower sensitivity (69-74%) and higher specificity (82-95%), and both techniques demonstrated a similar diagnostic accuracy ranging from 82 to 86% for CFVR and from 81 to 84% for wall motion score index (Rigo et al, 2003; Lowenstein et al., 2003; Nohtomi et al., 2003). So, the data for flow and function may be complementary in predicting the underlying angiographic findings and increase the diagnostic accuracy up to 94%(Nohtomi et al., 2003), because abnormal wall motion may confirm and negative CFVR may exclude CAD more accurately. So, CVFR measurement is more important in patients with negative stress echocardiography by wall motion criteria.

Similarly, transthoracic CFVR by Doppler is associated with perfusion abnormalities, as it has been shown with exercise single photon emission computed tomography thallium imaging. Most patients with abnormal perfusion in the LAD territory had abnormal CFVR in the LAD; those with perfusion defect in the RCA territory had abnormal CFVR in the PDA; vise versa, patients with normal exercise perfusion had CFVR greater than 2.0. A transthoracic CFVR in the LAD <2.0 provided data consistent with those obtained by single photon emission computed tomography for physiologic estimation of stenosis severity with 94% sensitivity and 100% specificity (Hirata et al., 2006). Tokai et al., 2003, showed the equivalent potential of transthoracic CFVR in the PDA for detecting myocardial ischemia in the left ventricular inferior region and RCA stenosis. As has been reported by Fujimoto et al, 2004, CFVR in the Cx could be obtained in 72% of patients and CFVR<2.0 had 92% sensitivity and 96% specificity for predicting the perfusion defect in the Cx territory.

In one comparative study (Osorio et al., 2007) with simultaneous CFVR assessment and quantitative analysis of myocardial contrast perfusion, CFVR was a much better index for detecting LAD stenosis than myocardial blood flow reserve (sensitivity 92% and 84%, specificity 94% and 87%, and accuracy 93% and 86% for CFVR and myocardial reserve, respectively).

In summary, CFVR measurement may be a useful tool for detecting myocardial ischemia in the LAD distribution and, probably, in the RCA and Cx territory.

4.4 CFVR in moderate and severe coronary artery stenosis

Evaluation of patients with angiographic moderate coronary artery stenosis is challenging. Neither visual assessment of an angiogram nor quantitative coronary angiography can accurately predict the significance of most moderate stenoses (50–70%). Rest coronary flow is preserved until severe narrowing occurs (>80%), but according to the experimental study

in mice (Wikstrom et al., 2005), there is a close correlation between CFVR in the left coronary artery and coronary minimal lumen diameter of this vessel ($r=0.87$, $p<0.005$), and CFVR depends on the diameter % stenosis. In clinical studies, the degree of angiographic stenosis also is related to CFVR in both the LAD and RCA (Lambertz et al.,1999; Pizzuto et al., 2001; Voci et al., 2002, 2003; Sicari et al., 2009). With insignificant LAD stenosis (<50%), CFVR in the LAD is normal (Lambertz et al., 1999; Voci et al., 2002), equal to 2.6±0.6 in subjects with totally normal coronary arteries, 2.6±0.6 in subjects with LAD stenosis of 0-20% and 2.2±0.5 in subjects with LAD stenosis of 20-40% (Sicari et al., 2009). In cases with moderate stenosis, overall group CFVR is intermediate (from 2.23± 0.20 to 2.33±0.32); and only in high-grade stenosis CFVR is low (from 1.12±0.49 to 1.64 ± 0.30). But according to individual analysis data, CFVR reduction is found upstream in the classical ischemic cascade, and can well detect stenosis unable to induce regional wall motion abnormalities during stress echo (see 4.3). CFVR is often <2.0 when stenosis is still moderate (50%-60%) (fig. 15) mirroring the decrease of subendocardial blood flow without total wall perfusion deficit and wall motion disturbance. So, demonstrating a higher negative predictive value in the detection of ischemia, compared with wall motion score index or nuclear cardiac imaging, CFVR is very useful in moderate stenosis to assess its functional significance and to help consider the necessity of revascularization (when CFVR<2.0).

In severe stenosis, CFVR is more preserved in subjects with stenosis of 70-90% than in subjects with stenosis greater than 90% (Voci et al., 2003). Pizzuto et al., 2001 and Voci et al., 2003, found the cut-off value of CFVR <1.0 to be a marker of coronary flow steal effect in patients with severe LAD stenosis (>90%) with 92%sensitivity, 98%specificity, and 97%diagnostic accuracy. This finding is in agreement with the experimental work of Gould et al., 1974, who showed that CFVR in over 90% stenosis of the lumen diameter is blunted mainly due to either stenosis site collapse or development of coronary steal through collateral vessels.

So, CFVR by Doppler TTE may be a useful tool for: 1) selection of patients with moderate stenosis and CFVR<2.0 for revascularization and 2) detection of patients with >90%stenosis basing on coronary steal effect (CFVR<1.0).

4.5 CFVR after intracoronary interventions

Another important application of TTE is assessment of early efficacy and remote coronary restenosis after intracoronary interventions. In the study of Pizzuto et al., 2001, CFVR measurement was performed before and within 1 day after LAD stenting. Prestent impaired CFVR increased after success stent implantation from 1.45±0.5 to 2.58±0.7 in groups with 70-90% and >90% stenosis, and an absence of CFVR change was revealed only in 3 patients with >90%stenosis due to reactive hyperemia, microvascular stunning or post-procedure vasoconstriction. Unlike intracoronary Doppler studies with the guide wire or guiding catheter, the influence of which on the vascular endothelium and coronary flow dynamics is unpredictable and may be a cause of high rate of impaired CFVR after balloon angioplasty or stenting (50% and 30%, respectively) even in the absence of any residual angiographic stenosis, TTE, being a non-invasive technique, is deprived of this effect, and can more accurately reflect changes of coronary flow.

In the study including 53 patients, coronary angiography and CFVR detection were performed 6 months after PTCA of the LAD (Lethen et al., 2003). The investigators observed CFVR to significantly differ in the groups with and without restenosis, and CFVR with the cut-off value <2.0 demonstrated 89% sensitivity and 90% specificity for predicting

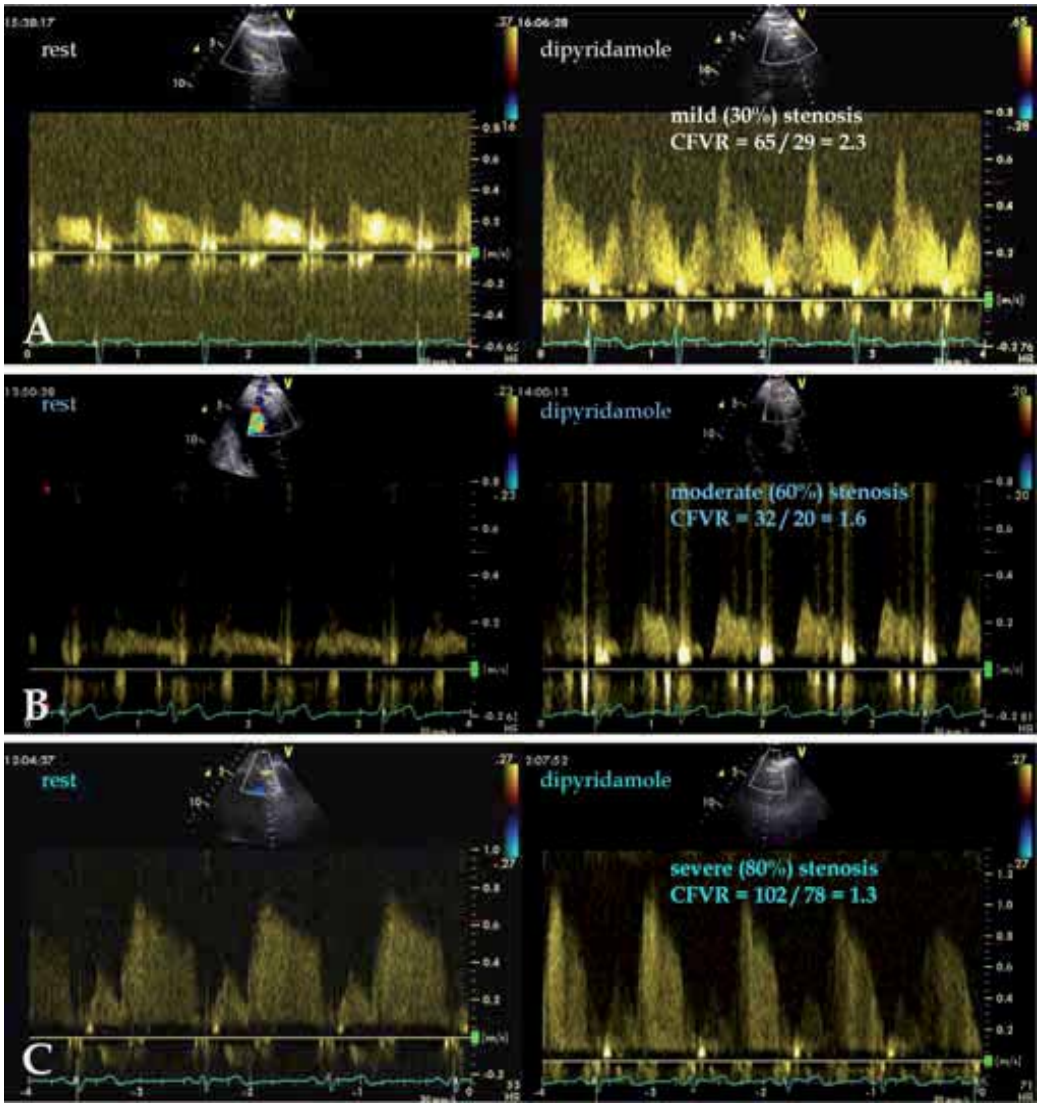

Fig. 15. Examples of CFVR in distal LAD in patients with LAD stenosis are presented. A - case with mild proximal LAD stenosis, CFVR is 2.3; B - case with moderate LAD stenosis; the basal peak diastolic flow velocity is normal, but it increases less than 2 times after dipyridamole, and CFVR is decreased (1.6); C - case with proximal severe LAD stenosis and basal poststenotic acceleration of coronary flow with high peak diastolic flow velocity in distal LAD at rest; after dipyridamole the increase of peak diastolic flow velocity is slight, CFVR is equal to 1.3.

significant LAD restenosis. Similar studies were carried out after LAD stent, and CFVR with the cut-off value <2.0 also demonstrated sensitivity ranging from 77% to 93% and specificity ranging from 78% to 100% for predicting LAD restenosis (Ruscazio et al., 2002; Pizzuto et al, 2003a; Hirata et al., 2006). One of the criticisms of TTE is that assessment has been limited to the LAD, but progress is being made in examining other vessels. High success rate of CFVR

measurements in the PDA permits to hope for the forthcoming appearance of CFVR studies before and after RCA intracoronary interventions.
Thus, noninvasive CFVR assessment by TTE is a very promising tool in monitoring early efficacy of LAD angioplasty or stenting and detecting remote restenosis after LAD intracoronary interventions.

4.6 Factors influencing CFVR

Besides stenosis, several factors can influence the baseline or hyperemic coronary flow and cause decreased CFVR. Elevation of the basal flow can occur with hypertension, tachycardia, anemia, thyrotoxicosis, or valvular disease, or after caffeine intake (coffee, tea, cola, etc.). The conditions which can decrease the maximum hyperemic flow include not only epicardial coronary stenosis but also microvascular disease with impaired structure, function, rheology, and lower density of capillaries, polycythemia and elevated left ventricle end-diastolic pressure (Baumgart et al., 1998; Hirata et al., 2001; Neishi et al., 2005; de Grigorio et al., 2005; Sherrid et al., 2006; Kaul & Jayaweera, 2008).

4.7 CFVR and prognosis in CAD patients

According to the data of studies in patients with acute anterior myocardial infarction, CFVR is able to reflect an absence of microvascular reperfusion in the presence of a patent epicardial coronary artery, and is a negative determinant for myocardial viability (Ueno et al., 2002a; Rigo et al., 2004; Meimoun et al., 2009). Ueno et al., 2002a, reported that CFVR measured in open infarct-related LAD on day 1 after success primary angioplasty was a good predictor of the recovery of regional left ventricle function at discharge, whereas Rigo et al., 2004, found CFVR in the LAD <2.0 to be useful also in predicting an unfavorable long-term (6-month) outcome. In the study of Meimoun et al., 2009, a cut-off CFVR value of 1.7 was an independent predictor of both the left ventricle recovery at a 3-month follow-up, and in-hospital adverse cardiac events including death, recurrent myocardial infarction, and acute heart failure.
CFVR<2.0 was associated with an unfavorable outcome in an unselected cohort of patients with positive and negative stress echocardiograms by wall motion criteria (Rigo et al., 2008), in patients with intermediate LAD stenosis (Rigo et al., 2007), and in patients with chest pain and normal or near-normal coronary arteries (Sicari et al., 2009). CFVR<1.8 provided independent information for prognostic stratification in patients with inducible ischemia during stress echocardiography, and it was associated with an increase of event rate (death and myocardial infarction) by 46% during 19-month follow-up (Cortigiani et al., 2010). But LAD CFVR demonstrated an extremely high additional prognostic value (up to 160%) in patients with negative stress echocardiography by wall motion criteria, and a reduced CFVR was associated with a less benign long-term outcome (Rigo et al., 2006; Cortigiani et al., 2010). In the study of Rigo et al., 2006, CFVR<1.92 was the best predictor of future events (sensitivity = 77%, specificity = 85%), and the 3-year event-free survival was higher in patients with normal CFVR (>1.92) compared with patients with reduced CVFR (98% versus 64%, P<0.0001). The main reasons for CFVR importance in the prognosis in patients with negative stress echocardiography are the following: (1) CFVR can detect moderate coronary stenoses inducing no wall motion abnormalities during stress test (see 4.3, 4.4); (2) CFVR can be useful for the detection of severe stenosis in patients on antiischemic therapy. When

ischemia-dependent wall motion abnormality is the diagnostic end point, concomitant antiischemic therapy modifies the prognostic value of stress echocardiography, markedly reducing TTE sensitivity; in fact, a positive test result in on therapy patients is more prognostically malignant, and a negative test result is less prognostically benign. The flow information in patients with known or suspected coronary artery disease is relatively unaffected by concomitant antiischemic therapy, and does not influence CFVR significantly. In the study with 1779 patients performed by Sicari et al., 2008, the symptoms-free survival was higher in patients with normal CFVR (>2.0) and lower in patients with abnormal CFVR (87% versus 34%, P =0.0001), and it was comparable to that in patients with normal CFVR on and off therapy and in patients with abnormal CFVR on and off therapy; so, antiischemic therapy at the time of testing did not modify the prognostic value of Doppler CFVR. (3) CFVR can identify severe microvascular disease (Rigo et al., 2006; Cortigiani et al., 2007; Sicari et al., 2009). Coronary microvascular dysfunction does not normally induce wall motion abnormalities but has been linked to an adverse outcome, which can be accurately detected by abnormal CFVR. So, in a prospective study in 394 patients with angiographically normal or near-normal coronary arteries and preserved regional and global left ventricular function at baseline and during stress, CFVR <2.0 adds incremental value to the prognostic stratification achieved with clinical and angiographic data (Sicari et al., 2009), and selects a subgroup of patients with a less benign prognosis. During a median follow-up of 51 months, long-term survival estimated for adverse events (death and myocardial infarction) showed an extremely better outcome for the patients with a normal CFVR compared with those with an abnormal CFVR (96% versus 55%).

In summary, CFVR provides independent information for prognostic stratification, and a reduced CFVR is associated with a less benign long-term outcome in patients with acute anterior myocardial infarction, known or suspected CAD with positive and particularly negative stress echocardiography by wall motion criteria. CFVR measurement is offered to be added to routine stress echocardiography.

5. Transthoracic visualization of internal thoracic artery grafts and venous grafts

We do not have our own sufficient experience of coronary graft assessment. But in published studies TTE visualization of the left internal mammary artery (IMA) graft has varied from 70% to 100%, the venous grafts to the LAD could be assessed in 91%, to the RCA – in 96%, to the Cx – in 90% (Chirillo et al., 2001, 2004; Chong et al., 2004). The Nyquist limits should be set at 20 cm/s for the IMA grafts and 10-12 cm/s for the venous grafts (Youn & Foster, 2004). In the proximal part next to the subclavian artery, patent IMA grafts demonstrate a flow pattern with predominant systolic velocity, and next to distal anastomosis site the flow pattern has predominant diastolic velocity similar to that of the coronary artery. Some researchers made an effort to detect dipyridamole-induced CFVR in the IMA and venous grafts. Meyer et al., 2004, compared CFVR in the proximal left IMA graft with the help of simultaneous TTE and intracoronary Doppler guide wire assessment. TTE reflected invasive measurement of CFVR accurately, and the agreement between the two methods was 0.97, and patients with CFVR>2.1 showed patent IMA graft. According to the data of Chirillo et al., 2001, 2004, CFVR<1.9 in proximal IMA grafts and CFVR <1.6 in

venous grafts demonstrated a higher sensitivity in the detection of 50-100% graft stenoses in comparison with wall motion score index. But recently, Pizzuto et al., 2005 have determined distal CFVR in the IMA grafts to depend on flow interrelation in the graft and native LAD, and to be a better predictor of LAD function than IMA graft patency in the presence of flow competition. So, TTE value for identification of graft patency is not yet established, and further studies are required.

6. Conclusion

In summary, with the advent of harmonic imaging, contrast agents and high-frequency transducers, TTE can be used for the diagnosis of coronary narrowing as a noninvasive, inexpensive, non-X-ray, high time resolution and widely used in clinical practice method. After a period of investigators' training, detection and measurement of distal LAD and RCA flow and CFVR by TTE is feasible in more than 90% of patients and correlates well with invasive measurements. Clinical applications of TTE for direct assessment of coronary stenoses and occlusions are limited due to partial artery visualization, and TTE can not be accepted as an alternative to coronary angiography. But Doppler TTE in CAD patients can be a helpful method providing additional information on the coronary artery function for:

- noninvasive clinical repeated or serial measurements of coronary flow velocity at rest after stress which are necessary for understanding the physiology and pathophysiology of coronary flow; in this case the LAD can be used as a reference vessel;
- preliminary identification of coronary stenosis in the LMCA, LAD and PDA;
- detection of restenosis after percutaneous intracoronary interventions in the LMCA, LAD and PDA;
- diagnosis of reperfusion in the acute phase of myocardial infarction in the LAD territory with evaluation of no-reflow after recanalization;
- identification of chronic occluded LAD and RCA;
- assessment of endothelial function;
- measurement of CFVR in the following groups of patients: (1) with suspected or confirmed CAD in those who undergo stress echocardiography (dobutamine or dipyridamole) for reflecting perfusion, function, prognosis-risk stratification, and selection of significant coronary stenosis (CFR<2.0) of the LAD and RCA; (2) with intermediate-grade coronary obstruction where the functional assessment of the lesion is doubtful, and CFVR can be used in considering *pro et contra* of coronary interventions; (3) with not reliable stress tests due to left bundle-branch block or right bundle-branch block because of the high rate of the false-positive results where CVFR may be used for noninvasive detection of LAD or RCA stenosis; (4) after coronary angioplasty, stent or coronary bypass graft of the LAD and RCA where serial follow-up for the assessment of efficacy and early or remote restenosis is required; (5) with ongoing drug therapy for serial follow-up, particularly if the drug effect is uncertain; (6) with typical or atypical chest pain and angiographically normal coronary arteries where microvascular disease is possible (syndrome X, left ventricle hypertrophy, diabetes mellitus, etc.); and (7) with acute anterior myocardial infarction for the assessment of viability, prognosis and prediction of improvement of the regional left ventricular function.

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3

Contrast Echocardiography in Coronary Artery Disease

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1. Introduction

Conventional echocardiography is widely used and well documented in evaluation of patients with stable and unstable coronary artery disease (Mollema et al., 2009). In particular, assessment of left ventricular function, volumes and ejection fraction adds important prognostic information in individual patients. In addition, echocardiography may detect any concomitant valvular heart disease as well as acute complications in unstable coronary syndromes. Stress echocardiography has through several studies established its role in diagnosis of stable coronary artery disease and assessment of myocardial viability (Sicari et al., 2008).

However, introduction of ultrasound contrast agents and contrast specific imaging modalities have significantly improved the usefulness of echocardiography in diagnosis and assessment of coronary artery disease (Dijkmans et al., 2006). Indications for use of ultrasound contrast are implemented in guidelines for assessment of left ventricular function at rest and during stress echocardiography (Senior et al., 2009; Mulvagh et al., 2008). Ultrasound contrast is recommended for assessing left ventricular ejection fraction at rest when image quality is suboptimal and for stress echocardiography when the endocardial boarder is not visualized in 2 or more left ventricular segments (Senior et al., 2009; Mulvagh et al., 2008).

In contrast echocardiography regional myocardial function and perfusion may be assessed simultaneously, thereby optimizing the non-invasive diagnostics of coronary artery disease. The incremental value of assessing myocardial perfusion in diagnosing coronary artery disease is emphasised by the ischemic cascade (Fig. 1), demonstrating that hypoperfusion precedes functional impairment, ECG changes, symptoms and myocardial necrosis as depicted in Fig.1. (Crossman, 2004; Leong-Poi et al., 2002).

Diagnosing distribution and extent of myocardial ischemia by contrast echocardiography can give information on the total ischemic burden and has become a supplemental tool in evaluation of the physiological impact of an angiographic coronary artery stenosis. Consequently, myocardial perfusion assessment by contrast echocardiography may also be used for risk prediction in patients with known coronary artery disease and in prioritizing the need for urgent revascularization among patients with acute coronary syndromes (Jeetley et al., 2007; Rinkevich et al., 2005; Lønnebakken et al., 2011). It has the potential to become a future tool to tailor and evaluate the effect of treatment on myocardial perfusion in patients with different clinical syndromes of coronary artery disease. Furthermore,

contrast echocardiography can be used to identify myocardial ischemia in patients with non-obstructive coronary artery disease i.e. microvascular disease which cannot be diagnosed by routine coronary angiography.

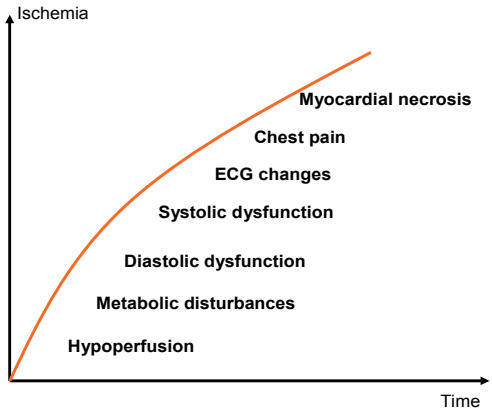

Fig. 1. The ischemic cascade

2. Methodology

Contrast echocardiography has several advantages compared to other non-invasive imaging techniques like cardiac magnetic resonance imaging and cardiac computer tomography. First, it can be performed without the radiation exposure of computer tomography and without the potential nephrotoxisity of the gadolinium contrast agent necessary to assess myocardial perfusion by magnetic resonance imaging. Second, it can be performed bed-side and give immediate answers to important clinical questions in management of patients with known or suspected coronary artery disease. Contrast echocardiography requires intravenous administration of a second or third generation ultrasound contrast agent during contrast specific ultrasound imaging.

2.1 Ultrasound contrast agents and imaging modalities

Ultrasound contrast agents consist of microbubbles with an inert gas core surrounded by a shell. Due to the microbubble size and stability, they can pass the pulmonary circulation without destruction and intravenous administration as bolus dosages or continuous infusion can therefore be used (Senior et al., 2009). Importantly, the contrast microbubbles act as isolated intravascular tracers and are therefore ideal for perfusion assessment. Future possibility of targeting contrast microbubbles against specific disease processes, including inflammation in unstable plaque, activated platelets in thrombus formation or against factors involved in angiogenesis may allow even more specific diagnoses (Kaufman & Lindner., 2007; Chadderdon & Kaul., 2010).

Ultrasound contrast agents in coronary artery disease have been shown to be safe, but allergic anaphylactic reactions have been observed (Senior et al., 2009; Wei et al., 2008). Therefore, patients should be observed closely with continuous recording of heart rhythm and frequent measurement of blood pressure during contrast echocardiography and for at least 20 minutes after the examination. Emergency equipment should always be available in the examination room during contrast echocardiography. Contrast echocardiography has

few absolute contraindications, except for known allergy against the contrast agent. However, caution and close observation should be performed in patients with unstable coronary artery disease or decompensated heart failure. Ultrasound contrast agent should also be used with caution in patients with severe pulmonary disease. The gas in the contrast microbubbles is excreted through the lungs, and in patients with severe pulmonary disease, clearance is delayed, causing increased halftime of the gas in the circulation. In patients with mechanical valve prosthesis contrast echocardiography should be avoided due to extensive destruction of contrast microbubbles by the prosthesis. An overview of current commercially available ultrasound contrast agents is given in Table 1.

Contrast agents	Gas core	Shell
SonoVue	Sulphur hexafluoride	Phospholipid monolayer
Luminity/Definity	Perflutren	Phospholipid monolayer
Optison	Perflutren	Albumin
Albunex	Air	Albumin

Table 1. Gas core and shell composition in ultrasound contrast agents available for clinical use

Contrast microbubbles have unique acoustic properties when exposed to ultrasound. At very low mechanical index, the ultrasound microbubbles have a linear response to the ultrasound exposure. At low mechanical index (MI 0.08-0.3) the ultrasound microbubbles start to oscillate giving rise to a non-linear response contrasting the linear response of the myocardial tissue at low mechanical index. Contrast specific ultrasound imaging modalities remove the linear tissue response and enhance the contrast microbubble response. Different techniques may be used to emphasis the contrast microbubbles acoustic signals and to filter the tissue signals, the main techniques being power modulation, pulse inversion or coherent contrast imaging.
Low-mechanical index imaging is the most commonly used modality, often combined with a high energy ultrasound flash causing microbubble destruction, known as destruction-replenishment imaging or flash imaging (Fig. 2). By this technique real-time contrast echocardiography with simultaneous assessment of myocardial function and perfusion can be performed.
High mechanical index imaging causes microbubble destruction. By high mechanical index triggered imaging, myocardial perfusion can be assessed, but myocardial function can not be assessed simultaneously using this imaging modality. The advantage of this imaging modality is a better reproducibility for quantification of myocardial perfusion.

2.2 Performance and image interpretation

Ultrasound contrast may be used to improve endocardial border delineation, a technique known as left ventricular opacification (LVO) (Fig.3) (Chahal & Senior, 2010), which has been demonstrated to optimize assessment of left ventricular volumes and ejection fraction by echocardiography compared to cardiac magnetic resonance imaging, the current gold standard (Malm et al., 2006). In patients with poor acoustic windows, left ventricular ejection fraction is often underestimated if ultrasound contrast is not used (Kurt et al., 2009; Plana et al., 2008). Using ultrasound contrast significantly improves echocardiographic reproducibility and accuracy in patients with poor acoustic windows, and use of contrast echocardiography in such cases for accurate assessment of left ventricular ejection fraction is

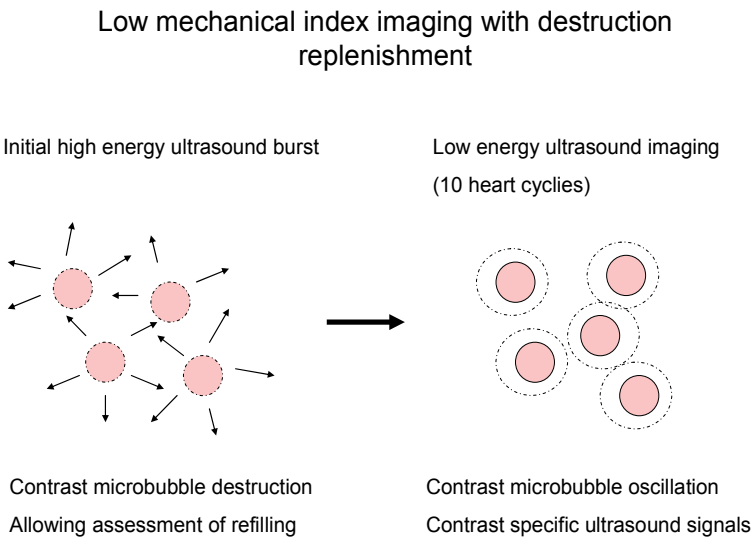

Fig. 2. Destruction replenishment contrast echocardiography, where a high energy ultrasound burst causes ultrasound microbubble destruction, followed by low mechanical index ultrasound imaging assessing only the non-linear ultrasound refection from oscillating contrast microbubbles by contrast specific ultrasound imaging allowing assessment of contrast enhancement and hence myocardial perfusion.

recommended in current guidelines (Senior et al., 2009). Similarly, during stress echocardiography, adding ultrasound contrast allows a complete evaluation of wall motion in all myocardial regions in almost every patient (Hoffmann et al., 2007).
In a study of 632 patients with poor acoustic windows, adding ultrasound contrast not only avoided the need of further expensive and time consuming examinations but also had direct impact on patient's treatment (Kurt et al., 2009).

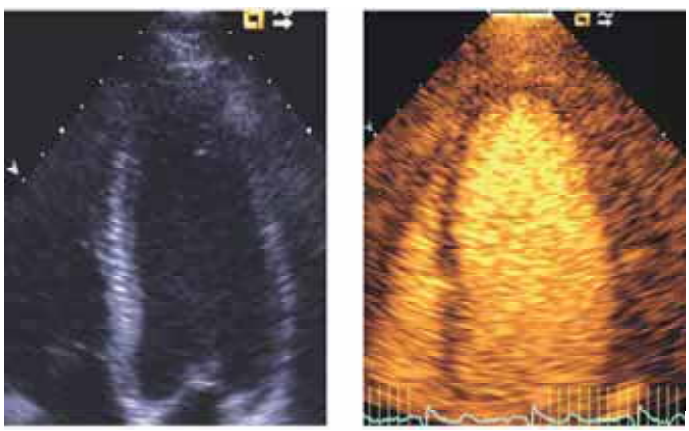

Fig. 3. Left ventricular opacification (LVO) by contrast echocardiography illustrating the improved endocardial border delineation in particular in the apical part of the left ventricle in an apical 4-chamber view compared to conventional echocardiography.

In myocardial contrast echocardiography (MCE), contrast is not only used for enhanced endocardial border delineation, but also for assessment of regional perfusion with high spatial and temporal resolution (Fig. 4) (Elhendy & Porter., 2005). MCE has the potential to significantly improve non-invasive evaluation of coronary artery disease (Elhendy et al., 2004; Lønnebakken et al., 2009). Contrasting other non-invasive imaging modalities, MCE visualizes the capillary filling in the myocardium and can give information on regional myocardial perfusion including subendocardial hypoperfusion, which is the first sign of ischemia (Dijkmans et al.,2006). Consequently, MCE increases the sensitivity to detect ischemia. In addition, myocardial microvascular integrity can be evaluated and myocardial viability assessed.

Myocardial contrast echocardiography is mainly performed using apical 4-chamber, apical 2-chamber and apical 3-chamber views. Parasternal imaging is more difficult due to contrast attenuation, but additional parasternal long- and short axis images may be useful in individual patients, in particular at peak stress. By combining rest-imaging with an exercise or pharmacological stress test, myocardial function and perfusion can be evaluated not only at rest but also during stress, which is particularly important in diagnosis of stable coronary artery disease, evaluation of viability and in evaluating the result after coronary revascularization. Image analysis is performed using a standardized 17-segment left ventricular model, in which the different left ventricular segments are assigned to the three main coronary arteries using a standardized scheme (Fig. 5) (Lang et al., 2006). However, the considerable variation in coronary artery anatomy must be taken into account when comparing MCE results to coronary angiography.

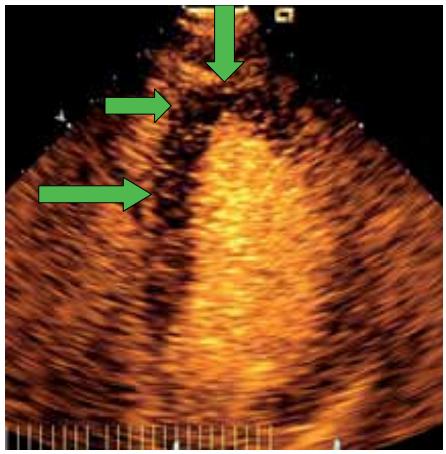

Fig. 4. Myocardial contrast echocardiography with low mechanical index demonstrating the delayed contrast enhancement in the distal septum and apex of the left ventricle (green arrows) compared to the proximal septum and lateral wall in an apical 4-chamber view.

2.2.1 Wall motion scoring

Myocardial regional function or wall motion is evaluated from active myocardial thickening and scored according to current guidelines as normal (1), hypokinetic (2), akinetic (3) or dyskinetic (4) (Sicari et al., 2008). In addition, there should be a further increase in wall thickening during stress testing to be scored as normal. In viability assessment, an akinetic

segment that starts to function at low stress level but ceases to function again at higher stress level is indicative of viable myocardium with ischemia, known as the biphasic response, typically for stunned or hibernating myocardium. Such findings indicate that the regional myocardial function will improve from revascularisation. Akinetic myocardial segments that remain akinetic during stress indicate infarct scarring which will not benefit from revascularization.

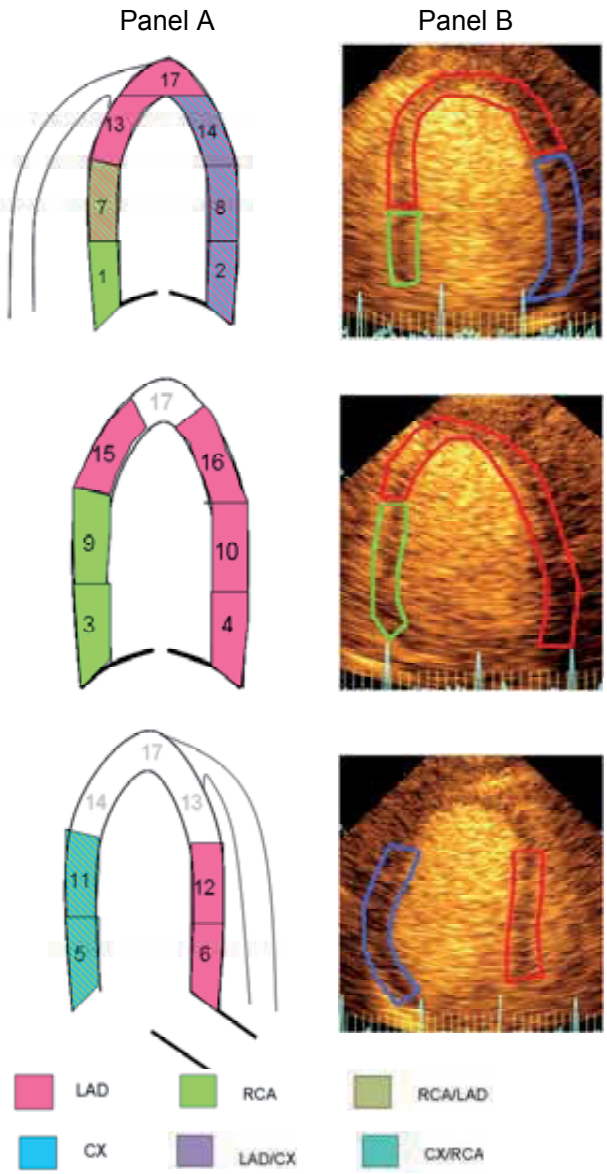

Fig. 5. Left ventricular model for wall motion and perfusion scoring during contrast echocardiography and standardized attribution to the main coronary arteries.

2.2.2 Perfusion scoring

Regional perfusion scoring is based on visual evaluation of contrast enhancement in the myocardium. At rest the myocardium should be filled with contrast during 5 heart beats, while at peak stress the myocardium should be filled in 1-2 heart beats. Delayed enhancement is consistent with hypoperfusion or ischemia, while lack of enhancement is consistent with myocardial fibrosis of infarct scaring. However, artefacts like contrast destruction in the near field may cause false perfusion defects in the apical myocardium, while attenuation may cause false perfusion defects in the basal parts. In addition, perfusion defects in thin fibrotic myocardium may be underestimated because of shine-through effect.
In patients with stable coronary artery disease, perfusion scoring by contrast stress echocardiography has been demonstrated to identify prognostically important angiographic coronary artery disease (multivessel disease and proximal stenosis in the left anterior descending artery) significantly better than wall motion scoring (Lønnebakken et al., 2009). However, anatomical variations in coronary anatomy as well as collateral circulation and coronary artery bypass grafting will influence the perfusion area of the individual coronary artery. Therefore, except for stenosis in the proximal left anterior descending artery, the anatomic culprit lesion can usually not be identified by MCE.

2.2.3 Quantification of myocardial perfusion

Quantification software assessing contrast enhancement from increase in video intensity over time has been developed (Agati et al., 2005). From quantitative analysis, typical contrast enhancement curves can be obtained for blood flow velocity (β), perfusion rate (Axβ), refilling time (rt) and total blood volume (A) (Fig.6.). In normally perfused

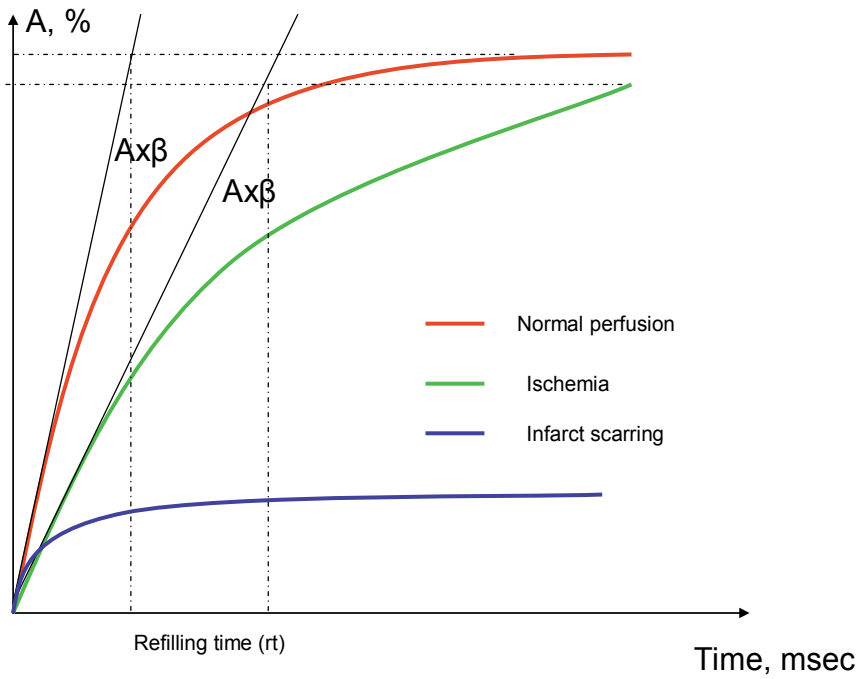

Fig. 6. Contrast enhancement curves: for normally perfused myocardium (red curve), ischemic/hypoperfused myocardium and for infarct area (blue curve), respectively.

myocardium, the blood flow velocity is about 0.5 ml/s and the peak intensity level is rapidly reached (Fig.6 red curve). In ischemic myocardium, both the blood flow velocity and perfusion rate are reduced, the refilling time is increased while the total blood volume remains normal (Fig.6 green curve). This contrasts the reduced total blood volume characterizing the myocardial contrast enhancement in infracts scarring and myocardial fibrosis (Fig.6 blue curve) (Wei et al., 1998; Toledo et al., 2006).
Using quantification, it is theoretically possible to compare myocardial perfusion in different regions and settings. However, previous studies have demonstrated that it is difficult to compare between different patients due to large interindividual variation. In a study of 20 healthy subjects with normal wall motion and coronary angiography, both considerable inter-individual and also inter-regional variability in perfusion parameters were noted, suggesting that quantitative perfusion parameters currently are best suited for with-in patient repeated assessment, for instance during stress testing (Malm 2005). This was confirmed in a follow-up study of patients who underwent quantitative contrast stress echocardiography prior to and 9 months after percutaneous coronary revascularization. In this study, stress induced perfusion but not absolute perfusion parameters were improved in patients with angiographic successful result, while a lack of improvement in stress induced perfusion was associated with angiographically confirmed restenosis irrespective of patient symptoms (Lønnebakken et al., 2009). However, standardisation and assessment of optimal cut-off values for myocardial perfusion has to be derived from larger trials before quantification of myocardial perfusion can be used in clinical assessment of coronary artery disease (Abdelmoneim et al., 2009).

3. Clinical applications

Contrast echocardiography has documented important clinical impact on diagnosis, risk prediction and follow-up of patients with different clinical syndromes of coronary artery disease as well as in detection of thrombotic complications in patients with ischemic heart disease. In addition assessing the total ischemic burden and viability by contrast echocardiography adds prognostic information in individual patients.

3.1 Stable coronary artery disease

Diagnosing stable coronary artery disease may be challenging, in particular since atypical symptoms are not uncommon. The most used diagnostic test in coronary artery disease, the exercise electrocardiogram, is associated with a low accuracy to detect significant angiographic coronary artery disease. Invasive coronary angiography is according to current guidelines the diagnostic gold standard. However, it is invasive and associated with potential risk for severe complications and allergic reactions, and includes radiation exposure. Furthermore, coronary angiography does not give information on the functional importance of a coronary stenosis. Stress echocardiography has an overall diagnostic sensitivity of 85% and specificity of 90% in detecting significant angiographic coronary artery disease from meta-analyses (Senior et al., 2005; Picano et al., 2008). However, in 33% of patients referred for conventional stress echocardiography the image quality does not allow adequate evaluation. By adding ultrasound contrast during stress echocardiography almost all patients can be satisfactory examined by stress echocardiography and by simultaneous assessment of both myocardial function and perfusion (MCE) the sensitivity of detecting significant angiographic coronary artery stenosis may be increased to 90% but the specificity is reduced (Senior et al., 2009).

In a meta-analysis of 8 studies the sensitivity and specificity of detecting coronary artery disease by myocardial contrast stress echocardiography was 83 and 80 %, respectively. In patients with known or suspected coronary artery disease, perfusion was significantly better than wall motion analysis in detecting angiographic coronary artery stenosis, in particularly at intermediate stress level (Elhendy et al., 2004). In addition, in patients with known coronary artery disease awaiting percutaneous coronary intervention, perfusion scoring was significantly better than wall motion scoring in identifying patients with prognostic significant angiographic coronary artery stenosis, like triple-vessel disease and proximal stenosis in the left anterior descending artery (Lønnebakken et al., 2009). Of note this could be achieved at intermediate stress level. Failure to achieve adequate stress level is an important limitation in assessing coronary artery disease by stress electrocardiography or stress echocardiography. It has been demonstrated that using contrast stress echocardiography with perfusion assessment (MCE) seems to overcome this limitation.

The weak association between the degree of angiographic coronary artery stenosis and quantitative myocardial perfusion by contrast stress echocardiography has been noted in several studies (Malm et al., 2006; Peltier et al., 2004; Perez et al., 2004; Lønnebakken et al., 2009). This may be explained by the fact that many other factors than coronary artery lumen diameter reduction is important for myocardial perfusion, including coronary flow autoregulation, collateral circulation, stenosis length and serial stenosis in addition to hemodynamic condition are important for myocardial perfusion. Although, mainly due to anatomic variation in coronary anatomy, contrast stress echocardiography has limited power to predict the anatomical localisation of angiographic coronary artery stenosis, the method is accurate to predict proximal stenosis in the left anterior descending coronary artery and to identify patients with multivessel disease. In addition, assessing the total ischemic burden in the individual patient may be clinical important for choosing the optimal treatment. It has been demonstrated that only patients with an ischemic burden >20% will benefit prognostically from revascularization, otherwise revascularization will only have symptomatic effect, suggesting that asymptomatic or low-symptomatic patients will have no or little effect and may be equally well off treated medically.

3.2 Acute coronary syndrome

Patients with acute coronary syndrome are a heterogeneous group with varying disease severity and prognosis, from unstable angina pectoris, non-ST elevation myocardial infarction (NSTEMI) and ST-elevation myocardial infarction (STEMI). It is well known that both short- and long-term prognosis in NSTEMI patients is as severe as STEMI patients, although the incidence of acute coronary artery occlusions varies. It has been demonstrated that contrast echocardiography can be used for risk assessment in acute coronary syndrome patients (Senior et al., 2004; Khang et al., 2005).

3.2.1 Unstable angina pectoris

In patients hospitalized with acute chest pain but having normal serum troponin level, contrast echocardiography has been shown to be a useful tool to distinguish between patients with acute coronary syndrome and non-cardiac chest pain (Jeetley et al., 2007; Rinkevich et al., 2005; Kaul et al., 2004). Another study in 957 patients with acute chest pain and a non-diagnostic electrocardiogram demonstrated that myocardial perfusion by

contrast echocardiography was better than other commonly used risk score models like the Thrombolysis in Myocardial Infarction (TIMI) risk score (Antman et al., 2000) to discriminate between patients with intermediate or high risk for reinfarction or death (Tong et al., 2005). Based on current documentation, advanced echocardiography is underused in diagnostics and management of patients with acute chest pain.

Variables in TIMI risk score	score
Age ≥65 years	1
≥3 risk factors of CAD (family history of CAD, hypertension, diabetes, current smoking, hypercholesterolemia)	1
Prior CAD (previous MI, CABG, PCI or known angiographic stenosis ≥50%)	1
ST-segment depression ≥0.05 mV in ≥2 ECG leads	1
≥2 episodes of chest pain the last 24 hour	1
Aspirin the last 7 days or unfractionated heparin the last 24 hour	1
Elevated serum cardiac markers	1

Table 2. Thrombolysis In Myocardial Infarction risk score (TIMI).

3.2.2 Non-ST elevation myocardial infarction

Current guidelines for management of NSTEMI are diverging, recommending invasive risk stratification and revascularization within 12-72 hours in patients at intermediate and high risk (Bassand et al., 2007; Smith et al., 2006). In clinical risk assessment, TIMI risk score is one of the most recommended and widely used models in NSTEMI patients (Table 2). Of note, these clinical risk score models may underestimate angiographic coronary artery disease severity, in particular in patients scored as intermediate risk (Volat et al., 2008). In a recently published series of 110 patients with NSTEMI, the extent of myocardial ischemia assessed by contrast echocardiography was a better predictor of angiographic severe coronary artery disease than the TIMI risk score, in particular in identifying patients with severe disease like left main stem stenosis, trippel-vessel disease or multi-vessel disease including proximal stenosis in the left anterior descending artery and also better than wall motion scoring analysis (Fig. 7) (Lønnebakken et al., 2011). In another study in NSTEMI patients, about 30% of the patients had an acute occlusion of a main coronary artery despite normal electrocardiogram. In this study, deformation analysis by echocardiography has proven useful in identifying NSTEMI patients with severe angiographic coronary artery disease (Grenne et al., 2010). In particular serial assessment of regional left ventricular strain may identify these patients while awaiting coronary angiography and revascularisation. Theoretically, detection of these patients by either contrast echocardiography or other advanced imaging techniques represents new tools for identification of patients with high subclinical ischemic burden that may benefit from earlier revascularization.

3.2.3 ST elevation myocardial infarction

In acute STEMI the recommended treatment is immediate coronary angiography and revascularization. Contrast echocardiography can assess area at risk and help in diagnosing acute myocardial infarction in patients with acute chest pain and a non-diagnostic ECG, particularly common in patients with acute occlusion of the circumflex artery (Hayat &

Senior., 2008). But contrast echocardiography will not be indicated in pre-catheterization evaluation of most patients with STEMI.

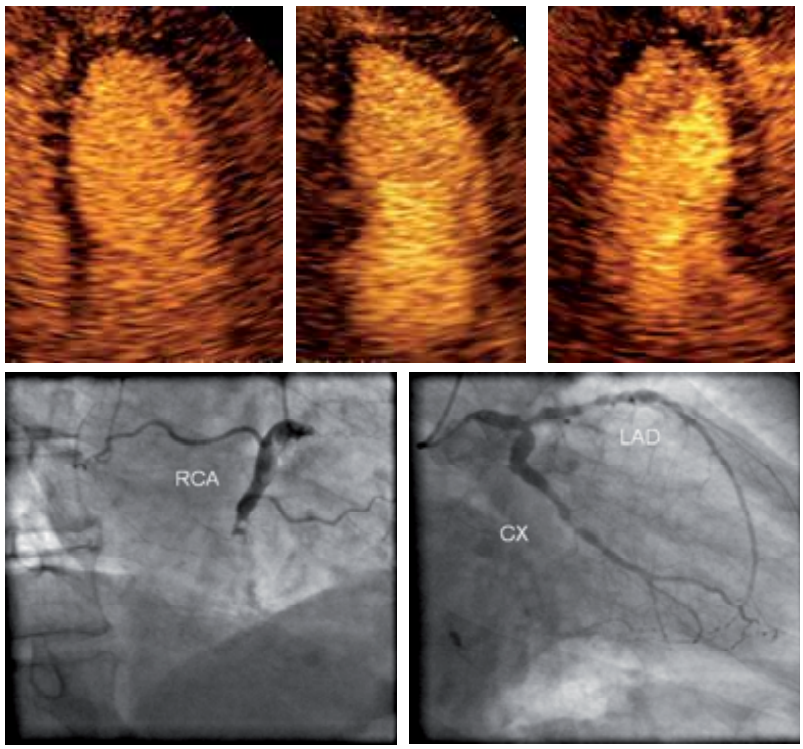

Fig. 7. Contrast echocardiography in apical 4-chamber, 2-chamber and 3-chamber views (upper panels) demonstrating the extensive reduction of myocardial perfusion in a NSTEMI patient with angiographic trippel-vessel disease including acute occlusion of the right coronary artery and left main stem stenosis (lower panels).

In spite of successful reopening of the infarct related artery by percutaneous coronary intervention, some STEMI patients still develop unexpectedly large myocardial infarctions due to the no-reflow phenomenon. The no-reflow phenomenon is caused by impaired microcirculation which can be a consequence of peripheral embolization during the percutaneous revascularization procedure or revascularisation damage due to inflammation and oedema causing microvascular obstruction and subsequent myocardial necrosis. The no-reflow phenomenon after revascularization can be diagnosed by MCE (Kaul., 2006). Lack of reperfusion after coronary intervention predicts myocardial necrosis, reduced left ventricular function, left ventricular remodelling and subsequent development of heart failure. Thus, MCE may be used in STEMI patients to identify successful reopening of the infarct related artery and to give prognostic information by identifying patients with no-reflow who need additional treatment in the acute and chronic phase of a STEMI (Dwivedi et al., 2008; Niccoli et al., 2009; Galiuto et al., 2010)

In addition to guide and evaluate treatment, an ongoing study evaluates the effect of ultrasound contrast enhanced thrombolysis in acute treatment of STEMI. The ongoing Sonolysis trial uses a combination of ultrasound induced contrast microbubbles destruction

at high mechanical index ultrasound and thrombolysis, where destruction of microbubbles causes streaming and thereby improves the effect of thrombolysis in reopening of the infarct related artery (Slikkerveer et al., 2008).

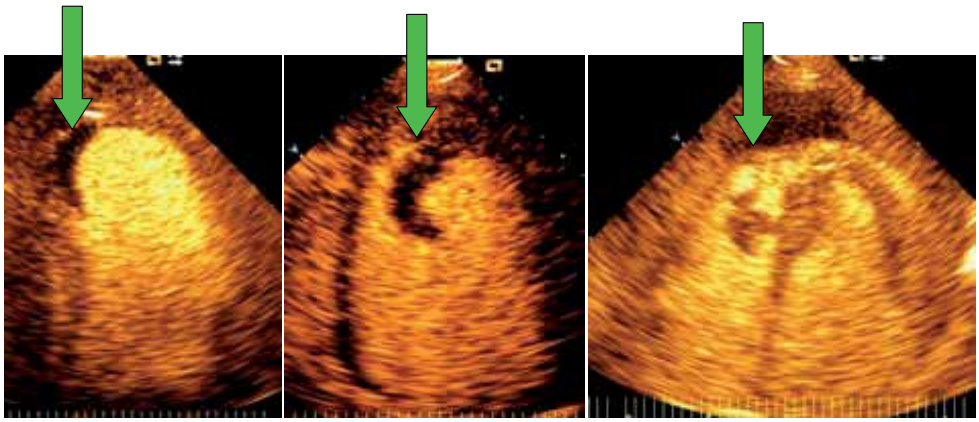

Fig. 8. Complications in acute myocardial infarction. Myocardial mural thrombus in the apex of the left ventricle, with lack of contrast enhancement due to the thrombus avascular characteristics (Panel A and B). In comparison, the typical contrast enhancement in a patient with pulmonary carcinoma and a myocardial metastasis in the right ventricle (Panel C).

Development of intraventricular mural thrombus is a feared complication to acute myocardial infarction which untreated may lead to severe thromboembolic episodes. A magnetic resonance study demonstrated that mural thrombus formation in patients with acute coronary syndromes may be more common than previously anticipated (Solheim et al., 2010). However, suspected mural thrombus may be ruled out in about 90% of patients by contrast echocardiography (Kurt et al., 2009; Hamilton-Craige et al., 2010). Diagnosing a mural thrombus with contrast echocardiography is simple and can be performed with a single ultrasound contrast bolus injection. A mural thrombus is characterized by a lack of contrast enhancement due to its avascular nature (Fig 8 Panel A and B). In contrast, a myocardial tumor is characterized by contrast enhancement which is particular high in malignant tumores that are highly vascularised structures (Fig.8 panel C).
In acute myocardial infarction, myocardial rupture is a rare and deadly complication. A rupture of the free ventricular wall is usually associated with sudden death, but occasionally, epicardial coverage occurs and subsequent formation of a ventricular pseudoaneurysm. Ventricular pseudoaneurysms can be difficult to diagnose by conventional echocardiography (Fig. 9 left panel), but are easy to recognize after injection of an ultrasound contrast agent during imaging (Fig. 9 right panel).

3.3 Restenosis after revascularization

In patients undergoing percutaneous coronary intervention with stent implantation, 10-30% will develop significant angiographic restenosis in spite of initial successful treatment. Restenosis is caused by intimal hyperplasia and is asymptomatic in 50% of patients (Giedd & Bergmann., 2004). However, even in asymptomatic patients development of restenosis is associated with a poorer prognosis (Pfisterer et al., 1993; Zellweger et al., 2003). Non-

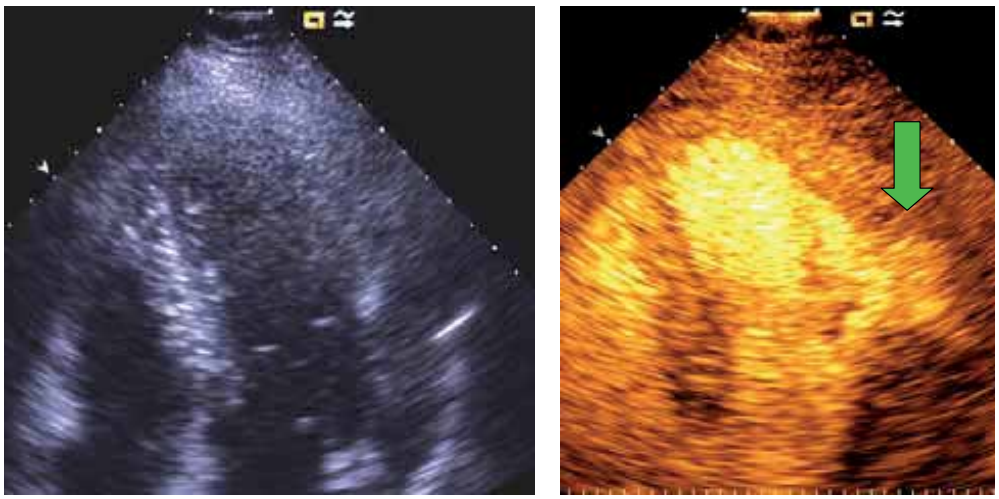

Fig. 9. Extracardial contrast enhancement due to a pseudoaneurysm in the lateral wall of the left ventricle (right panel) not visible with conventional echocardiography (left panel).

invasive diagnosis of restenosis can be challenging. Previous SPECT studies have demonstrated that normalization of regional myocardial perfusion usually occurs after successful revascularization (Manyari et al., 1988; Zhang et al., 2004). In a follow-up study using quantitative contrast stress echocardiography in 33 patients with stable angina pectoris treated with percutaneous coronary intervention and stent implantation, there was no improvement in stress-induced myocardial perfusion during follow-up in patients who had developed a significant angiographic restenosis, while the stress-induced perfusion was improved in patients with successful revascularisation at 9 months (Lønnebakken et al., 2009).

At present, quantitative contrast stress echocardiography is not recommended in routine assessment of coronary artery disease due to inter-individual variation and lack of data on normal values and cut-off values indicating ischemia for this method. Still, serial assessment in individual patients may be useful.

3.4 Non-obstructive coronary artery disease

Although coronary angiography remains the gold standard for diagnosis of coronary artery disease, it should be kept in mind that myocardial ischemia may be present in spite of angiographically open epicardial coronary arteries, a condition known as non-obstructive ischemic heart disease. This condition cannot be diagnosed using angiography alone, but requires additional use of perfusion assessment with cardiac magnetic resonance or MCE. In patients with acute coronary syndrome, non-obstructive ischemic heart disease is present in 15% of women and 9% of men (Berger et al., 2009). Cardiac magnetic resonance studies in NSTEMI patients have demonstrated myocardial infarction in up to 34% of patients with normal coronary arteries by coronary angiography. Clot autolysis and recanalisation of the infarct related artery are the main reasons for this finding as well as microvascular disease that can not be detected by coronary angiography, the current diagnostic gold standard. In patients with recurrent hospitalisation for chest pain and "normal" coronary arteries by coronary angiography additional non-invasive cardiac imaging should be performed. MCE

can be used to diagnose myocardial ischemia in such patients and thereby distinguish between patients with non-cardiac chest pain and patients with non-obstructive ischemic heart disease. Non-obstructive ischemic heart disease most often is caused by microvascular disease associated with diabetes mellitus, obesity and hypertension, but also hemodynamic changes like increased left ventricular filling pressure and increased arterial stiffness can cause reduced myocardial perfusion pressure and hence myocardial ischemia despite angiographically normal epicardial coronary arteries (London et al., 2004). Chronic myocardial ischemia in such patients may promote development of myocardial fibrosis and secondary structural changes in the left ventricle, finally leading to functional impairment and heart failure (Niccoli et al., 2009).

Another recently recognized condition mainly affecting women is the Takotsubo cardiomyopathy, mimicking an acute myocardial infarction. The exact pathophysiological mechanism remains unknown in Takotsubo cardiomyopathy, but it involves myocardial hypoperfusion that can be diagnosed by contrast echocardiography causing functional impairment mainly in the apical part of the left ventricle with the characteristic apical ballooning (Fig. 10) (Abdelmoneim et al., 2009). The microvascular involvement is also confirmed by early cardiac MRI demonstrating late gadolinium uptake suggesting diffuse microcirculation damage (Avegliano et al., 2011).

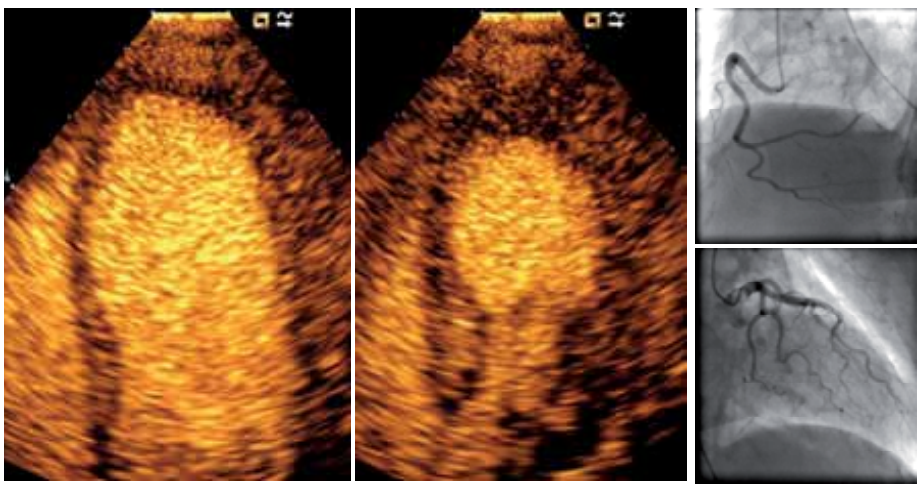

Fig. 10. Takotsubo Cardiomyopathy. Contrast echocardiography in diastole and systole illustrating the apical akinesia and ballooning of the left ventricle in an apical 4-chamber view, in addition there is a delayed contrast enhancement in the apical segments of the left ventricle. The right panel shows the normal coronary angiogram confirming the diagnosis Takotsubo cardiomyopathy.

4. Conclusion

Contrast echocardiography allows simultaneous assessment of regional myocardial function and perfusion, improving non-invasive diagnosis and assessment of coronary artery disease. Contrast echocardiography gives information on the physiological impact of the coronary artery stenosis, reveals the ischemic burden, detects viable myocardium and may

act as a supplemental tool to coronary angiography in management of coronary artery disease and in follow-up after treatment. In addition, the ability to diagnose myocardial ischemia in patients with no-reflow phenomenon or microvascular disease and angiographically normal coronary arteries may help distinguishing patients with non-obstructive ischemic coronary artery disease from patients with non-cardiac chest pain. Future studies using targeted contrast microbubbles against specific disease processes may further improve diagnosis in ischemic coronary artery disease, and on-going studies explore the use of ultrasound contrast agents to potentiate the effect of thrombolysis in acute coronary artery occlusions.

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4

Non-Invasive Imaging in Approaching Ischemic Coronary Artery Disease

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1. Introduction

Ischemic coronary artery disease represents one of the major contemporary health problems because of its elevated vital risk. Nowadays, atherosclerosis-related cardiovascular disease is the most significant non-traumatic mortality factor and the prime "killer" of the 21st century.

Increased morbidity and mortality in coronary artery disease (Rosamond et al., 2008), the significant impact on quality of life, negatively affecting the physical, psychological and social well-being of the patient (Xie et al., 2008) as well as the high costs of diagnosis and treatment for this disease (Rosamond et al., 2008) have lead to developing new imaging techniques in order to explore such patients more efficiently.

Cardiac imaging has developed greatly since the chest radiograph was first used to describe the heart's shape. Nowadays, non-invasive imaging, including echocardiography, Single Photon Emission Computer Tomography (SPECT), coronary angiography and, more recently, Positron Emission Tomography (PET), multidetector computed tomography angiography as well as cardiac magnetic resonance imaging (MRI), represent standard procedures in clinical practice (Budoff et al., 2008) (Gaemperli et al., 2008).

The latter, especially, have a crucial importance in approaching patients with suspected or known coronary artery disease, as well as in assessing risk stratification, prognosis and reperfusion indications in patients with ischemic coronary artery disease (Mark et al., 2010).

The purpose of this chapter is to help promote new ways of integrating the non-invasive diagnostic imaging in the diagnosis and management of coronary artery disease patients.

2. The value of non-invasive cardiac imaging in patients with coronary artery disease

Epicardial arteries visualization is essential for confirming coronary artery disease and for accurately establishing its severity. Over the last 40 years, coronary angiography has been the key exploration in assessing coronary artery obstruction, as well as the need for reperfusion, despite its invasive character and high costs. Nowadays, invasive coronary angiography is still considered the "gold standard" for diagnostic coronary testing, although it has a few major disadvantages and limitations (patient exposure to ionizing radiation, two-dimensional image acquisition of coronary arteries, inappropriate atherosclerotic plaques assessment).

16-channel computed tomography coronary angiography has recently become a leading procedure in cardiac imaging, as it allows an accurate visualization of the coronary artery lumen, as well as an appropriate assessment of stenosis severity; computed tomography coronary angiography results can now be used as an essential tool for prognosis assessment in patients with coronary heart disease (Mark et al., 2010).

2.1 The diagnostic value of non-invasive cardiac imaging in patients with coronary artery disease

However, non-invasive imaging techniques (e.g. coronary multidetector computed tomography angiography and magnetic resonance imaging angiography), which are valuable and comparatively cheaper, can currently be used for studying the coronary artery wall. Both contribute to the diagnosis of coronary artery ischemic disease and the image quality produced by coronary multidetector computed tomography angiography favorably compares to that of coronary angiography. These explorations have their own limitations, including poor image acquisition due to cardiac motion or important calcium deposits, but, most of the times, good quality images are obtained.

Coronary multidetector computed tomography angiography allows outer-luminal plaque visualization and can assess subsequent luminal stenosis. As with invasive coronary angiography, visual grading of coronary segment narrowing by ranges of stenosis is the current standard of practice and has been shown to provide useful clinical information relative to invasive coronary angiography (Cheng et al., 2008) (Miller et al., 2008).

Quantitative coronary multidetector computed tomography angiography is a high accuracy procedure which allows noninvasive detection of suspected obstructive coronary artery disease; it has been used in some research applications but is not currently a routine part of clinical interpretation. This promising technology has potential as an additional diagnostic tool, which will most likely complement invasive coronary angiography in routine clinical care (Hoffmann et al., 2005).

In a recent multicenter study, visual and quantitative assessments of stenosis severity by coronary multidetector computed tomography angiography were quite similar (Miller et al., 2008). Technical progress in coronary multidetector computed tomography technology (increased number of detectors – 64, prolonged rotation time and, consequently, good spatial resolution) a careful patient selection and multi-slice coronary analysis led to a current sensitivity of 90-100%, a specificity of 95-100% (Hamon et al., 2006) and a negative predictive value of 97-100% (Yang et al., 2010) in coronary artery disease evaluation. The use of coronary multidetector computed tomography versus conventional coronary angiography and intravascular ultrasound for diagnostic purposes has been assessed in several studies and meta-analysis.

In older studies, in which the computed tomography apparatus had less than 64 detection channels, coronary multidetector computed tomography seemed to provide less useful and less accurate information than conventional coronary angiography. However, the accuracy improved over the years, while the number of detectors continued to increase from 4 to 16 and then to 64, allowing a more faithful description of a larger number of coronary artery segments, previously impossible to visualize. (Hamon et al., 2007) (Vanhoenacker et al., 2007) (Janne d'Othec et al., 2008). Later studies affirmed that coronary multidetector computed tomography might allow a more accurate atherosclerosis evaluation, despite their lower efficacy in assessing stenosis severity.

Recently, other studies tried to compare the diagnostic performance of 64-channel computed tomography with that of invasive coronary angiography in identifying obstructive coronary disease in various populations; the average sensitivity per-patient for identifying obstructive coronary artery disease was 98%, with an average per-patient specificity of 88% (Stein et al., 2008). The mean prevalence of obstructive coronary artery disease in these studies was 61%. The negative predictive value for multidetector computed tomography was estimated at 96%, while the positive predictive value proved to be rather insignificant, with large variations within the interval 64% to 100%.

Recently, most multicenter studies which compared 64-channel coronary multidetector computed tomography with conventional angiography have proved that multidetector computed tomography angiography had excellent sensitivity and very good negative predictive value in detecting coronary artery disease (Mowatt et al. 2008). Nevertheless, the positive predictive values are less important, since coronary artery stenosis is often overestimated by coronary multidetector computed tomography angiography, when compared to coronary angiography. This tends to happen because calcium blooming artifacts diminish the signal acquisition accuracy, whilst invasive coronary angiography fails to appreciate the extent of positive vessel remodelling by atherosclerosis.

In the prospective ACCURACY (Assessment by Coronary Computed Tomographic Angiography of Individuals Undergoing Invasive Coronary Angiography) study, 230 patients with typical or atypical chest pain were enrolled, all of which were referred for invasive coronary angiography and agreed to have a coronary multidetector computed tomography prior to their catheterization (Budoff et al., 2008). After having carefully interpreted the data, the sensitivity, specificity, as well as positive and negative predictive values for detecting ≥50% or ≥70% stenosis were 95%, 83%, 64%, and 99%, respectively, and 94%, 83%, 48%, 99%, respectively. In this prospective multicenter trial of chest pain patients without known coronary artery disease, 64-multidetector row coronary multidetector computed tomography proved a very high diagnostic accuracy in detecting obstructive coronary stenosis for both 50% and 70% stenosis thresholds. Moreover, the high (99%) negative predictive value of coronary multidetector computed tomography angiography pleads for its current use as an effective noninvasive alternative to invasive coronary angiography to rule out coronary artery obstructive stenosis.

The CORE 64 (Coronary Artery Evaluation Using 64-Row Multidetector Computed Tomography Angiography) study was conducted in 9 international centers and enrolled 316 symptomatic patients, aged 40 years or more, with suspected or known coronary disease. All patients whose calcium scores were less than 600 were referred for invasive coronary angiography, 291 (92%) of which completed coronary multidetector computed tomography prior to invasive coronary angiography. Obstructive coronary artery disease was diagnosed in 56% of the patients. Quantitative computed tomography angiography showed a sensitivity of 85%, a specificity of 90%, a positive predictive value of 91% and a negative predictive value of 83% in detecting or ruling out significant (>50%) coronary artery stenosis when compared to conventional angiography, thus proving that it can very accurately assess the presence and severity of obstructive coronary artery disease. However, the negative and positive predictive values estimated by this study are not significant enough to consider completely replacing conventional coronary angiography by multidetector computed tomography. Only two patients in the study group had contrast agent-related side effects after computed tomography angiography. (Miller et al., 2008) (Dewey et al., 2010). One study conducted in the Netherlands showed that multidetector computed

tomography has a considerable value in diagnosing obstructive coronary disease, but is slightly less valuable for stenosis severity assessment when compared to both conventional coronary angiography and intravascular ultrasound. In this study, 100 patients underwent coronary multidetector computed tomography angiography followed by both conventional coronary angiography and intravascular ultrasound. Only those segments in which intravascular ultrasound imaging was performed were included for coronary multidetector computed tomography and quantitative coronary angiography analysis. On coronary multidetector computed tomography angiography, each segment was evaluated for significant stenosis (defined as ≥50% luminal narrowing, as in conventional coronary angiography). Secondly, on coronary multidetector computed tomography angiography, each segment was evaluated for the presence of atherosclerotic plaques. Atherosclerosis was defined on intravascular ultrasound as a plaque burden covering ≥40% from the vessel's cross-sectional area. Multidetector computed tomography angiography correctly ruled out significant stenosis in 53 of 53 (100%) patients. However, 19% patients were incorrectly diagnosed as having significant lesions on coronary multidetector computed tomography angiography, which finally led to 100% sensitivity, 85% specificity, 81% positive and 100% negative predictive values. (van Velzen et al., 2011).

Nowadays, coronary multidetector computed tomography angiography is used to diminish the large number of normal coronary angiographies (by 25-30 %) (Blanchard et al., 2002).

Although coronary multidetector computed tomography angiography results prior to 64-channel technology were once considered adequate, the current trend in cardiac imaging is to ignore such studies, which are only limited to assessing coronary calcium (Otero et al. 2009). To this day, the incremental diagnostic value of 128 -, 256 - and 320-channel coronary multidetector computed tomography systems over 64-channel systems has not been proven. (Mark et al., 2010) (Hein et al., 2009). However, non-invasive methods might completely replace the expensive and rather unpleasant coronary angiography in the future.

64-slice coronary angiography can be as effective as conventional coronary angiography in patient selection for coronary artery by-pass graft surgery, especially when 3-vessel or left main coronary artery disease and its equivalents are present. The overall sensitivity, specificity, positive predictive value and negative predictive value of coronary multidetector computed tomography angiography for coronary artery bypass graft surgery candidates selection were 85.9%, 96.0%, 93.8%, and 90.7%, respectively. (Lee et al., 2011).

Despite all its advantages, all cases should be thoroughly selected, as coronary multidetector computed tomography angiography exposes the patient to a fairly large amount of radiation. Since coronary artery disease has become the main cause for mortality in recently developed countries, a noninvasive test that would not imply patient exposure to radiation is greatly needed.

In addition to coronary multidetector computed tomography, magnetic resonance imaging angiography can also provide accurate data concerning cardiac morphology, as well as coronary and cardiac functions (Hoffmann et al., 2008). Over the last 20 years, cardiac magnetic resonance imaging techniques have developed greatly, but their current use in coronary artery disease assessment is rather limited, despite the high initial expectations. In an expert consensus document on "appropriate" indications for cardiac multidetector computed tomography (Tayloret al., 2010) and magnetic resonance imaging published in 2006 (Hendel et al 2006), only coronary multidetector computed tomography angiography is recommended as an "adequate" imaging method for coronary artery disease assessment, while cardiac magnetic resonance imaging angiography is firmly rejected.

As far as spatial resolution and study success rates are concerned, coronary multidetector computed tomography angiography is superior to coronary magnetic resonance imaging angiography. The latter also has the major disadvantages of long image acquisition times and operator dependency, which limit its use to diagnosing congenital abnormal coronary artery origin and coronary artery aneurysms in patients with Kawasaki disease (Sakuma et al., 2011).

In a recent study, multidetector computed tomography and magnetic resonance imaging angiography were compared. 7516 patients were evaluated by multidetector computed tomography, whilst 989 underwent magnetic resonance imaging angiography. Bivariate analysis of data yielded a mean sensitivity and specificity of 97.2% and 87.4% for computed tomography and 87.1% and 70.3% for magnetic resonance imaging. In other studies which only included patients with suspected coronary artery disease, sensitivity and specificity of computed tomography were 97.6% and 89.2%. Covariate analysis showed that 16-detector row scanners had a greater sensitivity (98.1%) in diagnosing coronary artery disease than older-generation scanners (95.6%) (Schuetz et al., 2010).

When using steady-state free precession magnetic resonance imaging angiography, no contrast agents are needed; however, the accuracy of this approach has not yet been determined in a multicenter trial. So far, in a patient-based analysis, the sensitivity, specificity, positive and negative predictive values of magnetic resonance imaging angiography were 88%, 72%, 88%, and 79%, respectively, all significantly lower than for multidetector computed tomography. The non–contrast-enhanced whole-heart coronary magnetic resonance imaging angiography at 1.5-T can noninvasively detect significant coronary artery disease with high sensitivity and moderate specificity. A negative predictive value of 88% indicates that whole-heart coronary magnetic resonance imaging angiography can rule out coronary artery disease (Kato et al., 2010).

A single MRI study can provide very useful information regarding ischaemic heart disease. This technique allows the assessment of rest function, as well as gadolinium chelate contrast evaluation. The latter comprises three postcontrast temporal phases: phase one provides information regarding microvascular obstruction, which is the equivalent of the no-reflow phenomenon, at rest and during stress; early perfusion is used to evaluate microvascular obstruction and to track down thrombi, while late gadolinium enhancement technique provides data regarding the presence of myocardial infarction and focal myocardial damage. In most cases of acute chest pain, biomarkers and electrocardiographically can identify high risk patients; however, in some circumstances, coronary artery disease cannot be detected by such methods, thus imposing the evaluation by contrast cardiac MRI; first-pass perfusion or early enhancement techniques (Nijveldt et al., 2008) can be used for this purpose. Ingkanisorn et al, apealed to MRI examination after adenosine stress perfusion to investigate patients with typical angina and negative troponin; in such cases, adenosine stress results proved to have a 100% sensitivity and 93% specificity and thus a very high negative predictive value (Ingkanisorn et al., 2006). Cardiac MRI-measured microvascular obstruction is frequently associated with extensive myocardial damage and poor left ventricular function and, by consequence, with a poor functional recovery and clinical outcome (Rubenstein et al., 2008).

A multi-center clinical trial proved that delayed-enhancement cardiac magnetic resonance imaging can provide solid evidence for the presence of acute and chronic myocardial infarction, particularly in patients with negative troponins. Moreover, this type of investigation can accurately discriminate between ischemic and non-ischemic myocardial

injury, which helps identify the correct diagnosis in patients with normal angiographic results. This technique is useful even in cases of confirmed coronary artery disease, as it allows the examiner to identify details such as microvascular damage, myocardial stunning, residual viability or right ventricular infarction. Post-myocardial infarction complications such as pericarditis or left ventricular thrombi can also be identified, which can help in choosing the right therapy for these patients. MRI evaluation also allows the quantification of infarct size by delayed-enhancement techniques and can be a valuable surrogate end point for clinical trials, with significant reductions in sample size when compared to other methods (Kim et al., 2010).

Cardiac magnetic resonance imaging can complete or replace echocardiographic findings in patients with dilated ischemic cardiomyopathy and can provide useful information concerning myocardial perfusion and viability (Maceira et al., 2006). Left ventricular dysfunction after myocardial infarction may be due to necrosis, to post infarction stunning or hibernation of viable myocardium (Schinkel et al., 2007). Recurrent stunning may lead to myocardial hibernation and requires revascularization for function recovery.

Regional myocardial function can be evaluated by regional kinesis or regional parietal thickening assessment using standard or post-apnoea cardiac magnetic resonance imaging techniques, in normal conditions or after pharmacologically induced stress (using adenosine or dobutamine) (Kuijpers et al., 2004). Stress cardiac magnetic resonance imaging is particularly useful for assessing lesions in stable coronary patients who have no indication for coronary angiography (Kuijpers et al., 2004).

By using cine-cardiac magnetic resonance imaging in post-apnoea, major coronary arteries images can be obtained, while morphology and tissue analysis of the atherosclerotic plaque allows an accurate assessment of its composition and rupture risk. It is possible to evaluate the atherosclerotic plaques while using echo-spin sequences with T_1, T_2 pondering or by measuring proton density. However, the low temporal resolution of this technique limits its current use. In this case, MRI signal improvement by using contrast agents, especially gadofluorine (Sirol et al., 2004), allows a more appropriate examination of the atherosclerotic plaque because of its fast and persistent (as long as 24 hours) accumulation in lipid-rich atherosclerotic plaques. The gadofluorine-M-enhanced MRI can accurately identify atherosclerotic plaque inflammation and neovascularization in animal models of atherosclerosis, which can help identify high rupture risk atherosclerotic plaques (Sirol et al., 2009).

Since macrophage activity is an essential factor in atherosclerosis development and complications, macrophage imaging can be used as a biomarker for subclinical inflamed lesions, allowing risk assessment and therapy guiding. A sufficient T_2 signal intensity after superparamagnetic phagocytosable nanoparticles administration can help identify inflamed plaques and closely monitor early tissue response to adequate therapy (Morishige et al, 2010). Cardiac magnetic resonance imaging can also highlight neoformation blood vessels in the atherosclerotic plaque by using Gadolinium, which binds $\alpha v\beta 3$ vessel wall integrin. Other contrast agents, with high affinity for lipidic structures, muscular and inflammatory cells, can also be used for assessing subclinical coronary artery disease by cardiac magnetic resonance imaging (Barkhausen et al., 2003).

Velocity encoding techniques in post-apnoea can be used to measure the coronary flow with the purpose of establishing the coronary flow reserves. Last, but not least, the fast echo-gradient with phase contrast technique allows perfusion evaluation in normal and stress conditions (using vasodilators) and normal or pathological coronary flow velocities. Other

technologies are able to provide an accurate image of diseased vessel walls, such as intravascular ultrasound, magnetic resonance imaging, and optical coherence tomography (Mark et al., 2010).
Cardiac magnetic resonance imaging allows a fast, radiation-free investigation of the myocardial function, perfusion and viability and can provide useful data concerning coronary arteries and atherosclerotic plaques. However, precise indications for the use of this technique, based on a balanced risk-advantage ratio, are yet to be established.

2.2 Prognostic value of non-invasive cardiac imaging in patients with coronary artery disease

Coronary calcifications are vascular degenerative phenomena determined by active vascular remodeling processes. The calcium burden is an important risk factor for vascular remodeling (Schmermund et al., 2001) and plaque instability (Burke et al., 2002). Histo-pathological studies (Rumberger et al., 1995) have shown a significant relationship between calcified areas and the level of arterial atherosclerosis, indicating that coronary calcifications are direct markers of coronary artery disease.
Coronary multidetector computed tomography is a sensitive method for detecting coronary artery calcium. Multidetector computed tomography coronary angiography allows a pseudo-quantitative assessment of coronary artery calcifications using the Agatston calcium score (Corti et al., 2001) as well as calcium mass and volume calculations for certain coronary segments (Ulzheimer et al., 2003). Although the latter method proved to have higher reproductibility, the Agatston score is more frequently used in clinical practice. The prognostic value of coronary calcification assessment by multidetector computed tomography coronary angiography has been reported in several studies, but with mixed results (Chow et al., 2010) (Carrigan et al., 2009) (Hay et al., 2010).
Coronary multidetector computed tomography is also important for identifying high risk asymptomatic patients, especially if calcium scoring is used (Detrano et al., 1996) and has proved to be more accurate than the Framingham score (Greendland et al., 2004) or the SCORE method. In asymptomatic patients, low detectable coronary artery calcium scores are significantly less reliable in predicting plaque burden due to their association with high overall noncalcified coronary artery plaque prevalence and nearly a 10% rate of significantly occlusive noncalcified coronary artery plaque (Cheng et al., 2007). In one study, in which 632 subjects were enrolled, Raggi et al. have demonstrated that elevated coronary artery calcium can increase the odds of acute myocardial infarction and cardiac death in asymptomatic patients (Raggi et al., 2000). The MESA study also proved that the coronary artery calcium score was a better predictor for cardiovascular events in 6772 asymptomatic patients, when compared to classic risk factors (Detrano et al., 2008). The target is to emphasize risk stratification and primary risk prevention in asymptomatic patients in order to decrease cardiovascular mortality and morbidity. The Framingham Risk Score only predicts coronary heart disease events moderately well when family history is not included as a risk factor. There is a current preoccupation for developing new risk stratification tests and establishing new and more accurate risk factors. While the Framingham Risk Score, European Systematic Coronary Risk Evaluation Project and European Prospective Cardiovascular Munster Study still provide excellent tools for risk factor modification, the coronary artery calcium score may bring an additional benefit in risk assessment. There have been several studies supporting the role of coronary artery calcium score in predicting myocardial infarction and cardiovascular mortality, which proved to be significant for risk

stratification in asymptomatic patients presenting in the emergency room (Sharma et al., 2010). In CORE study done on 291 patients (5% patients had low, 75% had intermediate, and 20% had high pre-test probability of obstructive coronary artery disease) was observed that the absence of coronary calcification does not exclude obstructive stenosis or the need for revascularization among patients with high enough suspicion of coronary artery disease to be referred for coronary angiography, in contrast with the published recommendations (Gottlieb et al., 2010).

Also, the total coronary calcium score measured by multidetector computed tomography can be used as an independent prediction factor for major acute coronary events in patients with chest pain. A recent study on 263 patients presenting in the emergency room with acute chest pain and low-to-intermediate cardiovascular risk, defined by traditional risk factors, showed that computed tomography coronary artery calcium assessment is a powerful tool for prognosis evaluation. The absence of coronary artery calcium suggests an excellent long-term (5-year) prognosis, with no primary or secondary cardiac outcomes occurring in study patients at 5-year follow-up (Laudon et al., 2010). However, it has not yet been established whether there is a significant correlation between the total coronary calcium score and long- term clinical outcomes in patients who underwent percutaneous coronary intervention with drug eluting stents. In a recent study on 286 patients with coronary artery disease, Jeong showed that the total coronary calcium score measured by multidetector computed tomography was highly associated with chronic and significant coronary lesions, while the coronary calcium score ≥ 400 predicted lower procedural success rate and poor long-term clinical outcomes (Jeong et al., 2010).

One recently published meta-analysis (Hay et al., 2010) established the prognostic value of multidetector computed tomography in 9592 symptomatic patients with suspected coronary artery disease, reporting major adverse cardiovascular events such as death, myocardial infarction and revascularization. The sensitivity was 0.99 for major adverse cardiovascular events after normal multidetector computed tomography findings, with a specificity of 0.41. Stratifying by no coronary artery disease, nonobstructive coronary artery disease, or obstructive coronary artery disease, there were incrementally increasing adverse events. Adverse cardiovascular events among patients with normal multidetector computed tomography findings are very rare and comparable to a baseline risk among healthy patients. Increasing burden of coronary artery disease on multidetector computed tomography is associated with an increasing rate of revascularization necessity, myocardial infarction, and death. In predicting the incidence of adverse clinical events, normal findings in multidetector computed tomography are comparable to reported values for stress myocardial perfusion scan or stress echocardiography.

The critical analysis of these studies shows that there is a moderate correlation between the Agatston score and coronary artery stenosis assessed by coronarography (Detrano et al., 1996); secondly, the total calcium scoring seems to have a rather low positive predictive value, which tends to vary according to sex and age, but a significantly high negative predictive value (>95% in asymptomatic patients and 98% in patients presenting with chest pain) (McLaughlin et al., 1999). Some of these studies were not adequately powered to detect differences in rates of clinical outcomes such as death, myocardial infarction, and coronary revascularization. Adequate outcome evaluation studies require the submission of large samples and often long periods of follow-up. A recent multidetector computed tomography expert consensus statement sponsored by the American College of Cardiology and 6 additional medical societies points to the need for continued collection and

assessment of prognostic data after multidetector computed tomography (Mark et al., 2010).

2.3 Therapeutic value of non-invasive cardiac imaging in patients with coronary artery disease

It is quite difficult to establish whether there is a perfect diagnostic test, since there are quite many indirect issues to consider. A diagnostic test can only be considered valuable if it provides additional information that can help alter the clinical approach. So far, stress testing has not proven useful in such patients. However, some tests were validated by clinical trials as useful tools for every day practice. For instance, the choice between early emergency revascularization versus elective angiography seemed to favor the latter, as the clinical outcome tends to be better (Mark et al., 2010). Although it is reasonable to assume that fully validated diagnostic tests that show a direct impact on the clinical outcome are better, such level of validation is quite difficult to obtain, since large trial funding is scarce and the studies themselves are time consuming. Some statistical models including chi-square, p values with adjusted hazard ratios or calculation of c-indexes and proportion of subjects with reclassified risk have shown that some anatomic imaging tests are more likely to provide useful additional information in patients with acute coronary syndromes. However, favorable statistical analysis does not imply that a certain diagnostic test should alter clinical outcomes more than others and usually does not influence clinical decisions, as the test's value is often overestimated (Mark et al., 2010).

2.4 Non-invasive cardiac imaging in clinical practice of patients with coronary artery disease

The evolution of atherosclerosis is progressive and clinically silent for a long period of time before stable angina is installed, followed by non-stable angina and other acute coronary syndromes. The term "acute coronary syndrome" includes unstable angina pectoris, non-ST-segment elevation myocardial infarction and ST-segment elevation myocardial infarction, all of which are life-threatening events (Figure 1). Over the past decades, following the use of evidence-based guidelines for the diagnosis and treatment of these conditions, the prognosis has improved considerably, as mortality rates became lower.

Current guidelines provide vital information which can help assess the appropriate use (Taylor et al.; 2010), performance (Abbara et al., 2009), and interpretation (Raff et al.; 2009) of multidetector computed tomography and magnetic resonance imaging (Hendel et al.; 2006) in order properly establish the diagnostic and therapeutic approach towards patients with coronary artery disease.

2.4.1 Asymptomatic patients with coronary artery disease

In asymptomatic patients with high risk for developing silent or manifest coronary artery disease, including acute myocardial infarction or coronary heart disease death, risk stratification is essential and three different classes have been established (Taylor et al.; 2010):

- Low risk of coronary artery disease development; the age-specific risk level is below average and correlates with a 10-year absolute coronary heart disease risk <10%.
- Intermediate risk of coronary artery disease development; the age-specific risk level is average or above average and correlates with a 10-year absolute coronary heart disease risk between 10% and 20%.

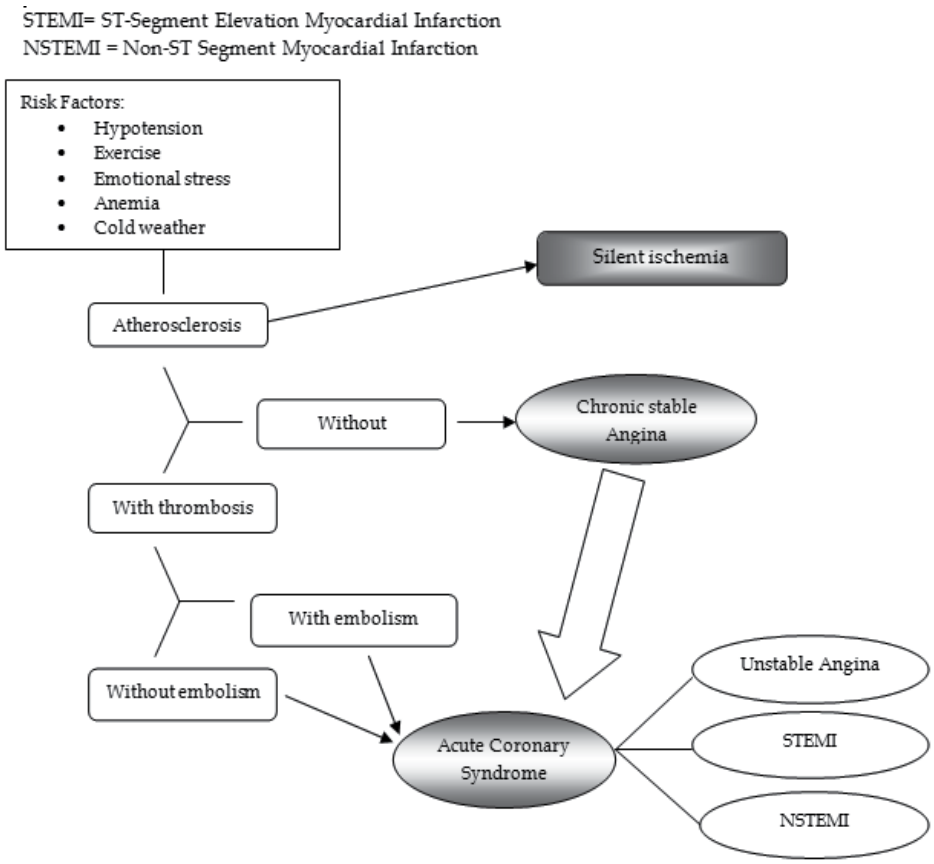

Fig. 1. Algorithm of diagnostic in coronary artery disease

- High risk of coronary artery disease development; in patients >40 years of age, with diabetes mellitus, peripheral arterial disease or other coronary risk equivalents; it correlates with an absolute or 10-year risk of coronary heart disease >20%.

Multidetector computed tomography angiography (64-slice scanner) has high sensitivity and specificity in patients presenting to the emergency department with atypical chest pain, normal cardiac biomarkers and/or non-diagnostic ECG (Hoffmann et al., 2009).

Symptomatic patients with coronary artery disease are stratified in three classes according to the risk of major cardiovascular events development:

- Low risk for developing cardiovascular events (<10%).
- Intermediate risk for developing cardiovascular events (10% to 90%).
- High risk for developing cardiovascular events (>90%).

2.4.2 Symptomatic patients with stable angina pectoris

Several studies have shown that multidetector computed tomography helps assess non-significant, but possibly unstable atherosclerotic plaques with high risk for rupture and embolisation. This is the more important as the negative predictive value of stress tests with regard to major cardiovascular events is rather low (Vignaux, 2008). The role of cardiac magnetic resonance imaging in such patients is not established (Figure 2).

CAD = coronary artery disease

MDCT = multidetector computed tomography

MRI = magnetic resonance imaging

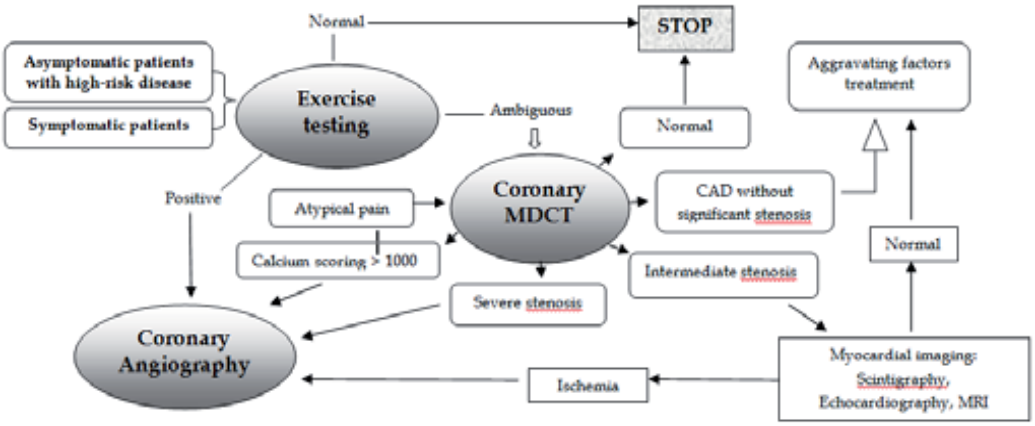

Fig. 2. Non-invasive imaging in stable coronary artery disease detection algorithm

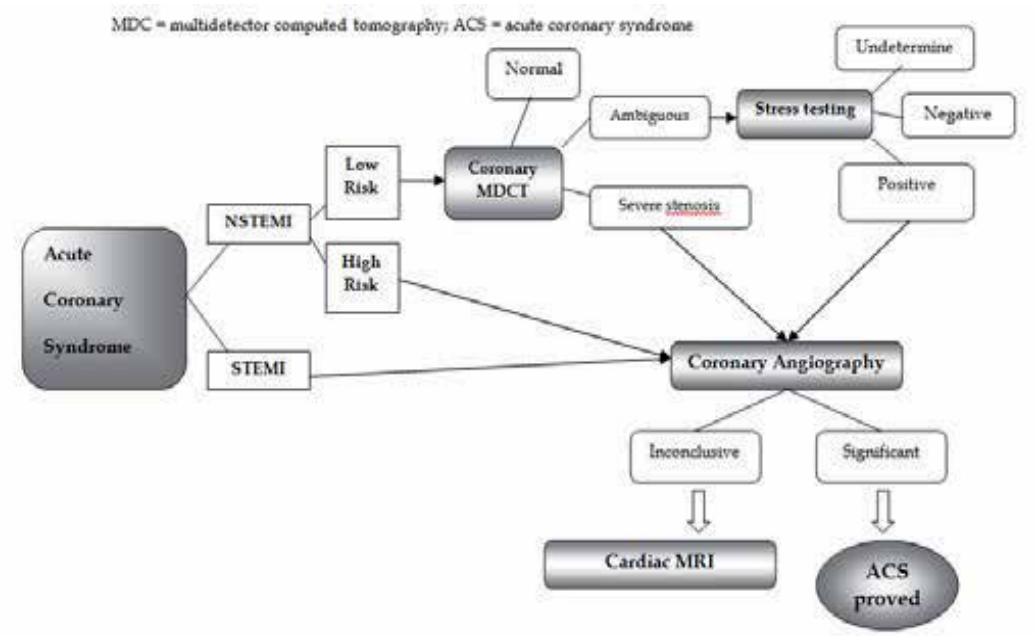

Fig. 3. Algorithm for non-invasive imaging use in detecting coronary artery disease in patients with Acute Coronary Syndrome

2.4.3 Symptomatic patients with acute coronary syndromes

Non-invasive imaging techniques should be employed according to the type of coronary syndrome (ST-segment elevation myocardial infarction, non- ST-segment elevation myocardial infarction or UI) or the possible risk (whether high or low) for developing major cardiovascular events (Figure 3).

2.4.3.1 ST-segment elevation myocardial infarction patients

In ST-segment elevation myocardial infarction patients, non-invasive cardiac imaging is used only in accordance with the intended moment for revascularization (before of after revascularization), with the time passed from the onset of pain until hospitalization (acute or recent myocardial infarction) or with invasive coronary angiography results (coronary lesions versus normal arteries). In ST-segment elevation myocardial infarction patients which present within the first hours from the onset of pain, emergency revascularization is required and non-invasive imaging is of no use. However, non-invasive imaging can prove useful in patients with previously revascularized ST-segment elevation myocardial infarction for assessing residual ischaemia and establishing the need for late revascularization. Multidetector computed tomography is particularly useful if common trunk or right coronary artery sinus lesions, non-detected by invasive techniques, are suspected or in quantifying lesion length, as well as the vascular bed down-stream in order to provide the necessary information pending aorto-cornary by-pass. Cardiac magnetic resonance imaging in post-revascularization ST-segment elevation myocardial infarction patients is useful for morphology and global and segmentary left ventricle systolic function assessment, mechanical complication evaluation, infarctus size, stress and rest myocardial perfusion. Cardiac magnetic resonance imaging is particularly efficient in establishing the cause of acute coronary syndromes in ST-segment elevation myocardial infarction patients with normal coronary arteries, who sometimes have a Tako-Tsubo syndrome or suffer from myocarditis. In some cases, coronary multidetector computed tomography can help identify intra-stent restenosis or unstable atherosclerotic plaques, as well as coronary artery abnormalities that have not been identified during the invasive procedure. At patients with ST-segment elevation myocardial infarction and normal coronaries (Vignaux, 2008), cardiac magnetic resonance imaging have an important role in establishment of infarctus diagnosis and for excluding Tako-Tsubo syndrome or myocarditis.

2.4.3.2 Non-ST-segment elevation myocardial infarction patients

Myocardial revascularization is not a top priority in patients presenting with non-ST-segment elevation myocardial infarction, since no major long term benefit has been proven. The most appropriate approach is a late (48 hours) angiography. Multidetector computed tomography angiography could help select patients at risk who have normal myocardial biomarker values and non-diagnostic ECGs and allow the early discharge of subjects with normal coronary arteries. (Goldstein et al., 2007) (O'Connor et al, 2010).

2.4.3.3 Patients with unstable angina and atypical chest pain

Multidetector computed tomography is very useful in patients with atypical thoracic pain and normal cardiac enzymes, as well as in patients with valvular or vascular disease, dilated cardiomyopathy, congenital heart disease or left ventricular aneurysms, intracardiac thrombi and confirmed coronary artery disease. In addition to that, only 15-25% of hospitalized patients presenting with nonspecific acute chest pain prove to have genuine acute coronary syndromes (Yoo et al., 2010)

Coronary artery disease can also provide additional data when common trunk or right coronary ostium stenosis are present, in case of myocardial infarction with normal coronary arteries or in patients with confirmed coronary artery disease. Cardiac magnetic resonance imaging can be used for confirmation and risk stratification in patients with coronary artery disease and for sustaining differential diagnosis with myocarditis or pericarditis.

3. Conclusion

Over the last few years, new non-invasive imaging techniques that allow heart exploration such as coronary multidetector computed tomography and cardiac magnetic resonance imaging have demonstrated their diagnostic and prognostic value, as well as their impact on clinical outcomes, when properly used. However, despite convincing evidence concerning their high efficiency, sustained by several studies, clinical trials and meta-analyses, there is still an acute need for proper algorithms which can be used for basic approach in relation to cardiovascular risk and coronary artery disease severity.

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5

Non-Invasive Coronary Angiography

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1. Introduction

Computed Tomography (CT) scanners essentially consist of a rotating X-ray tube emitting a fan-beam of X-rays mounted on a gantry opposite a set of curvilinear detector rows. The X-ray beam, collimated at source and prior to detection, rotates around the patient who lies on a table that passes through the gantry. The gantry may either move sequentially down the table (step and shoot) or the table and the gantry move together (helical scanning) thereby reducing scan times and improving temporal resolution.

Early CT scanners, with only one detector and a pencil beam, took approximately 3 minutes to complete one 360^{o} rotation around the patient. Fan shaped x-ray beams, increasing the number of detectors and the advent of slip-ring technology allow modern CT scanners to have speeds in excess of 330ms per rotation (with their absolute mechanical limit being between 50 and 200ms) and has allowed cardiac CT to flourish, and in particular allows motion free images of the coronary arteries to become a reality. (Kalender, 2000, as cited in Nicol & Padley, 2007a).

The detectors sense and record the attenuation of the X-ray beam for any given point in the imaged slice. In Cardiac CT (CCT) images are obtained with slice thicknesses as thin as 0.4mm. The X-ray attenuation is translated into a numerical value (Hounsfield Units (HU)). Multiple attenuation values are obtained from any given point during the rotation of the X-ray tube. Filtered back projection is then automatically performed to achieve a final attenuation value. These values are converted and mapped to form a grey-scale image.

Magnetic resonance uses a strong static magnetic field to effectively magnetise the protons in the body. Radiofrequency pulses are transmitted to excite the protons in the tissue being imaged and an echo signal is produced and recorded in the receiver coils and these are used to produce an image. Different types of pulse sequences can be used to take advantage of the different relaxation characteristics of the tissue to help generate image contrast. Typically a 1.5 tesla (T) MRI scanner is used for cardiac MR (CMR) and superior image quality is achieved by using higher numbers of receiver channels.

2. Technical aspects of CT and MR coronary angiography

CT and MR coronary angiography (CTCA and MRCA) depend on three main factors – spatial resolution, temporal resolution and contrast resolution.

2.1 Spatial resolution

Spatial resolution is defined as the ability to distinguish two separate objects in close proximity (Fig. 1) (Smith, 1997).

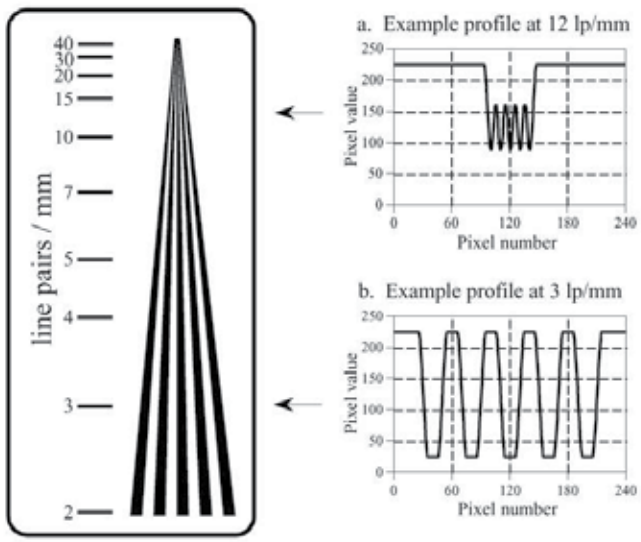

Fig. 1. Spatial resolution , expressed as line pairs/mm (lp/mm), is considered the point at which the individual strips cannot be readily distinguished by the eye. A line pair gauge such as this one is typically used to measure this. Reproduced with permission (Smith, 1997).

This is critically important in coronary artery imaging as coronary arteries have small luminal diameters, approximately 5mm at the ostia, tapering distally (or within the branches) to < 1mm. As CT and MR values for any given point is represented as a voxel (a three dimensional pixel), the smaller the voxel, the higher the spatial resolution. Other factors that affect spatial resolution may be fixed or variable. Fixed (non-modifiable) factors include scanner capabilities and patient size whilst variable factors include heart rate and motion artefact that can, to large extent, be mitigated.

2.1.1 Fixed factors

Current CT scanners generate images with isotropic voxel sizes as small as $0.4mm^3$. Importantly, the detector thickness of the scanner determines the z-axis "in-plane" resolution which varies between manufacturers from 0.4 to 0.7mm (Nicol & Padley, 2007a). As a result of this limitation CTCA can currently only distinguish stenoses to within 30% accuracy, compared with 10% on invasive coronary angiography (ICA) with a spatial resolution of 0.1-0.2mm. The spatial resolution of MR is typically 1-1.5mm but high resolution black blood images may be as low as 0.6mm.

2.1.2 Variable factors

In CT and MR the attenuation or signal values within each voxel are averaged out before being displayed on a grey scale image. Slice thickness is also modifiable; the thicker the slice, the greater the volume averaged and therefore the lower the spatial resolution. The trade-off with higher spatial resolution is increased noise. In both CCT and CMR spatial resolution can be improved by reducing the field-of-view, akin to zooming into an image. In CT coronary angiography, thin-cuts are obtained with the field of view reduced to just larger than the cardiac boundaries (Fig. 2).

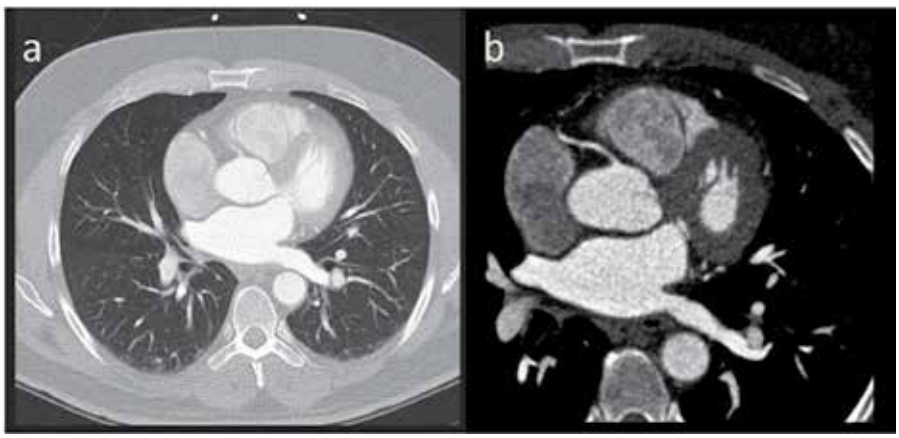

Fig. 2. The acquired scan is reconstructed to give a wide field-of-view (FOV) to include the lungs (a) and a smaller FOV to increase spatial resolution of cardiac structures (b).

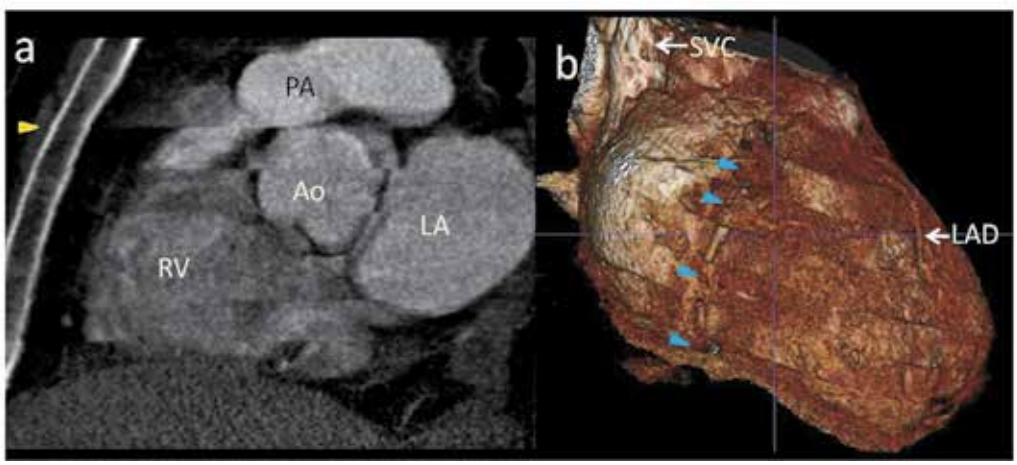

Fig. 3. Both cardiac and respiratory motion can lead to step artefact. These appear as horizontal lines on the sagittal dataset (panel a) and missing sections of the right coronary artery (blue arrowheads) on the volume rendered reconstruction (panel b). Respiratory rather than cardiac motion artefact can be distinguished by the involvement of the sternum (yellow arrowhead) in the former (panel a). (RV=Right Ventricle; Ao=Aorta; PA=Pulmonary Artery; LA=Left Atrium; SVC=Superior Vena Cava; LAD=Left Anterior Descending artery.)

Motion artefact impairs spatial resolution in both CCT and CMR. A well-prepared and co-operative patient who is able to comply with the breathing instructions will reduce the chance of step artefact (in CCT) (Fig. 3) and blurring (in CMR) due to respiration or movement. Reducing the heart rate reduces cardiac motion by increasing the diastolic phase during which coronary arteries move least. Arrhythmias, especially if irregular, may make prospectively gated studies impossible.

2.2 Temporal resolution

Cardiac motion artefact can also be reduced by acquiring images faster. In CCT this is achieved by increasing the speed of rotation of the gantry and the pitch of the table. This is similar to selecting a faster shutter speed on a camera and enables fast-moving structures such as coronary arteries to be captured with minimal blurring. Standard single source scanners with temporal resolution of 165 to 250 ms require heart rates to be <65bpm for optimal coronary image quality, and pharmacological rate control, usually with β-blockers, is ubiquitous. Dual source CT scanners have reduced the temporal resolution in CCT to 75ms with each detector array requiring only a quarter scan of data. This has made acquisition of CTCA possible at almost any heart rate (Flohr et al., 2006); however image quality is still improved at lower heart rates.

The temporal resolution of CMR is typically 50ms. It is preset by the technician and is not constrained by MR hardware as with CT. However, tachycardia does adversely affect image quality and lower heart rates are more desirable as the scan time is reduced and more k space is filled during each cardiac cycle (Kato et al., 2010).

2.3 Contrast resolution

Contrast resolution is the ability to distinguish between objects of different attenuation or signal when they are next to each other. In CT the coronary arterial wall and lumen have similar attenuation values and administration of intravenous contrast is therefore required. Adequate and well-timed opacification enables differentiation of the vessel wall from the lumen. Various components of atherosclerotic plaque also have different densities and are able to be characterised. This is an advantage of CTCA when compared with the pure lumenography of invasive catheter angiography. Coronary calcium can be readily identified on an unenhanced CT scan. However lipid-rich soft plaque, that is more prone to rupture and vessel remodelling are not visible without contrast administration (Fig. 4).

In CMR, exogenous contrast agents are usually not required. In 2D black blood sequences, a dual inversion recovery prepulse is used to make the blood appear black with persisting signal within the walls of the coronary arteries, producing images with reasonable contrast. For bright blood sequences, prepulses make the blood appear bright with adjacent tissues including myocardium and fat appearing dark. The prepulses used include T2 preparation pulses and fat saturation techniques, pre-programmed into the CMR sequence.

2.4 Patient preparation

Patient preparation is probably the most vital part of ensuring diagnostically adequate studies in both CCT and CMR. The patient selection process should identify those who would benefit from CTCA or MRCA and those who would be suitable to have the scan. Attention should be paid to patient factors such as excessive body mass index, arrhythmias, potential inability to keep still or follow breathing instructions or claustrophobia. If present, alternative means of coronary assessment should be considered.

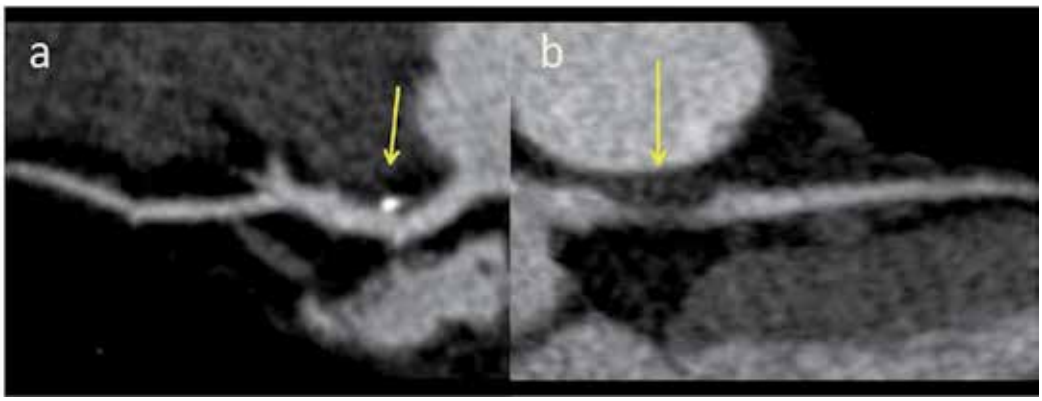

Fig. 4. Eccentric plaque (yellow arrows) can lead to positive remodelling (panel a) where the vessel expands to preserve lumen size, however continued plaque accumulation eventually leads to stenosis (panel b).

All patients referred for CCT or CMR should receive a patient information leaflet outlining the process of their scan. Patients are usually told to take their usual medications, including cardio-active medications, and to avoid consuming caffeine for twelve hours prior to the scan. On arrival, baseline observations including a heart rate and blood pressure should be taken. Patients should complete a questionnaire about allergies, relevant medical conditions and medications.

For CCT, contraindications to β-blockade and glyceryl trinitrate (GTN) are also ascertained. Intravenous access in the right antecubital fossa that allows rapid flow of contrast should be sited (18G or 20G cannula). The right side is used as it prevents high density contrast traversing the thorax and obscuring the cardiac structures through streak artefact (Nicol et al., 2008a).

For both CT and CMR, ECG electrodes are placed in the appropriate positions on the patient's chest to obtain a good amplitude R wave on the ECG trace. For CCT, where a low heart rate is critical, the heart rate is monitored and if just greater than 70 beats per minutes (bpm), breathing instructions alone may reduce the heart rate to < 65bpm. If the heart rate remains greater than 70bpm, negative chronotropic agents should be considered to reduce the heart rate.

For CCT the commonest drug used to reduce the heart rate is metoprolol. It is cheap, has a short half-life and is available in oral and intravenous (IV) forms, both of which are equally efficacious. Ideally, patients should be rate controlled prior to attendance at the CT department; however, if the heart rate remains high, IV metoprolol can be given immediately before acquisition. Ivabridine (Procoralan) can be used as an alternative to β-blockade in those with contra-indications. Sublingual GTN can be administered to promote vasodilatation of the coronary arteries and improve image quality but the patient should be warned about headaches as possible side effects (McParland et al., 2010).

2.5 ECG gating

Once the patient's heart rate is optimised, the appropriate CCT or CMR gating protocol is selected for the acquisition.

For CCT gating may be prospective or retrospective depending on the clinical scenario and information required. Cardiac motion is usually least in diastole, usually between 60-80% of

the R-R interval (Fig. 5). However, in patients with heart rates greater than 70bpm, imaging the heart in end-systole (35% of the R-R interval) may be better (Hoffmann et al., 2005).

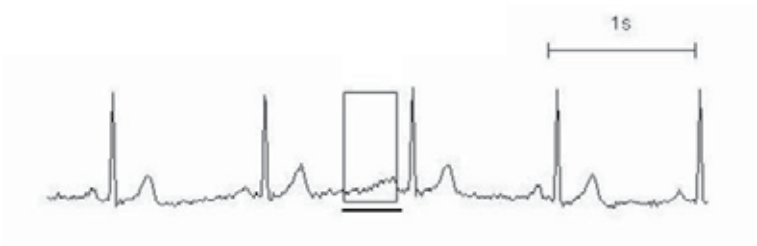

Fig. 5. Sample gated ECG where the heart is scanned during 60-80% of the cardiac cycle (diastole). This is when cardiac motion is likely to be at its minimum.

In order to minimise radiation dose, prospective gating (with variable temporal padding) is usually preferred if the heart rate is between 55 and 70bpm. However, if the heart rate cannot be optimised to less than 70bpm, or is irregular, a retrospectively gated study should be considered. Even with retrospective gating, newer scanning algorithms are able to limit the higher dose delivered to diastole (dose modulation). However, even with this, the retrospectively gated acquisition confers a higher radiation dose to the patient. However, as the heart is imaged throughout the whole cardiac cycle, additional information on cardiac output, ejection fraction and wall motion analysis can be obtained. With increasing experience, it may also be possible to perform diagnostically adequate prospectively gated studies in patients with certain arrhythmias as long as the heart rate variability is not too extreme. In CMR, prospectively triggered and gated scans are acquired.

3. Acquiring CTCA and MRCA

3.1 CTCA acquisition

The CT coronary angiogram is acquired in several steps – topogram, coronary calcium score, test bolus and contrast enhanced coronary angiogram.

3.1.1 Coronary calcium scoring

The presence of calcium is a surrogate marker for atherosclerosis and an independent risk factor of future coronary risk. It is used as an adjunct to conventional risk stratification. To obtain a coronary calcium score, an un-enhanced scan is performed from the carina to just below the diaphragm. Good contrast resolution with CT enables quantification of the overall burden of disease. The usual scoring system is the Agatston calcium score (Agatston et al., 1990). Software used in coronary calcium scoring automatically detects any structure >130HU. The aggregate score of all detected calcium which lies within the coronary arterial tree is used to calculate the overall coronary calcium score.

3.1.2 Contrast administration (test bolus and CTCA)

Intravenous contrast administration improves the contrast resolution of the coronary angiogram and is essential for lumenography. For CTCA a high iodine concentration (300-370 mg/ml) is required for appropriate opacification. Usually the contrast injection is given

as a timed bolus followed by a saline push to concentrate the dye in the left heart and aorta. The minimisation of contrast within the right heart and SVC reduces the likelihood of streak artefact interfering with the interpretation of the proximal right coronary artery.
The accuracy of the timing of the bolus may be improved by the use of a test bolus or bolus tracking to accurately determine the time taken to achieve peak concentration in the ascending aorta prior to full coronary assessment.
The test-bolus method determines the time to peak concentration of a small bolus of contrast in the aortic root. This time plus an additional 3-5 seconds (to allow adequate coronary opacification) is then used for the CTCA acquisition. The test bolus allows the patient to be aware of the common side effects of flushing and hopefully negates the potential heart rate response during the full CTCA contrast administration. Bolus tracking is similar to the test bolus but the scan and contrast injections are activated simultaneously. Once the contrast opacification in the region of interest reaches a predetermined threshold (usually 100-150HU), and following a preset delay (usually 5-8 second) to allow for breathing instructions or table movement, the full scan is started and the CTCA is acquired. Whilst the total radiation dose is slightly less, the timed bolus does not allow much room for error.
Whilst newer contrast media have an improved allergenic profile, any cardiac CT imaging service must be equipped to handle any potential contrast reactions. Patients with contrast media allergy may still undergo an un-enhanced coronary calcium score so that some information about their coronary risk profile can be obtained.

3.2 Post processing

CTCA images are best viewed on dedicated post-processing workstations. The table below shows the commonly used post-processing display protocols highlighting their advantages and drawbacks (Table 1).

Protocol	Advantages	Disadvantages
Trans-axial or "raw" data scrolling (Fig. 6)	• Actual data set • Accurate indentification of stenoses • Multiple "stacks" at different phases allow selection of optimal phase	• Need to be viewed in standard stacks, not good for communicating detail of vessels to non-radiologists • No 3-D perspective
Multiplanar reformat (MPR) (Fig. 7)	• Interactive, semi-automated oblique or curved vessels plotted from axial, sagittal or coronal images to show vessel in its entirety • 360o rotations enables visualisation of eccentric and circumferential stenoses • Accurate quantification of stenoses	• Image quality highly dependent on resolution and isotropy of original data volume set.
Curved MPR (cMPR) (Fig 8)	• Curved vessels can also be shown in complete linear conformation • Advantages as for MPR	• Distortion of actual configuration of anatomical structures related to vessel of interest
Maximum intensity projection (MIP) (Fig. 9)	• Enhances differentiation between high attenuation structures such as enhanced vascular structures and lower attenuation structures such as soft tissue • Allow more accurate quantification of "soft plaque" stenoses	• Loss of differentiation in regions of heavy calcification and stents due to over-projection of high density structures into the lumen • No visualisation of the vessel wall
Volume rendered (Fig. 10)	• Intuitive, coloured images, allows assessment of whole 3D structures, good for communication of anatomy to non-radiologists • Can "grow" coronary tree	• Not suitable for plaque assessment or accurate measurements • Poor visualisation of calcium and stents

Table 1. Comparison of commonly used post-processing display protocols. (Reproduced with permission from Nicol & Padley, 2007b).

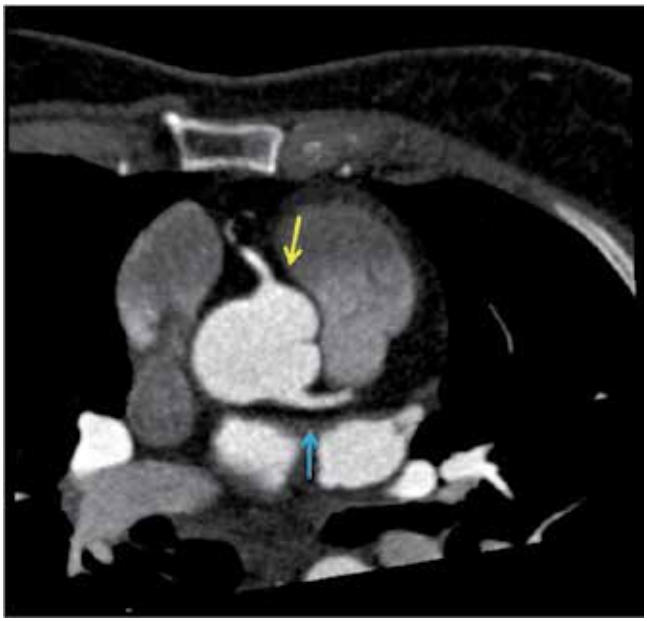

Fig. 6. Axial raw data through the heart showing the right and left coronary ostia (yellow and blue arrows respectively).

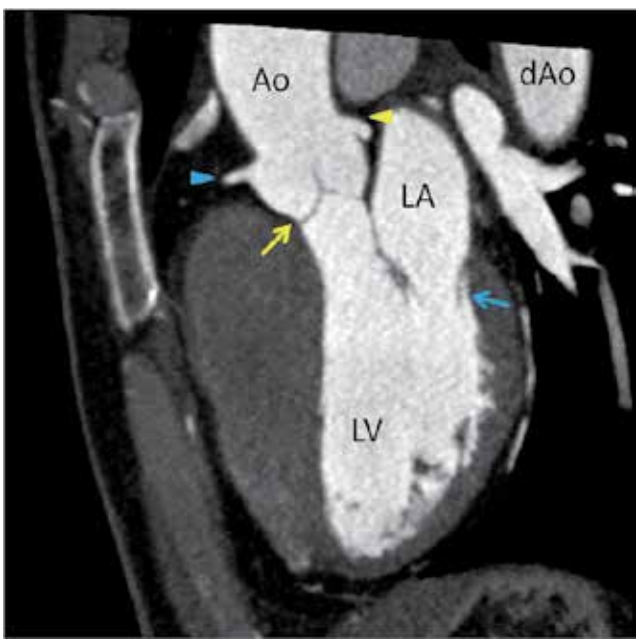

Fig. 7. Sagittal multiplanar reformat showing the closed aortic valve in profile (yellow arrow), open mitral valve (blue arrow) and right (blue arrowhead) and left (yellow arrowhead) coronary ostia. (LV=Left Ventricle; LA=Left Atrium;Ao=Aorta;dAo=descending Aorta).

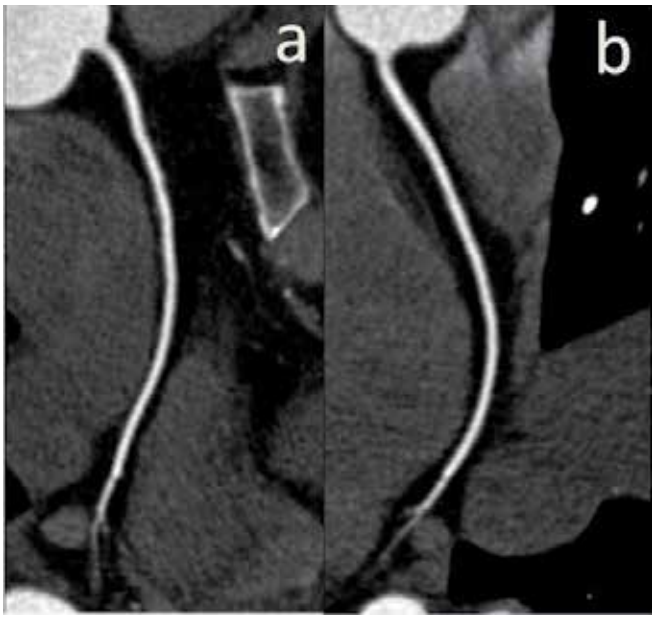

Fig. 8. Two curved multiplanar reformat images of the right coronary artery. These are obtained by rotating the image about a centreline through the artery.

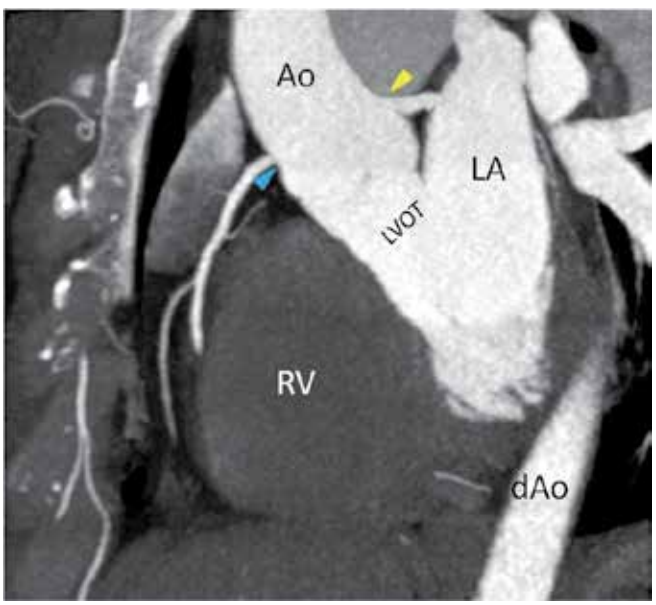

Fig. 9. Sagittal maximum intensity projection of showing the right (blue arrowhead) and left (yellow arrowhead) coronary ostia. (RV=Right Ventricle; Ao=Aorta;dAo=descending Aorta; LA=Left Atrium; LVOT=Left Ventricular Outflow Tract).

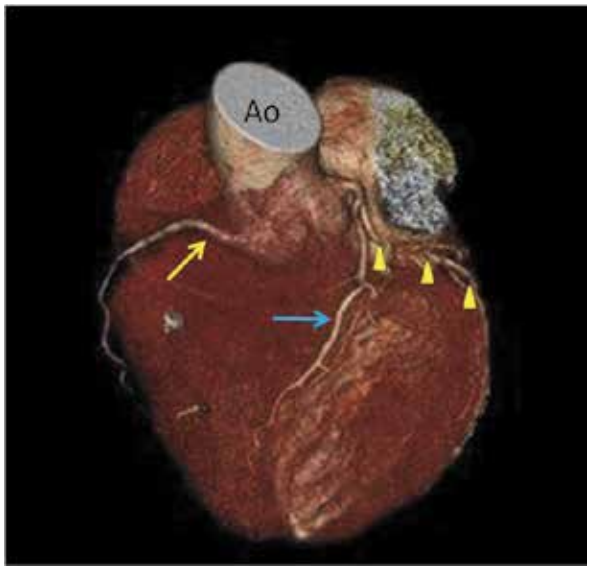

Fig. 10. Volume rendered image demonstrating a left anterior oblique view of the heart. The right coronary (yellow arrow), left anterior descending (blue arrow) and left circumflex (yellow arrowheads) arteries are clearly seen. (Ao=Aorta).

3.3 MRCA acquisition technique

There are two major hurdles to overcome when performing MRCA; respiratory and cardiac motion. Two methods are used to acquire MRCA images. These are breath-hold and free-breathing, coronary MRA. MRCA, as with CTCA, is further hampered by arrhythmias.

Breath-hold MRCA attempts to suppress respiratory motion by acquiring images in periods of apnoea. This technique allows both two-dimensional (2D) sequential images and subsequent shorter 3D imaging with first pass intravenous contrast. However, image quality is often suboptimal due to limited patient co-operation secondary to fatigue or inability to follow instruction adequately. Additionally breath holding is frequently associated with cranial drift of the diaphragm (of up to 1cm) (Danias et al., 1998), further limiting the final resolution of the images. These limitations may result in registration errors with apparent gaps in the coronary arteries that may be misinterpreted as signal voids from stenoses. As a result of these limitations free breathing navigator sequences are now most commonly used for MRCA.

Navigator sequences are used to correct for, and reduce the effects of, respiratory motion (Fig. 11). The position of the diaphragm is tracked and image data is only acquired at end expiration when respiratory motion is minimal or absent. Prospective ECG gating is used to correct for cardiac motion and data is only collected when coronary artery motion is known to be minimal. As with CTCA, this is usually mid-to-late diastole, however, at higher heart rates end-systole may be preferable. The disadvantages of this technique are that scan times are long with a full coronary dataset taking between 5 and 15 minutes to acquire (Sakuma et al., 2005, 2006). This is due to the fact that this technique is very inefficient with often less than 2% of the scan time being used to acquire data when there is neither coronary nor respiratory motion. The data acquisition is pre-programmed into the MRCA sequence,

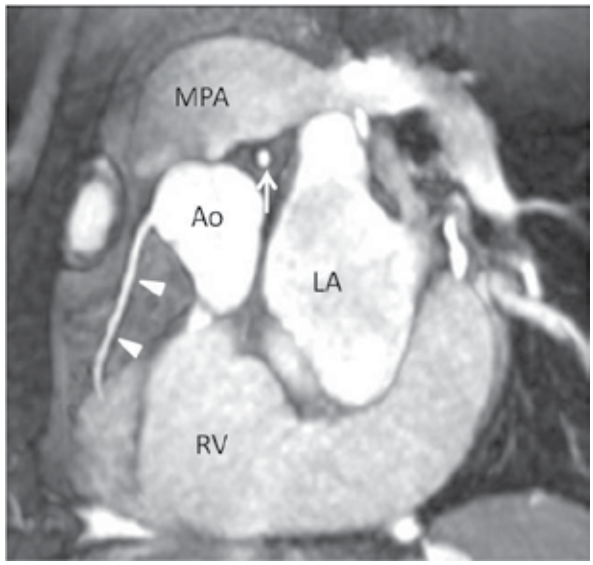

Fig. 11. Coronal view of the MRCA sequence showing the right coronary artery (arrowheads) as it passes through the AV groove. The left main stem is seen in cross-section (arrow) as it passes underneath the right pulmonary artery (RPA). (RV=right ventricle; MPA=main pulmonary artery; LA=left atrium).

although the user must define the time of least coronary motion at the time of acquisition. More recently 3D data acquisition during a single breath-hold using steady state free precession (SSFP) and parallel imaging has become possible (Deshpande et al., 2001) producing high resolution and high quality images with reduced scan times (Jahnke et al., 2005). Parallel imaging (with under sampling in two rather than one phase encoding direction) further reduces scan time but requires large coil arrays (Nehrke et al., 2006; Niendorf et al., 2006).

Newer self-navigated, free-breathing, whole heart MRCA techniques further improve image quality due to reduced respiratory and cardiac motion artifact. This technique uses a synchronous respiratory signal from the echoes acquired during imaging. The motion information is then retrospectively corrected, improving temporal resolution and producing stiller images (Stehning et al., 2005).

4. Clinical application of CTCA and MRCA

The significant technological improvements in CT imaging have brought CTCA into the forefront of coronary artery disease (CAD) assessment. With the improved temporal and spatial resolution, CTCA has become a viable alternative to invasive coronary angiography (ICA) in patients with low to intermediate likelihood of CAD (Schuijf et al., 2011). ICA however remains the most appropriate test in those with a high probability of severe CAD that may require intervention.

More broadly CCT can also be used to assess plaque morphology, and depending on the protocol selected, be used to assess cardiac function (wall motion and ejection fraction), cardiac chamber volumes, myocardial perfusion and be used to image the pericardium, cardiac valves, and pulmonary veins (Nicol et al., 2009). CCT is increasingly used to

examine acquired structural or congenital heart disease (Nicol et al., 2007), aberrant coronary vasculature, coronary artery bypass grafts (CABG) (Niemen et al., 2003) and intra-coronary stents (Gaspar et al., 2005).

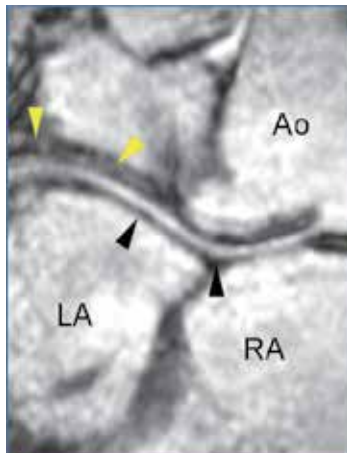

Fig. 12. Maximum intensity projection image from a navigator coronary MRA sequence demonstrating an aberrant circumflex artery (black arrowheads) arising from the right coronary artery passing between the aorta (Ao) and the right atrium (RA). Note also the resultant artefact (yellow arrowheads) due to cardiac motion. (LA=left atrium).

MRCA is most commonly used to investigate patients with suspected anomalous coronary arteries (Fig. 12) and fistulae, and in children and young adults with suspected coronary artery aneurysms such as in patients with Kawasaki's disease. It can potentially be used to assess graft patency in CABG; however the presence of surgical clips may limit graft visualisation. In patients with poor renal function, MRCA can be used to assess the patency of proximal coronary arteries in patients undergoing major cardiac surgery, such as valve replacement, as no contrast agent is used.

4.1 CT and MR coronary angiography

Unlike ICA that provides a "lumenogram", a good quality CTCA can demonstrate both the lumen and the wall. The real strength of CTCA is its negative predictive value (usually over 99% *cf*. ICA), effectively ruling out coronary artery disease in those with a normal study (Budoff et al., 2008; Meijboom et al., 2008). The positive predictive value of CTCA is less favourable due its comparatively limited spatial resolution. The diagnostic accuracy of CTCA is further impaired by the presence of heavy coronary calcification, which may lead to the overestimation of stenoses. All coronary stenoses should be viewed from multiple angles and appropriate window settings to reduce "blooming" artefact from calcium. If contrast is seen passing alongside a calcified lesion in any plane, then the stenosis is unlikely to be more than 50% on ICA. CTCA is as effective in determining soft plaque burden as intravascular ultrasound (IVUS) (Leber et al., 2006). Clinically this is important due to the higher prevalence of soft plaque in those patients with acute coronary syndromes than those who have stable angina (Korosoglou et al., 2010; Motoyama et al., 2007). An algorithm for the investigation of symptomatic patients based on their pre-test probability is suggested (Fig. 13).

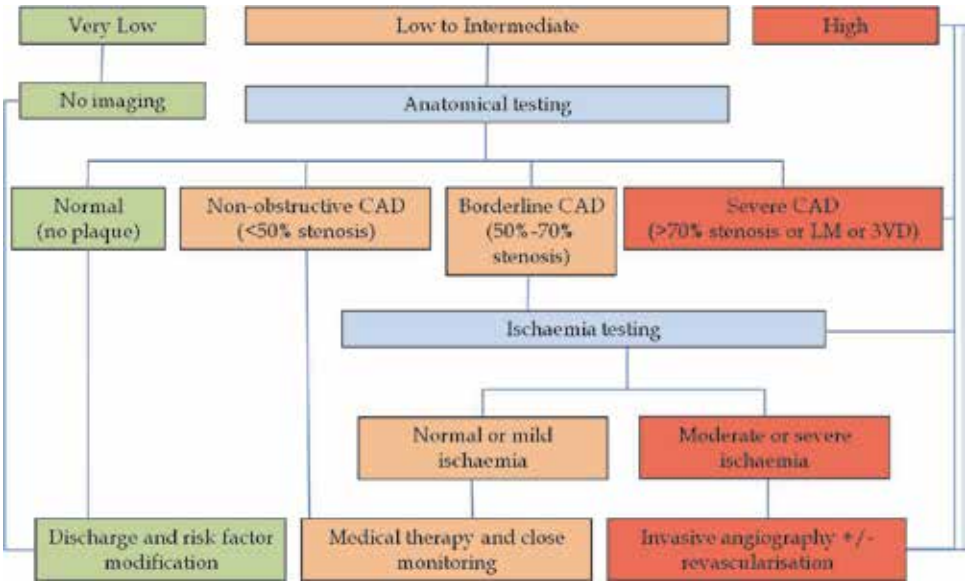

Fig. 13. Potential algorithm for sequential imaging of anatomy and function for diagnosis and management of coronary artery disease (CAD) based on pre-test probability in symptomatic patients. A low to intermediate pre-test probability favours initial evaluation of the presence or absence of obstructive stenosis, since the prevalence of obstructive CAD will be low. As a consequence only a few patients will have abnormalities that may require further testing and revascularisation. (LM = left main coronary artery; 3VD = triple vessel disease.) Adapted with permission from Schuijf JD et al., 2011).

Like CTCA, MRCA is able to demonstrate both the lumen and vessel wall. However CMR studies routinely provide additional cardiac anatomy and functional information. Compared with CTCA the speed of acquisition and spatial resolution of MRCA has so far limited its use clinically. There are important technical differences between CTCA and MRCA; in CTCA the right coronary artery is often the most difficult vessel to image due to movement artifact, especially in prospectively acquired imaging, but in MRCA the left circumflex artery is relatively difficult to image due to its distance from the receiver coil and its proximity to the great cardiac vein (Danias et al., 1999). As with CTCA, multiple studies have assessed the accuracy of MRCA for the detection of significant CAD. In essence MRCA, like CTCA, has a high negative predictive value but variable specificity and positive predictive value (Danias et al., 2004; Kato et al., 2010; Kim et al., 2001; Schuetz et al., 2010). MRCA is particularly useful in left main coronary and three vessel disease assessment (Kato et al., 2010; Kim et al., 2001) (Fig. 14) but overall 1.5T CMRA is comparable with 16 MDCT when assessing the entire coronary tree (Kefer et al., 2005).

Wall thickness and plaque characterisation are significant areas of research in both CTCA and MRCA as plaque rupture and myocardial infarction can occur in the absence of significant luminal narrowing. In MRCA, T1 weighted 2D and 3D black blood imaging can detect atherosclerotic plaque and determine wall thickness and thus positive remodelling (Fayad et al., 2000; Kim et al., 2002), whilst CTCA studies have demonstrated that the presence of positive remodelling and "spotty" plaque morphology (a predominantly soft plaque with some areas of calcification within it) are strongly associated with subsequent

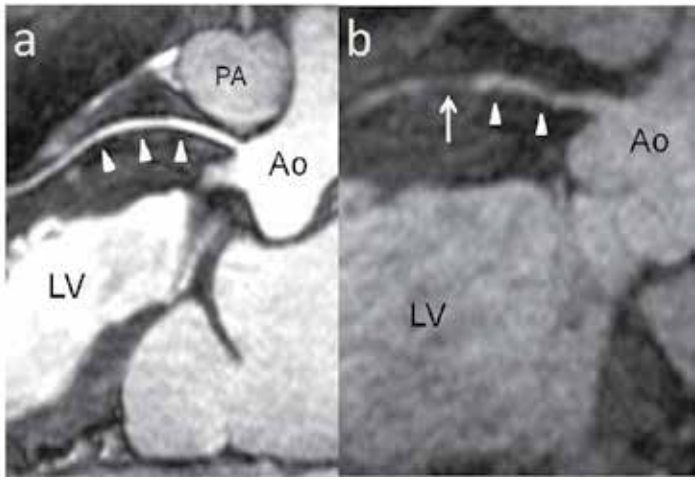

Fig. 14. Maximum intensity projection image (a) from a navigator coronary MRA sequence demonstrating a normal calibre left anterior descending artery (arrowheads). The second image (b) shows non-occlusive, non-calcified plaque (arrow) in the mid left anterior descending artery (arrowheads) on this coronary MRA. (Ao=aorta; LV=left ventricle; PA=pulmonary artery).

acute coronary syndrome (Motoyama et al., 2007). MRCA has been used to demonstrate positive remodelling in diabetic patients with nephropathy compared with those without (Kim et al., 2007) and recent evidence demonstrates that high signal on T1 weighted images seen in plaques in the walls of coronary arteries is associated with positive remodelling. This suggests MRCA may also be useful for investigating complex plaques non-invasively (Kawasaki et al., 2009). Late contrast enhancement of the coronary arterial wall in MRCA has been seen in areas of calcific plaque and significant stenotic lesions following recent infarcts (Yeon et al., 2007; Ibrahim et al., 2009) and it has been proposed that late contrast enhancement may be useful in visualisation of inflammatory activity in atherosclerosis associated with acute coronary syndrome. For early CAD assessment, recent studies have shown that MRCA can demonstrate endothelial loss of normal vasomotor tone prior to the development of any vascular remodelling (Hays et al., 2010).

4.2 Coronary stent assessment

Clinically all stents are susceptible to varying degrees of neo-intimal hyperplasia, in-stent re-stenosis and complete occlusion. The metal in the stents make them easily visible on CT (Fig. 15) however CTCA analysis of stents must be done cautiously due to "blooming" artefacts (Nicol & Padley, 2007b). Blooming is worse with bare metal stents than drug eluting stents but CTCA has been shown to be clinically reliable for stents >3mm in diameter and is clinically useful in the assessment of left main stem stents (Pugliese et al., 2008). Stents of smaller calibre are less easily assessed and caution is advised when attempting to determine the severity of stenoses.

4.3 Coronary artery bypass graft assessment

The inherent larger calibre of vessel grafts, relative immobility and lack of calcification, make them ideally suited to CTCA analysis (Fig. 16). Indeed, the sensitivity and specificity

of CTCA in graft patency analysis has been shown to be 95-100% and 94-100% respectively (Nieman et al., 2003).

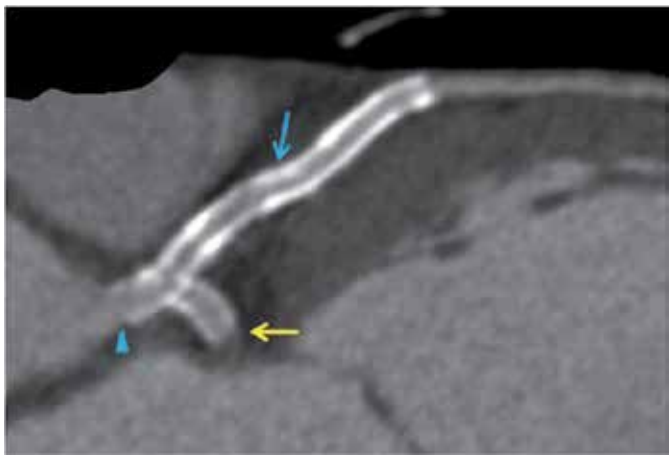

Fig. 15. Curved planar reformat of a metal stent extending from the left main stem (blue arrowhead) into left anterior descending (blue arrow) and left circumflex (yellow arrow) arteries.

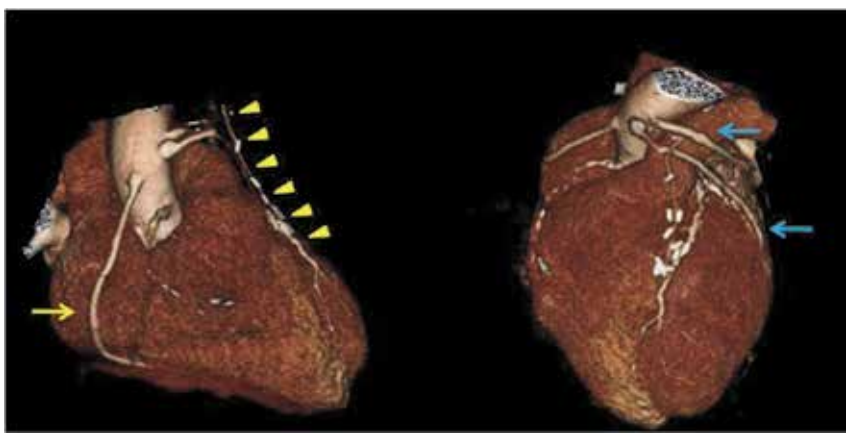

Fig. 16. Volume-rendered images demonstrating quadruple coronary artery bypass grafts. There are a vein graft to the right coronary artery (yellow arrow), two vein grafts supplying the acute marginal and lateral marginal branches of the LCx (blue arrows) and a left internal mammary artery graft supplying the left anterior descending artery (arrowheads).

However, complete examination of grafts and their patency should include assessment of their run-off which may be limited with CT. Limitations of CTCA include blooming artefact from surgical clips which can particularly affect distal LIMA anastomosis assessment. CABG patients often have significant and heavy calcification, which can also cause blooming artefact on CTCA. MRCA can also be used to assess CABG but again surgical clips may hinder full assessment.

4.4 Functional information

Whilst CMR remains the gold-standard for cardiac functional analysis, retrospectively gated CCT studies allow assessment of global and regional left ventricular function with good correlation with both CMR and transthoracic echocardiography (Nicol et al., 2008b). It is important to be aware of limitations of CCT however when assessing both global and regional wall motion abnormalities especially if not reconstructing 100% of the cardiac cycle. Whilst end-systole and end-diastole usually fall at around 35% and 65% respectively there is significant inter-patient variation and values from the analysis may not reflect that of CMR or echocardiography that routinely utilise the whole cardiac cycle. Importantly, when assessing functional CT data regional wall motion abnormalities in the absence of impaired systolic wall thickening should also be treated with caution as they may be artefactual (Nicol et al., 2008).

5. Clinical application of CTCA and MRCA in congenital and structural heart disease assessment

Both CCT and CMR are able to demonstrate complex anatomy in congenital cardiac disease. CMR remains the gold standard for adult congenital heart disease assessment but the increasing availability, speed of acquisition and superior spatial resolution of CCT makes it a viable alternative in many clinical situations (Nicol et al., 2007). CMR is generally contraindicated in patients with pacemaker and implanted defibrillator devices.

Unlike CMR, that offers complete cardiothoracic visualisation, CCT is only able to demonstrate both the coronary anatomy and the pulmonary arterial trunk with extended injection protocols that increase right heart and pulmonary opacification in addition to the coronary anatomy (Nicol et al., 2009). CTCA is the gold standard for the full delineation of aberrant coronary anatomy, however MRCA is, in most patients, adequate for delineation of the clinically important coronary ostia and using dedicated sequences can also sometimes produce diagnostic images of the entire coronary tree. As a general rule, MRCA should be considered first line if radiation exposure is likely to be higher than acceptable, i.e. in children or young females, and MRCA should certainly be considered in those requiring regular follow up such as Kawasaki's disease.

Increasingly CCT is used for acquired structural heart disease and assessment of valve disease using planimetry and assessment of valve function on cine images acquired in retrospectively gated studies is gaining clinical acceptance (Chheda et al., 2010). It is important to remember however that CCT is unable to assess flow and is therefore inferior to both CMR and echocardiography for the assessment of valve gradients.

Combined cardiac and non-cardiac angiography is now used in the assessment of trans-catheter aortic valve implantation (TAVI). Assessment of the aortic root size, aortic pathology (plaque burden, calcification, vessel tortuosity), access routes (ilio-femoral and subclavian arteries), coronary arteries and valve calcification can all be assessed using CT angiography (Ewe et al., 2011) (Fig. 17).

6. Future developments in CT and MR

6.1 CCT imaging

There have been significant advances in CT scanner technology over the last decade with the advent of increasing numbers of detectors (up to 320) allowing whole heart coverage

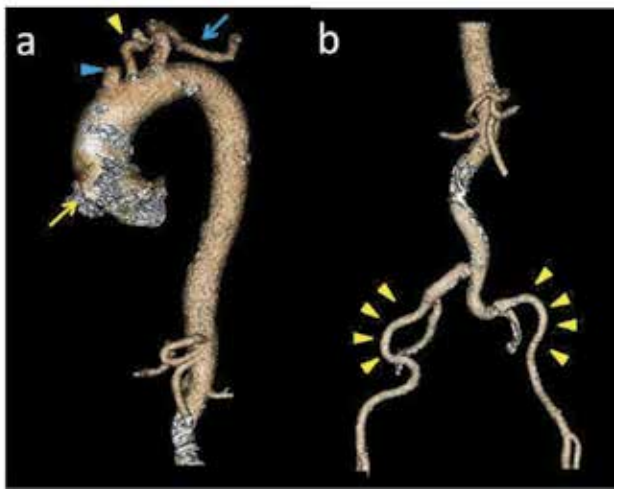

Fig. 17. Volume rendered images of the aorta obtained as part of the TAVI assessment protocol. The level of the aortic root (yellow arrow), right subclavian artery origin (blue arrowhead), right carotid artery (yellow arrow head) and left brachiocephalic artery (blue arrow) are shown in (a). The tortuosity of the iliofemoral arteries (yellow arrowheads) demonstrated in (b), will help with surgical planning.

without table feed, and fast pitch dual source CT allowing a full cardiac acquisition in a fraction of a second with radiation doses routinely <1mSv for a CTCA.
Future technological advances are likely to remain focused on rapid acquisition of cardiac data at low ionizing radiation doses. This may be achieved using a variety of techniques such as multi-source, multi-energy CT or inverse geometry CT.

6.2 Multi-source, multi-energy CT

By increasing the number of X-ray sources it may be possible to further reduce the temporal resolution by two-thirds with the addition of a third source (58ms) or three-quarters with a fourth (41ms), however the weight of each additional X-ray source may reduce the overall gantry rotation time negating any additional benefit. The use of air bearing systems may allow this but the ability to overcome the effects of high centrifugal forces remains a significant challenge.
The major advantage of dual source CT (DSCT) technology is the ability to acquire a complete dataset using one-quarter of a gantry rotation time, thereby reducing the temporal resolution by half. The second advantage of dual headed CT is the ability to acquire the dataset at differing energies (Dual energy CT (DECT)). It is also possible to perform DECT on single headed scanners by rapidly alternating kV from a single tube. Either technique fundamentally alters the penetration of the X-ray beam and therefore the attenuation by tissues. By subtracting one dataset from the other it is possible to, for example to artificially "remove" calcium or contrast, potentially spelling the end for non-enhanced CT preceding contrast studies. It may also be used to assess myocardial densities at different energies, paving the way for potential tissue characterization in infarct or ischaemic myocardium. DECT subtraction techniques are currently limited to large calibre vessels such as the aorta as the resolution of the current generation of CT scanners is not yet sufficient to apply this to the coronary arteries.

6.3 Inverse Geometry CT (IGCT)

IGCT is a novel system under investigation that employs a large array of X-ray sources opposite a smaller detector array (Fig. 18). It is anticipated to be able to image a thick volume in a single gantry rotation with isotropic resolution. The ability to image a volume is primarily determined by the size of the X-ray source array, in much the same way that it is determined by the size of the detector array in a conventional CT system. As well as demonstrating low wasted radiation (Mazin et al., 2007) (and therefore a much smaller radiation requirement), this technique also has the potential to maximise gantry rotation time further reducing spatial resolution.

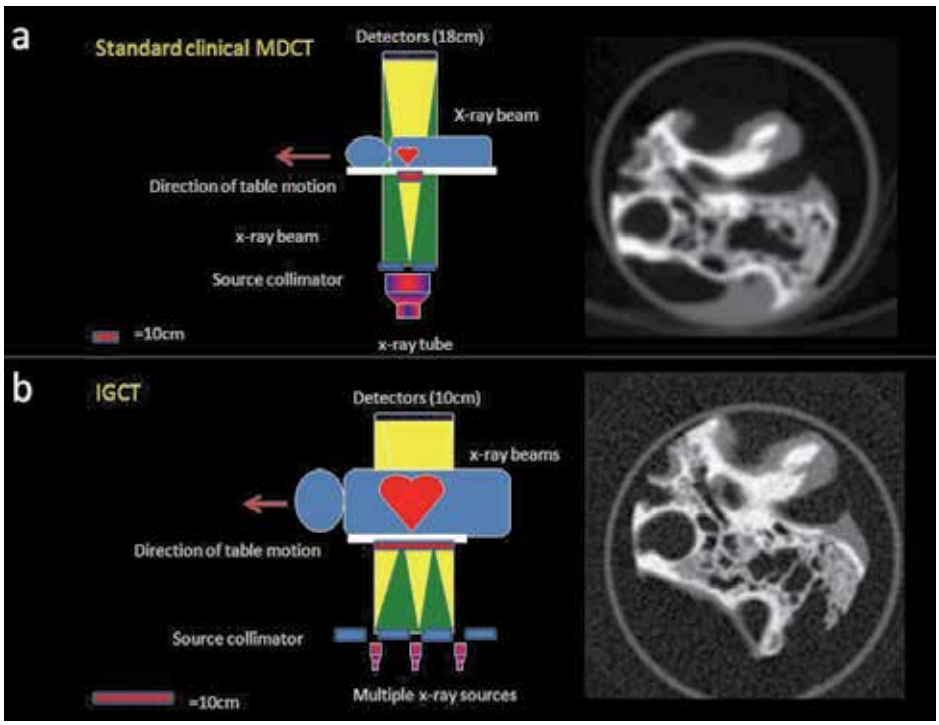

Fig. 18. Standard MDCT (a) requires an 18cm detector array when scanning a 10cm region of interest (due to cone beam) introducing artefacts at beam edges. Inverse Geometry CT (IGCT) (b) uses multiple x-ray sources and only requires a 10cm detector array for a 10cm region of interest, reducing detector size and weight, reducing cone beam artefact and producing greater signal to noise ratios for reduced radiation exposure.

Future advances in material technology will also advance CT imaging with flat panel technology already under investigation. The use of strong light weight materials may also overcome some of the mechanical limitations that prevent faster gantry rotation times today and may improve spatial and temporal resolution to nearer that of interventional coronary angiography.

6.4 3T CMR imaging

At 3T, there is improved signal to noise ratio (SNR) as SNR is proportional to the field strength of the static magnetic field (Singerman et al., 1997). 3T results in better spatial and

temporal resolution and shorter scanning time. There is a doubling of SNR and a 4-fold reduction in scanning time using 3T parallel imaging and spatial harmonics compared with 1.5T. Additionally, at higher field strengths the prolongation of the T1 values make spin labelling techniques more attractive. However triggering is more problematic at higher field strengths (3T and 7T) as the enhanced magneto-hydrodynamic effect produces an artifactual voltage that is overlaid on the T wave of the ECG. This can result in triggering off the T wave (rather than the R wave) making it difficult to reliably identify the time of least coronary motion. Using sophisticated R wave detection algorithms this problem can be overcome.

MRCA at 3T has been performed (Stuber et al., 2002) however although the contrast to noise ratio is improved, there is no overall improvement in image quality or diagnostic accuracy (25). 3T MRCA has been shown to have high sensitivity and specificity for the detection of significant (>50%) coronary stenoses (Yang et al., 2009), possibly even being comparable to 64 MDCT (Hamdan et al., 2011) when using contrast-enhanced methods that rely on double dose infusion of contrast media. This is required as gradient-echo sequences are used instead of SSFP sequences due to the need to overcome magnetic field inhomogeneity and radiofrequency energy deposition at high field strengths (Bi et al., 2007; Liu et al., 2008). It is hoped that the use of sophisticated shimming algorithms and adiabetic T2 preparations will further improve acquisition at 3T (Nezafat et al., 2006; Schär et al., 2004) in the future.

7. Future application of CTCA and MRCA

Both CCT and CMR continue to develop rapidly. The applications for CTCA continue to expand and with the development of myocardial perfusion and scar imaging the ability to look at the coronary lumen and vessel wall and gain functional information about both the blood flow and myocardial function will see this field continue to expand.

The recent UK NICE guidelines include CTCA within their recommendations for patients with chest pain (NICE, 2010) and the ever growing demand for rapid exclusion or confirmation of coronary artery disease are likely to see CTCA become a far more ubiquitous tool used in almost all hospitals.

As radiation doses continue to fall and as CTCA research produces outcome and cost effectiveness data, the role and utilisation of this rapidly evolving technology is likely to increase further. The combination of newer technology has raised the possibility of assessing flow; indeed venous and arterial phases of cerebral flow can already be imaged using CT and this opens up many possibilities for potential cardiac flow assessment in the future.

In MRCA, the greater availability of more powerful magnets, increased number of receiver coils and more sophisticated algorithms, may reduce imaging time. MRCA may become routine in the early detection of coronary artery disease at the positive remodelling stage or even earlier. Plaques at risk of rupture may be identified early and intervention undertaken before myocardial damage occurs.

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6

Coronary CT Angiography as an Alternative to Invasive Coronary Angiography

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1. Introduction

X-ray computed tomography (CT) was first invented in 1972 and its ability to obtain cross sectional images has proven to be a major advance in the field of medicine, garnering the Nobel Prize in medicine for Sir Godfrey Newbold Hounsfield and Allan McLeod Cormack in 1979. Since then, numerous advances have been made with the introduction of high quality scanners and new imaging protocols to enhance the quality of the images and reduce the amount of radiation. Inherent drawbacks of conventional CT imaging for cardiac imaging, such as low temporal resolution and the need for ECG gating, prompted the development of electron beam CT (EBCT). Further advances in the scanners have led to the introduction of multi detector CT (MDCT) scanners, which have increased spatial resolution and now utilize sequential imaging acquisition modes and other features to minimize radiation exposure. MDCT scanners for coronary imaging utilize a minimum of 16 slice, and now 64 - 320 slice scanners are widely used to get excellent, high-resolution images of the heart and the coronary arteries.

2. Basics of operation

The coronary CT angiogram (CCTA) is a relatively fast and simple procedure. Adequate patient preparation and co-operation are essential for good image acquisition. Beta-blockers are routinely given to slow the heart rate (Bluemke et al. 2008) in order to eliminate or reduce artifact from motion of the coronary arteries. This chapter deals primarily with the use of coronary CTA as an alternative to invasive angiography and another chapter describes the technical features in more detail. However, in brief, temporal resolution is an important limitation and is determined by the speed of the X-ray gantry(Kapoor and Thompson 2009). MDCT scanners improve this temporal resolution by acquiring a full CT slice in only half a rotation, and recent advances in scanner design have improved the speed of rotation and even added second tube sources in order to improve temporal resolution even more. The 64-slice MSCT instruments are now considered the minimum standard for CT scanners intended for coronary artery imaging. Images are most commonly acquired using prospective gating and only during diastole (for example at 70-80% of the R-R interval) in order to reduce the radiation dosage (Earls et al. 2008). Both spatial resolution and temporal resolution are less with CCTA than conventional invasive coronary

angiography and these limitations must be kept in mind when deciding which test to order. The maximum spatial resolution of MSCT is 0.4 mm, and is determined by the size of the picture elements of the CT detector. Smaller coronary segments frequently are not evaluable with CTA and coronary CTA may be unable to distinguish moderate from severe flow limiting stenosis, because of these limitations of resolution(Kapoor and Thompson 2009).

3. Application of coronary calcium scoring

Non-contrast CT scanning of the coronary arteries is a widely used technique for risk stratification and prognosis for individuals without know coronary artery disease. With this technique, calcium in the form of calcium hydroxyapatite in the artery wall is imaged and quantified in order to provide a rough measure of the presence of coronary atherosclerosis. There is a linear relationship between the amount of coronary calcium and coronary atherosclerosis, that is, the more coronary calcium the higher the likelihood of coronary stenosis(Rumberger et al. 1995), and proportionally prognostic – the higher the calcium score the greater the likelihood of coronary events(O'Rourke et al. 2000). Moehlenkamp and colleagues recently demonstrated that coronary calcium scoring not only adds prognostic value to the Framingham risk model, but also that it's value is greater than hsCRP (Mohlenkamp et al. 2003). Also the recent randomized Eisner Trial demonstrated that coronary calcium testing leads to better downstream control of coronary risk factors(Rozanski et al. 2011). Thus, the ability of coronary calcium scoring to non-invasively identify and quantify the amount of coronary calcium is invaluable to diagnose pre-clinical CAD and help to aggressively modify the risk factors. It should be emphasized that coronary CTA, while a very useful tool, is not a particularly appropriate test for patients with chest pain syndromes. CT coronary calcium scoring is performed without the use of iodinated x-ray contrast. In order to perform CT coronary angiography, the focus of this chapter, x-ray contrast is administered.

4. CCTA as an alternative to invasive angiography

While CCTA can sometimes provide images of truly impressive quality, the spatial and temporal resolution are significantly inferior to invasive angiography, and CCTA images are sometimes degraded by artifacts. A recent prospective multicenter-blinded study evaluated the diagnostic performance of 64-multidetector row CCTA compared with invasive coronary angiography (ICA) in patients with chest pain without known CAD who were referred for non-emergent ICA. The data revealed high diagnostic performance at both 50% and 70% stenosis thresholds. In addition, the 99% negative predictive value (NPV) of CCTA at the patient and vessel levels establishes it as a highly effective noninvasive alternative to ICA for the exclusion of obstructive coronary artery stenosis(Budoff et al. 2008). The strength of the CCTA lies in its high negative predictive value. A good quality negative CCTA without artifacts can rule out the presence of CAD with a great level of confidence. The high negative predictive value has been consistently demonstrated(Gopalakrishnan et al. 2008; Mowatt et al. 2008; Stein et al. 2008) and it is this population where coronary CTA might well be a substitute for invasive angiography. Figure 1 demonstrates a normal high quality coronary CT angiogram and figure 2 demonstrates one in which the diagnosis of high - grade coronary artery disease was made. In general, coronary CTA is much less accurate in the presence of heavy coronary

calcification and in patients with atrial fibrillation or other irregular cardiac rhythms. Figure 3 is an example of such a non-diagnostic study. Another chapter deals with the accuracy of CTA in greater detail.

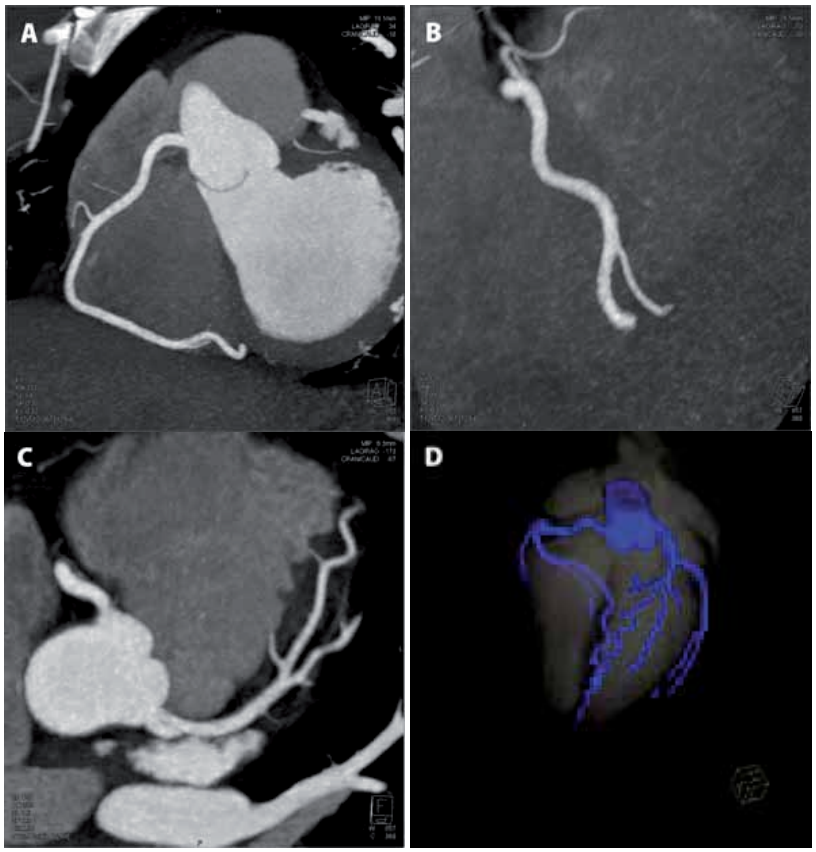

Fig. 1. CT coronary angiogram of a 39 year old male who complained of chest pain (maximum intensity image of the right coronary artery (A and B) and the left coronary artery (C) and reformatted image (D). The study was normal giving a high degree of confidence that coronary artery disease was not present.

Although coronary CTA has limitations, it is much less invasive than invasive coronary angiography and it is an appropriate alternative in selective cases. For example, according to the ACCF/ SCCT/ ACR/ ASNC appropriate use criteria for diagnosing CAD, patients who present with non-acute symptoms and possibly representing an ischemic equivalent, CCTA is considered an appropriate test in those with a low to intermediate probability of CAD whose ECG is uninterpretable and who cannot exercise(Taylor et al. 2010). CCTA is also considered appropriate in patients with a low to intermediate pre-test probability with acute symptoms suggestive of acute coronary syndrome (ACS) with a normal ECG and cardiac biomarkers(Taylor et al. 2010). However, in patients who have obvious severe cardiac ischemia, coronary CTA is not an appropriate alternative to invasive angiography as it will simply delay interventional therapy and add to the x-ray contrast load prior to the needed invasive procedure(Taylor et al. 2010).

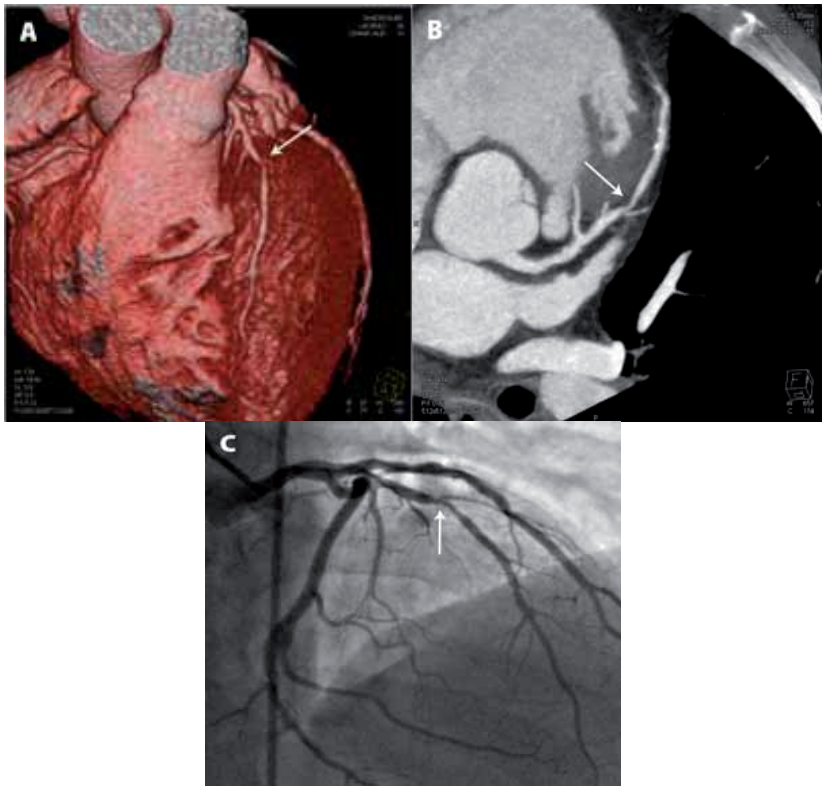

Fig. 2. Volume rendered (A) and maximum intensity projection (B) CT angiogram images from a 48-year-old male demonstrating a high-grade stenosis in the mid segment of the left anterior descending coronary artery. The patient had presented with persistent, intermittent chest pain and a myocardial perfusion image had been equivocal.

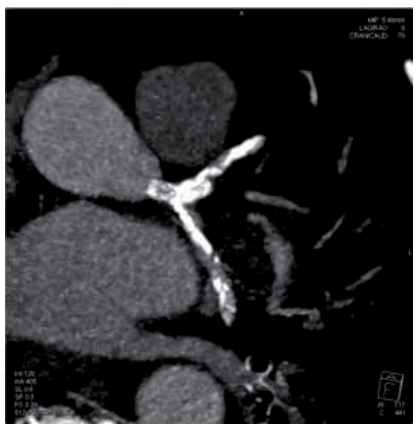

Fig. 3. Thin maximum intensity projection (MIP) coronary CT angiogram in a 65-year-old female patient showing the proximal left coronary artery branches. Heavy coronary calcium makes the study non-diagnostic. Image quality is also impaired by insufficient x-ray contrast in the aorta and coronary arteries at the time of acquisition.

Another category of patients in whom CCTA is used as an alternative to invasive angiography are those with an equivocal or low probability stress tests whose symptoms suggestive of angina persist despite low probability scans. These patients are often referred for cardiac catheterization, but CCTA is a less invasive test that can rule out obstructive CAD as a cause for their symptoms. The high negative predictive value of CCTA allows the clinician to confidently reassure the patient without invasive testing. This same rationale applies to those patients with suspected coronary artery disease who are scheduled to undergo non-cardiac surgeries. When applied to the appropriate subset of patients, i.e. those with relatively low pre-test probability of CAD, CCTA can prove to be the final test to confidently rule out the presence of CAD and these patients benefit from avoiding the higher cost and potential complications of invasive coronary angiography.

4.1 Etiology of cardiomyopathy

When patients present with a new diagnosis of heart failure / cardiomyopathy, further diagnostic evaluation is warranted. Coronary artery disease is the most common cause of cardiomyopathy and differentiating ischemic from non-ischemic etiology has important therapeutic and prognostic implications. In particular, since ischemic cardiomyopathy has the potential for improvement from revascularization, it is very important that the etiology be established. Treatment guidelines for patients with a new diagnosis of cardiomyopathy prominently feature the use of invasive coronary angiography, and it is considered the gold standard for establishing the diagnosis of cardiomyopathy of coronary artery disease. While myocardial perfusion imaging has some value in this population, there are important limitations in this group such as lower diagnostic accuracy and difficulty in performing stress in some of them. Also, while regional wall motion abnormalities identified by two dimensional echocardiography or radionuclide angiography have been proposed to be specific for coronary artery stenosis induced ischemia and infarction(Budoff et al. 1998), subsequent studies have shown similar abnormalities with dilated cardiomyopathies, not related to coronary artery disease(Andreini et al. 2007). With the use coronary CT angiogram, however, a diagnosis either in favor of or against coronary artery disease can be made fairly easily. Patients with acute congestive heart failure may not be suitable for CCTA immediately because of tachycardia and the need to avoid x-ray contrast. However, once the heart failure is compensated, coronary CTA can be performed and the accuracy in this setting has been established. One study that demonstrated excellent sensitivity (89%) and negative predictive value (99%), suggests that it could be used in patients with a cardiomyopathy to identify the etiology(Garcia et al. 2006). Also, a direct comparison with coronary angiography revealed excellent accuracy of the MDCT in correctly diagnosing the etiology of cardiomyopathy, ischemic or non-ischemic(Andreini et al. 2009). There are obvious advantages to non-invasively ruling in or out coronary artery disease in this group of patients. The ACC/ ASNC/ AHA consider it appropriate to evaluate for CAD in new onset HF and no prior diagnosis of CAD and reduced LVEF(Taylor et al. 2010).

4.2 Utilization prior to electrophysiology procedure

Atrial fibrillation is a common arrhythmia and is associated with significant morbidity. The options available for the treatment of atrial fibrillation, especially in symptomatic

individuals, include anti-arrhythmia drug therapy, surgery, and catheter based approaches. The use of radio frequency ablation to isolate the pulmonary veins is a growing therapy for atrial fibrillation and has emerged as an important alternative to conventional treatments. The majority of the atrial fibrillation foci appear to arise around the pulmonary veins and successful pulmonary vein isolation (PVI) can prove to be a permanent cure for atrial fibrillation. Coronary CT angiography is a key diagnostic tool in managing patients being considered for PVI(Cronin et al. 2004). For one, the presence of significant coronary artery disease has implications for adjunctive medical therapy since Vaughan-Williams class 1C drugs such as flecainide are contraindicated in patients with CAD. Coronary CTA can diagnose coronary artery disease in these patients. Essential components for a successful pulmonary vein isolation procedure include accurate anatomical mapping of the pulmonary veins. This is important because the pulmonary vein anatomy is variable, and common ostia and extra pulmonary veins are common. The knowledge of this anatomy is helpful to avoid pulmonary vein stenosis and necessary to avoid missing potential areas contributing to atrial fibrillation(Jongbloed et al. 2005). MDCT provides excellent visualization of the origins of the pulmonary veins, the diameter of the ostia, course of the pulmonary veins, presence of dual ostia and presence of additional supernumary veins(Schwartzman et al. 2003). The distance of the esophagus from the left atrium is also an important parameter that can also easily measured. Another important pre-requisite for the procedure is the absence of a left atrial appendage thrombus, a finding not uncommon in patients with atrial fibrillation. CT angiography allows for excellent visualization of the entire left atrium and the appendage and can be used to identify the presence or absence of a thrombus. Invasive angiography is not ordinarily needed in patients being evaluated for pulmonary vein isolation for treatment of atrial fibrillation; the exception being those with signs of ischemia who may need revascularization as part of the management strategy.

4.3 Coronary anomalies

The incidence of coronary anomalies in the general population is about 1%(Yamanaka and Hobbs 1990). A coronary anomaly can be defined as a pattern that is not routinely encountered in the general population. Coronary anomalies account for about 12% of the deaths in U.S high school and college athletes(Van Camp et al. 1995). The American Heart Association's (AHA) committee on sudden death attributed 19% of the deaths in athletes to coronary anomalies. Invasive coronary angiography is often used to diagnose coronary anomalies and they are routinely found unexpectedly when angiograms are performed for other reason, but ICA has inherent limitations to accurately study coronary anomalies because of its two dimensional display and the non-visualization of adjacent vascular structures. Computed tomography angiography, however, is inherently three dimensional and is ideal for providing extensive information about the spatial anatomy of the coronary artery, including the origin, course, presence of an intra-myocardial path as well as the relationship to the surrounding structures. The sensitivity of the CCTA in diagnosing coronary anomalies is nearly 100%(Kacmaz et al. 2008). Figures 4 and 5 demonstrate the superb display of coronary anomalies by CT angiography. Since many patients in whom the diagnosis of a coronary anomaly are made young, it is important to pay particular attention to keeping the radiation dose low in this group. For example, diagnostic tests to rule out a coronary anomaly are frequently ordered in young athletes who have symptoms. The

radiation dose with CCTA is heavily dependent on imaging technique and pristine image quality is not needed for this indication. Thus, lower than standard tube current and tube voltage along with other radiation sparing algorithms should be used for this group of patients. In fact, the origins of the coronary arteries can sometimes be seen quite well with a limited field-of-view, non-contrast CT at a very low radiation dose and such an exam should be considered.

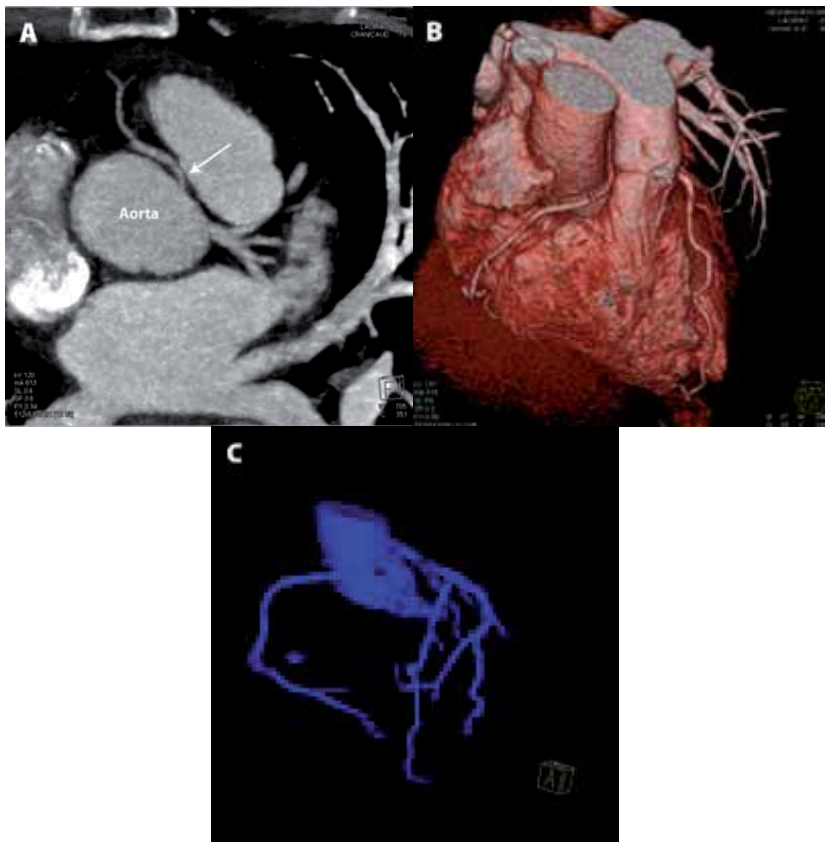

Fig. 4. CT angiogram maximum intensity projection (A), volume rendered (B) and reformatted image (C) from a 55 year old female with chest pain and problems during a coronary angiogram. The right coronary artery arises from the left coronary cusp (arrow) and courses between the aorta and the main pulmonary artery

4.4 Evaluation of left main coronary artery stent patency

The conventional treatment of left main coronary artery (LMCA) stenosis is coronary artery bypass graft surgery. However recent advances in percutaneous coronary intervention (PCI) have prompted utilization of stents to treat LMCA stenosis. The rate of angiographic restenosis on follow up studies varies from 22% to 40%(Silvestri et al. 2000; Park et al. 2003), although the use of drug eluting stents (DES) has markedly reduced the incidence of in-stent restenosis. However, because of the potential dire consequences of re-stenosis in the left main coronary artery, surveillance angiography is usually recommended 2-6 months after

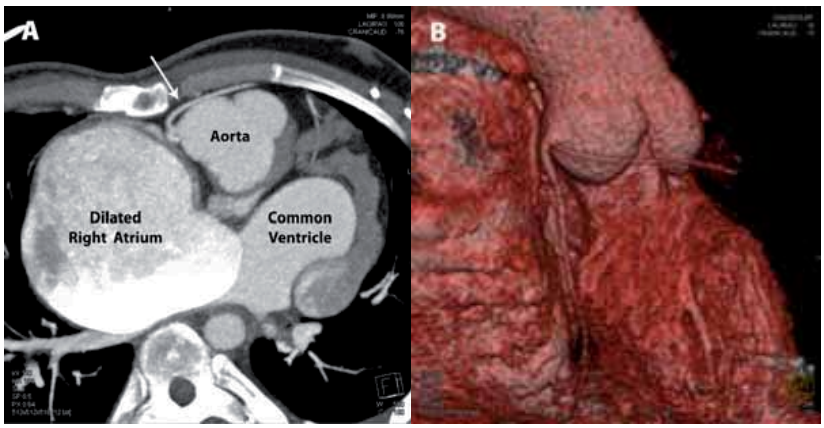

Fig. 5. Complex congenital heart disease in a 22-year-old male patient being considered for surgical intervention. The patient previously had a modified Fontan operation for a large ventricular septal defect and transposition of the great arteries. He developed a giant right atrium and very difficult atrial arrhythmia. Oblique maximum intensity projection (A) and volume rendered (B) CT coronary angiogram images also demonstrate anomalous coronary arteries (arrow) arising from the same coronary cusp to the right and posteriorly to the sternum.

intervention to this segment. Several studies have proposed CCTA as an alternative non-invasive modality to assess ISR in this patient group. Blooming artifacts caused by the stent struts are a major hindrance in the accurate CTA evaluation of ISR in other vessels(Mahnken et al. 2004), (Cademartiri et al. 2007). However LMCA stents tend to be large, usually with a diameter greater than 3.5mm. Also the left main segment is less prone to motion artifact on CT than other segments, and evaluation of stents in the LMCA is more accurate with CCTA than in other locations. One study prospectively evaluated the accuracy of stent patency in

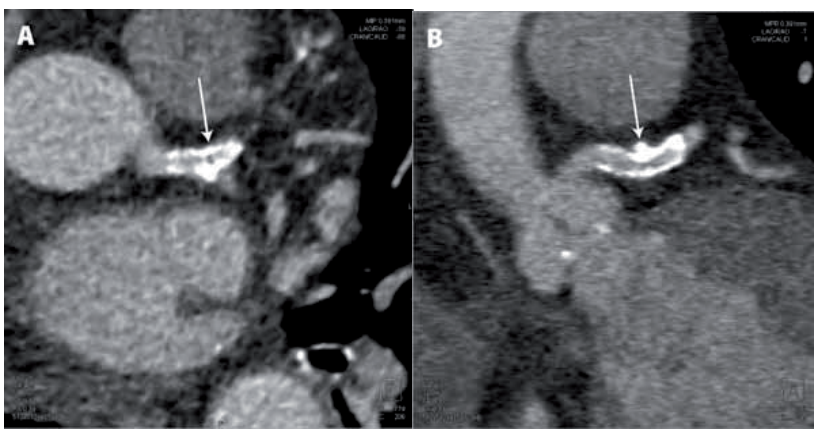

Fig. 6. Thin MIP oblique projections from a coronary CT angiogram in a patient with a stent in the left main coronary artery (arrow). Although there is suboptimal image quality related to artifact from the metallic struts and coronary calcium in this case, the stent appears to be patent. Coronary CT angiography is considered to be accurate in determining the patency of large stents, which have been placed in the left main coronary artery segment.

the LMCA by CCTA and compared it to conventional coronary angiography and intravascular ultrasound(Van Mieghem et al. 2006). In this study of 70 patients the sensitivity, specificity, accuracy, positive predictive value and negative predictive value of identifying ISR (defined as >50% narrowing) in LMCA stents was 100%, 97%, 98%, 86% and 100% respectively. The accuracy was lower (83%) for LMCA bifurcation stents. Conventional angiography identified 14% of the patients having ISR, which were also identified by CCTA. The 2010 appropriateness use criteria (AUC) consider it appropriate to use CCTA in asymptomatic patients for the assessment of left main coronary stent with a stent diameter greater than 3mm. The accuracy of CCTA in detecting in-stent restenosis depends on the type of stent and the complexity of the lesion and these should be taken into account before considering this modality for evaluating ISR (figure 6). Coronary CT angiography, and even non-contrast gated CT scans, can also be useful as an alternative or adjunct to invasive angiography if there is a question of mal-positioning of a left main stent (figure 7).

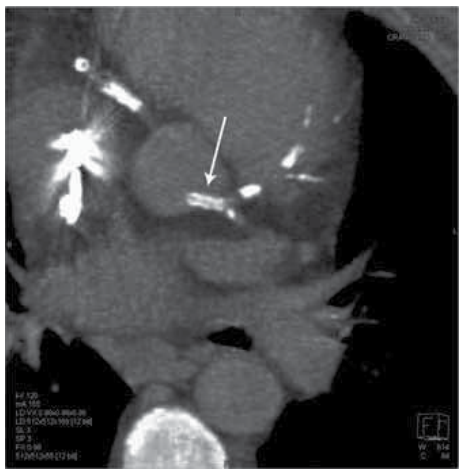

Fig. 7. Non-contrast CT of the heart demonstrating a mal-positioned stent in the left main coronary artery (arrow) that extrudes approximately 5 mm into the aorta. Invasive coronary angiography had been planned, but was deferred because of concerns that the stent might become dislodged.

5. Coronary CT angiography as complementary to invasive angiography

As discussed, coronary CT angiography is less invasive, although has lower resolution than invasive angiography. However, it is inherently three - dimensional and therefore provides diagnostic content which is different from and which can be complementary to the information derived from invasive angiography.

5.1 Defining coronary anatomy prior to CTO-PCI

Chronic total occlusions (CTOs) of coronary arteries are complex lesions present in approximately 30% of patients undergoing coronary angiography(Grantham et al. 2009). CTOs are defined, as lesions present for at least 3 months with grade 0 to 1 Thrombolysis in Myocardial Infraction (TIMI) flow on angiography. CCTA is being increasingly used as a

non-invasive modality to accurately define the characteristics of the CTO to assist in the interventional procedure.

There are several advantages to performing a CCTA prior to planned revascularization of a CTO. The length of the occluded segment, which is an important predictor of success of revascularization, can be easily obtained(Mollet et al. 2005). The course of the artery, occlusion length, collateral circulation, degree of calcium are important variables that can be obtained by CCTA, all of which assist in planning the procedure that translates into shorter procedure times. Foreshortening of the vessel and limited visualization of the distal vessel in the absence of collateral filling are important shortcomings of invasive coronary angiography, which are easily fixed by CCTA. It can also define bends and angles inside the occluded segment that prove to be extremely helpful to the operator to navigate the wire safely and successfully through the occlusion to the distal vessel. The degree of calcification also has an adverse effect on the success of the procedure. One study showed that the distribution of calcium within the lumen is an independent predictor of failed percutaneous revascularization of CTO(Soon et al. 2007). The ability of CCTA to characterize the distribution of calcium within the lesion is important in predicting the likelihood of PCI success. Cross-sectional calcification, which can only reliably be detected on CCTA, was noted to be more important than the length of calcium deposits in one study(Soon et al. 2007). CCTA information can be utilized to plan the strategy of CTO-PCI. In the evaluation of a CTO, the CT angiogram is able to provide complementary data to that of conventional angiography that may be relevant to the success of the CTO recanalization (figure 8).

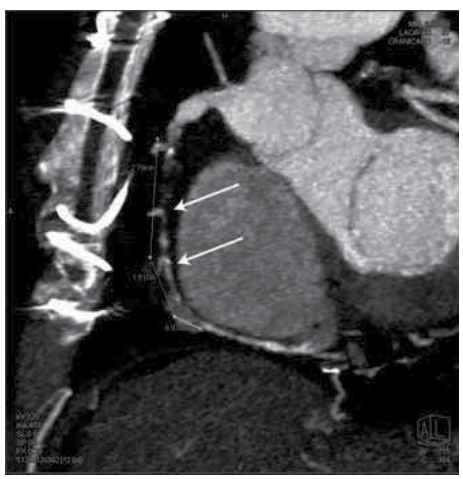

Fig. 8. Chronic total occlusion (CTO) of the right coronary artery (arrows) is seen with measurements of the occlusion length on MIP images of a coronary CT angiogram. The patient subsequently underwent PCI of the CTO. Knowledge of the occlusion length and the morphology of the occluded segment were felt to have been helpful in planning the interventional procedure.

5.2 CT angiography for coronary bypass grafts

Many coronary bypass grafts ultimately fail and the standard of care for assessing patients post aorto - coronary bypass has been invasive coronary angiography. Traditionally, this method has been used somewhat liberally, as these patients already have known significant

CAD and, in the presence of symptoms, a negative non-invasive functional study may not be completely reassuring. CCTA has been shown to be a useful alternative in evaluating by-pass grafts. The location and patency of grafts can be established with very high accuracy and the conduit can be visualized in a three dimensional fashion(Malagutti et al. 2007). Stenoses in the proximal anastamotic site and the body of vein grafts are accurately displayed with CCTA. However, distal anastomotic sites and especially the coronary artery just beyond the anastomosis are sometimes not easily discerned. Current CT technology is still limited in the presence of significant coronary calcifications and these patients often have heavy coronary calcifications. Metallic clips also sometimes obscure the distal anastomosis site. When patients are being considered for repeat coronary artery bypass surgery, potential injury to patent internal mammary artery bypass grafts can contribute to the hazard of the procedure. Sometimes this injury occurs during the median sternotomy if the bypass graft is adherent to the posterior sternum and inadvertently cut. This occurrence can lead to death or major morbidity. CT angiography can very effectively display the course of bypass grafts relative to the sternum and is considered highly appropriate in this patient population. It is considered to be a useful tool for planning these operations and helpful in avoiding this complication (figure 9).

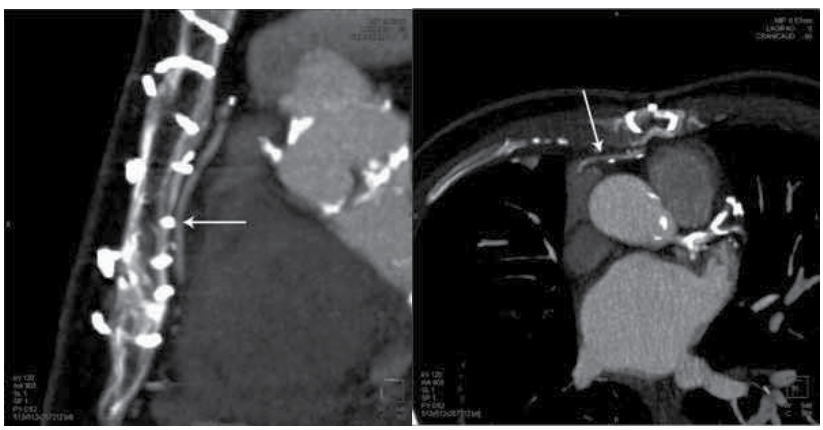

Fig. 9. Modified lateral and axial views of coronary CT angiogram MIP images were obtained in a patient scheduled for repeat coronary bypass surgery. The previously placed left internal mammary artery graft (arrows) is patent and seen to be located immediately posterior and to the right of the midline of the sternum. Based on the results of the examination, the planned approach to the subsequent sternotomy was modified in order to avoid injury to the bypass

5.3 Coronary aneurysms

Coronary artery aneurysms and ectasia are characterized by an abnormal dilatation of a coronary artery. Morgagni in 1761 first described a coronary artery aneurysm(Falsetti and Carrol 1976). Coronary artery aneurysms are defined as coronary artery segments that have a diameter that exceeds the diameter of normal adjacent coronary segments or the diameter of the patient's largest coronary vessel by 1.5 times and involves less than 50% of the total length of the vessel(Pahlavan and Niroomand 2006). The various etiologies of aneurysms include coronary atherosclerosis (50%), followed by congenital (17%) and infectious causes

(10%)(Pahlavan and Niroomand 2006). The pathogenesis is related to the underlying cause, but a pre-requisite to aneurysm formation is the presence of an abnormal tunica media in the vessel wall that results in enlargement and remodeling of an arterial segment(Diaz-Zamudio et al. 2009). Percutaneous intervention, especially with stents, has also been shown to be a cause for the development of aneurysms(Aoki et al. 2008). The diagnostic approach to coronary aneurysms depends on the clinical scenario, and coronary CT angiography is a useful adjunct to diagnose, evaluate, and follow up these coronary artery abnormalities. Figures 10-12 demonstrate close agreement between invasive assessment and CT angiography in 3 patients with coronary aneurysms and figure 13 demonstrates it in one with a saphenous vein graft pseudoaneurysm. While the natural history of these conditions is not well described and follow up algorithms are not worked out, coronary aneurysms appear to be displayed in accurate detail using CT angiography.

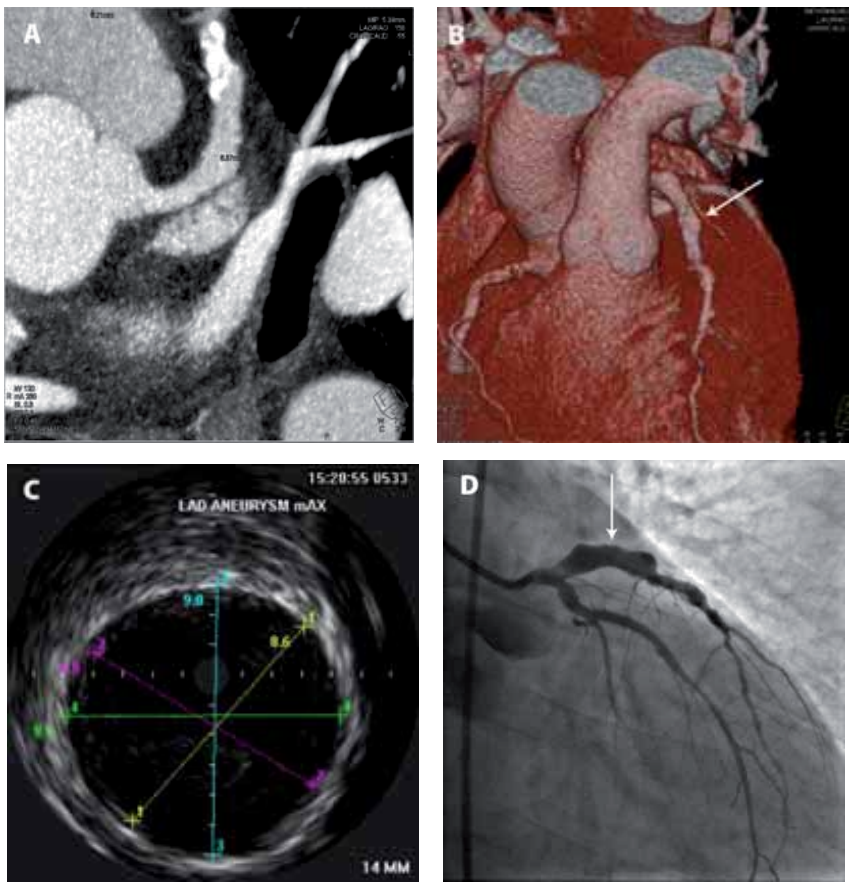

Fig. 10. Aneurysmal dilation of the proximal segments of the left anterior descending artery in a male patient who had recurrent coronary emboli. The aneurysmal segment is seen on oblique maximum intensity projection (A) and volume rendered (B) CT angiogram as well as on intravascular ultrasound (C) and right anterior oblique projection invasive coronary angiogram (D). The appearance and diameter of the dilated segment agrees closely on the three modalities.

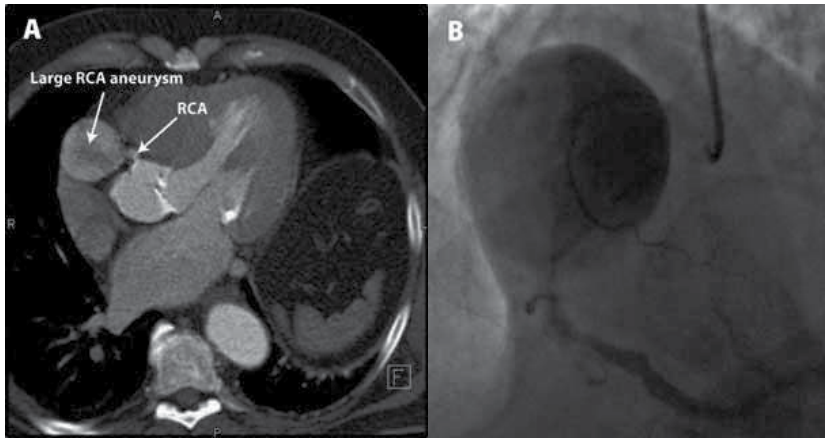

Fig. 11. Huge right coronary artery aneurysm seen on axial view MIP coronary CT angiogram (A) and on invasive coronary angiogram (B).

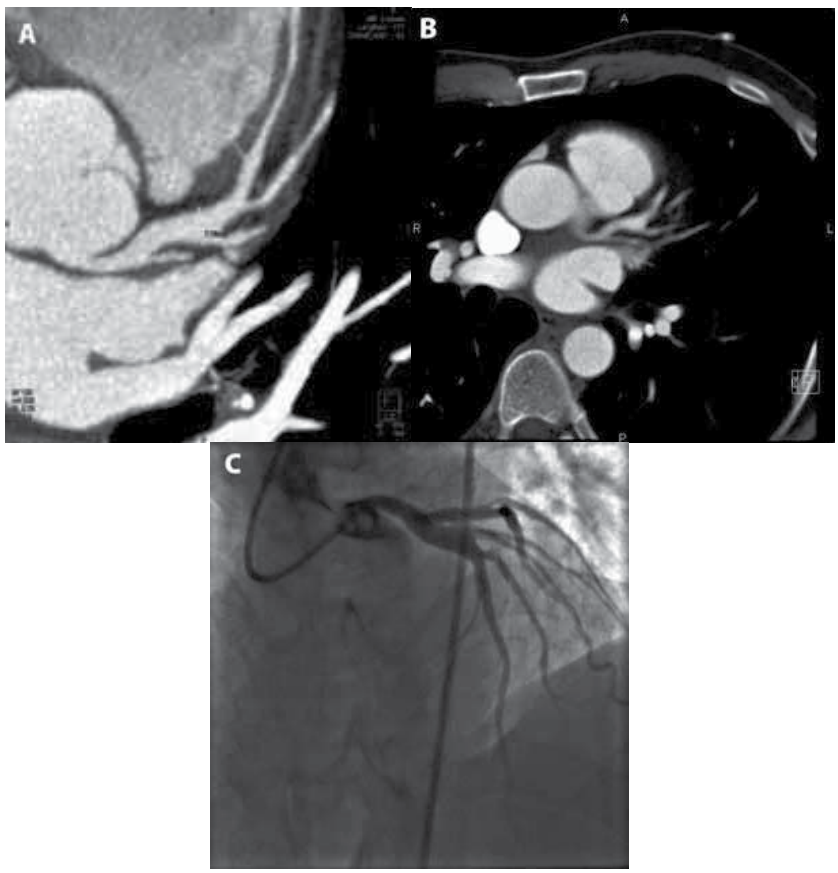

Fig. 12. Coronary CT angiogram MIP (A) and MPR (B) views and invasive coronary angiogram RAO projection showing aneurysmal dilation of the left anterior descending coronary artery.

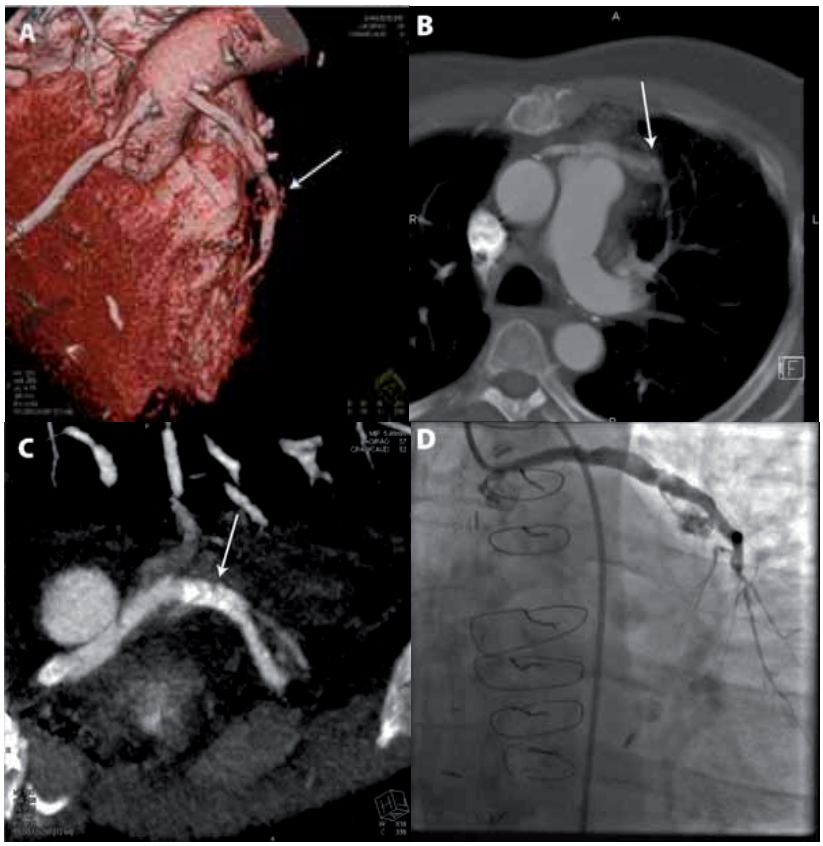

Fig. 13. Saphenous vein bypass graft which developed dissection and contained rupture / pseudoaneurysm post percutaneous intervention. The appearances on CT angiogram by volume rendered image (A), MPR (B), and oblique MIP (C) agreed closely with those on invasive coronary angiogram (D).

6. Prognostic implications of CCTA

CCTA has proven to be an excellent technique to diagnose CAD and is increasingly being used as a first line modality in low risk populations. Several prior diagnostic techniques used in the detection of CAD have proven to provide important prognostic information. CCTA, although a relatively newer imaging technique, has also been shown in recent studies to provide important prognostic information(Ostrom et al. 2008). In a recent report by Gaemperli and colleagues, patients without coronary atherosclerosis on CTA had an excellent prognosis where as the risk for cardiac events increased significantly in the presence of coronary plaques or obstructive lesions. The event rates in the first year were reported to be 34% and 59%, respectively(Gaemperli et al. 2008). There was a strong positive correlation between the extent of coronary atherosclerosis (i.e., the number of segments with coronary plaques) and an adverse cardiac outcome. The presence of three or more coronary plaques was the cut-off providing the highest accuracy to predict future cardiac events. Another study revealed the 1st-year event rate for patients with abnormal coronary arteries to be 30% compared to 0% in patients with normal coronary arteries emphasizing the importance of evaluation of coronary

plaque burden(Gaemperli et al. 2008). Recent angiographic and IVUS derived evidence suggests that assessing plaque phenotype is important in detecting vulnerable plaque(Stone et al. 2011). CCTA's can be used to evaluate the coronary lumen and vessel wall and provide information on obstructive lesions as well as non-stenotic plaques. The burden of atherosclerotic disease determined by CCTA was associated with all-cause mortality among patients with suspected CAD referred for evaluation in an ambulatory setting(Ostrom et al. 2008). CTA demonstrating the presence of luminal obstruction or non- obstructive, non-calcified plaque is a useful noninvasive modality that accurately predicts all-cause mortality with incremental benefit over traditional risk factor assessment and CACS.

7. Other applications of CTA in lieu of invasive angiography

In addition to the above common conditions, there are other, less frequent states when the relatively non-invasive nature of CTA makes it preferable to invasive angiography. For example, occasional patients have very challenging arterial access problems for direct angiography and noninvasively imaging the coronary arteries or bypass graft anatomy might be preferable, even if it would not otherwise be the approach of choice. Also, other patients in whom invasive angiography is risky, such as those with aortic valve endocarditis or heavy aortic atherosclerosis, might also benefit from the less invasive alternative. In these cases, the potential hazard of the invasive procedure is large enough to alter the decision.

8. Conclusion

Coronary CT angiography is an excellent non-invasive diagnostic modality that can be utilized to study in detail the coronary anatomy. It can sometimes supplant traditional invasive coronary angiography, especially in patients in whom the need for coronary intervention is felt to be unlikely. It's excellent negative predictive value makes it a useful, less invasive tool for ruling out coronary artery disease in patients who are at the lower risk of the spectrum for the presence of coronary atherosclerosis. The inherently three dimensional nature also makes coronary CT angiography useful for patients with coronary anomalies and in selected patients who have already had coronary angiography, such as those scheduled to undergo repeat coronary bypass surgery intervention for chronic coronary total occlusions, or those with incomplete procedures. While there has been considerable improvement in the temporal and spatial resolution of the CCTA, it is still inferior to invasive coronary angiography in these aspects and direct invasive angiography is usually needed to make detailed revascularization decisions.

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7

New Noninvasive Modalities in Coronary Angiography: Cardiac Computed Tomography Angiography

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1. Introduction

Coronary artery disease (CAD) is a leading cause of mortality and morbidity in most developed countries [1]. CAD is a common and sometimes disabling disorder, although medication therapy, percutaneous coronary intervention and coronary artery bypass grafting have developed recently. Medical doctors need to prevent from developing acute coronary syndrome. The development of non-invasive cardiac imaging tools (particularly, cardiac computed tomography, echocardiography and so on) for the diagnostic and prognostic assessments of patients is evolving evidence for various treatment strategies. Cardiac catheterization is golden standard for the diagnosis of CAD. Although the risk of adverse events for invasive coronary angiography is generally considered to be low, potential life-threatening complications can arise, including not only coronary artery dissection, but also arrhythmia, stroke, hemorrhage, myocardial infarction (MI), and death [2]. Non-invasive imaging devices for CAD have been developing, such as echocardiography, scintigraphy, computed tomography (CT) and magnetic resonance imaging (MRI) and so on. Particularly, the development of cardiac CT is remarkable in the last 10 years.

Prior report suggested a hierarchial model of efficacy to assess the contribution of diagnostic imaging to the patient management process. Level 1 is technical quality of the images. Level 2 is diagnostic accuracy, sensitivity, and specificity associated with interpretation of the images. Level 3 is whether the information produces change in the referring physician's diagnostic thinking. Level 4 is efficacy, which concerns effect on the patient management plan. Level 5 is effect of the information on patient outcomes. Level 6 is societal costs and benefits of a diagnostic imaging technology [3].

CT imaging was introduced in 1972 [4]. The ability to obtain cross-sectional images of the computer-assisted tomography, Sir Geoffrey N. Hounsfield and Allan M. Cormack were awarded the Nobel prize in Medicine in 1979.

Since a 4 detector row cardiac CT angiography was launched in 1998. Cardiac CT has experienced rapid improvement of imaging qualities with the ongoing evolution of cardiac CT. The diagnostic accuracy of the 64 detector cardiac CT to detect coronary stenoses is available. Cardiac CT is useful for the diagnosis and risk stratification of CAD. Cardiac CT presently has not been considered a routine replacement for invasive coronary angiography,

because the diagnostic accuracy of cardiac CT is not greater than that of invasive coronary angiography yet. Invasive coronary angiography is more appropriate than cardiac CT for patients with a high pretest likelihood of CAD. Cardiac CT is appropriate for stable patients with acute chest pain [5]. A recently published guideline reports several appropriate indications for cardiac CT. At First, they are symptomatic patients with intermediate likelihood of CAD. Particularly, the patients are indicated in whom stress testing, including in electrocardiography, echocardiography, and scintigraphy is not possible because of severe aortic stenosis, severe heart failure and aortic dissection etc., or the result of stress testing is equivocal or uninterpretable with acute chest pain. Secondly, they are patients with acute chest pain and intermediate likelihood of CAD but absence of ECG changes and normal myocardial enzyme levels [6]. Cardiac CT is useful for the diagnosis of CAD in complete left bundle branch block patients, because the diagnostic accuracy is slight lower with stress test of electrocardiography, echocardiography and scintigraphy [7].
The best approaches to the care of CAD, improving not only the efficacy and safety of treatments, but also the cost.
We discuss the usefulness of cardiac CT for the risk stratification of CAD.

2.1 The technique and limitation of cardiac CT imaging

The clinical value of CT for imaging of the heart has been very limited for the long time. Cardiac imaging requires a very high temporal resolution, because the heart is rapid motion. Therefore, dedicated scanner designs needed to be developed to increase acquisition speed. Furthermore, cardiac CT imaging requires providing contiguous cross-sectional images of the heart. Every displayed image must be of the same cardiac phase. Gaps may occur if adjacent images depict the heart in different phases of the cardiac cycle. Data acquisition must be triggered by the patient's electrocardiogram (ECG), image reconstruction must be synchronized to a function correlated to cardiac motion. The heart is subjected to intrinsic motion by cardiac contraction and to motion by breathing. CT imaging of the complete heart has to be performed within one single breath-hold.
Recently, the number of detectors expands from 64 to 320. With a detector width of 0.5 mm, this will be able to be coverage of about 160 mm. It can be obtained in one rotation and can be performed during one heartbeat [8-10]. This will decrease the length of the necessary breath-hold, decrease the amount of contrast agents to achieve intravascular enhancement during scan, and may be useful in patients with an inconstant heart rate or arrhythmia.

2.2 Atherosclerosis of the coronary artery

Cardiac CT has several potential applications for patients with CAD. Cardiac CT can demonstrate the morphological features of CAD and estimate ventricular function, perfusion. Recently, the visualization of coronary artery has been developing with coronary artery calcium (CAC), coronary artery stenosis.
The pathology of coronary artery is important in order to understand the image of cardiac CT, taking into account the pathology of coronary artery. CAC is a surrogate marker for coronary atherosclerotic plaque. In the coronary arteries, calcifications occur almost exclusively in the context of atherosclerotic changes [11,12]. In the most patients with acute coronary syndromes, CAC can be detected, and the amount of calcium in these patients is substantially greater than in matched control subjects without CAD [13]. With the exception of patients in the renal failure, nonatherosclerotic calcification of the coronary artery wall is

rare. The amount of CAC correlates moderately with the extent of atherosclerotic plaque burden in a coronary artery [11,12]. On the other hand, not every atherosclerotic coronary plaque is calcified. The presence or absence of CAC is not closely associated with an individual atherosclerotic plaque to rupture. And CAC is not associated with stability or instability of an individual plaque [12]. Plaques with healed ruptures almost invariably contain calcium, whereas plaque erosions are frequently not calcified.
Although there is a quantitative relationship between CAC and coronary plaque burden, there is only a weak correlation between the amount of CAC and the angiographic severity of coronary artery stenoses [11]. Cardiac CT has several potential applications for patients with coronary artery disease. Cardiac CT can demonstrate the morphological features of coronary artery disease and estimate ventricular function, perfusion. Recently, the visualization of coronary artery has been developing with CAC, coronary artery stenoses. Non-enhanced CT studies of the heart are almost exclusively performed to assess calcified structures within the heart and CAC.
The absence of detectable CAC rules out the presence of significant coronary artery stenoses with high negative predictive value [11].

2.3 Coronary calcium in cardiac CT

Non-enhanced CT studies of the heart are almost exclusively performed to assess calcified structures within the heart and coronary arteries.
Cardiac CT detects and quantifies the amount of CAC. It is a marker of atherosclerotic disease burden. Calcification does not occur in a normal coronary artery wall, it therefore indicates the presence of atherosclerosis, but is not specific for coronary artery stenoses. CAC scores predict the total atherosclerotic plaque burden.
However, the absence of detectable CAC rules out the presence of significant coronary artery stenoses with high negative predictive value [11]. Although these findings are consistent with the concept that the calcified plaque burden parallels the overall plaque burden, CAC testing is not appropriate as an alternative for angiographic disease detection, because of the modest relationship between CAC and coronary artery stenoses [14]. Because even coronary atherosclerotic plaque burden is not necessarily associated with significant coronary artery stenoses, even the detection of large amounts of calcium does not imply the presence of the significant stenoses. The CAC detection could be as a marker for CAD prognosis in asymptomatic patients. The presence and severity of CAC has independent and incremental value in the estimation of death or nonfatal MI [15]. CAC is important in the risk stratification, and noncalcified atherosclerosis is also important. Some studies against intravascular ultrasound have reported a sensitivity of 91% and 95% to detect calcified and 78% and 58% for noncalcified lesions by 16 slice CT [16,17] and increased the sensitivity to detect noncalcified lesions to 83% by 64 slice CT [18]. Because of the high CT attenuation of calcified lesions, their differentiation from fibrous and lipid-rich lesions is easy. Noncalcified plaque is consisted of lipid-rich plaques and fibrous plaques. Lipid-rich plaque (less than 50 HU) is lower CT Hounsfield attenuation numbers than fibrous plaques (50 to 120 HU) and calcified plaque (more than 121 HU) [17,19]. Patients with acute coronary syndromes were found to have a higher prevalence of noncalcified as compared with calcified plaque than stable coronary artery disease [20].
We show the algorithm to diagnose ischemic heart disease with cardiac CT in American Heart Association guideline and Japanese Circulation Society guideline.

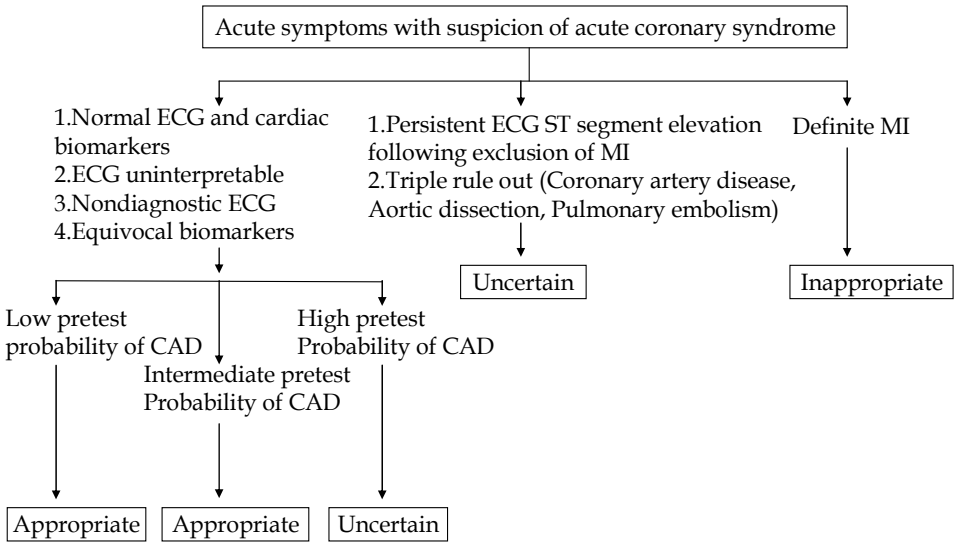

Fig. 1. Detection of CAD in symptomatic patients without known heart disease symptomatic acute presentation [21]. The stratification of pretest probability of CAD are shown in Table 1.

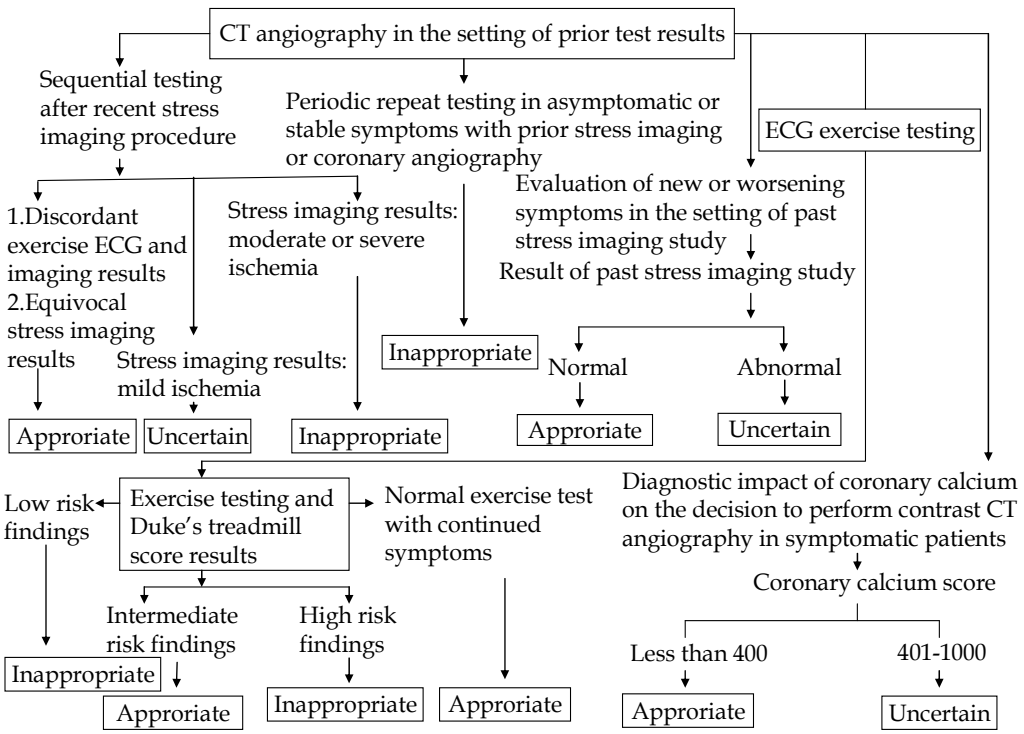

Fig. 2. Use of CTangiography in the setting of prior test results [6].

Age	Sex	Typical/Definite Angina Pectoris	Atypical/Probable Angina Pectoris	Nonanginal Chest pain	Asymptom
<39	Men	Intermediate	Intermediate	Low	Very low
	Women	Intermediate	Very low	Very low	Very low
40–49	Men	High	Intermediate	Intermediate	Low
	Women	Intermediate	Low	Very low	Very low
50–59	Men	High	Intermediate	Intermediate	Low
	Women	Intermediate	Intermediate	Low	Very low
>60	Men	High	Intermediate	Intermediate	Low
	Women	High	Intermediate	Intermediate	Low

1) Very low pretest probability: Less than 5% pretest probability of CAD
2) Low pretest probability: Between 5% and 10% pretest probability of CAD
3) Intermediate pretest probability: Between 10% and 90% pretest probability of CAD
4) High pretest probability: More than 90% pretest probability of CAD

Table 1. Pretest Probability of CAD by Age, Sex, and Symptom [22]

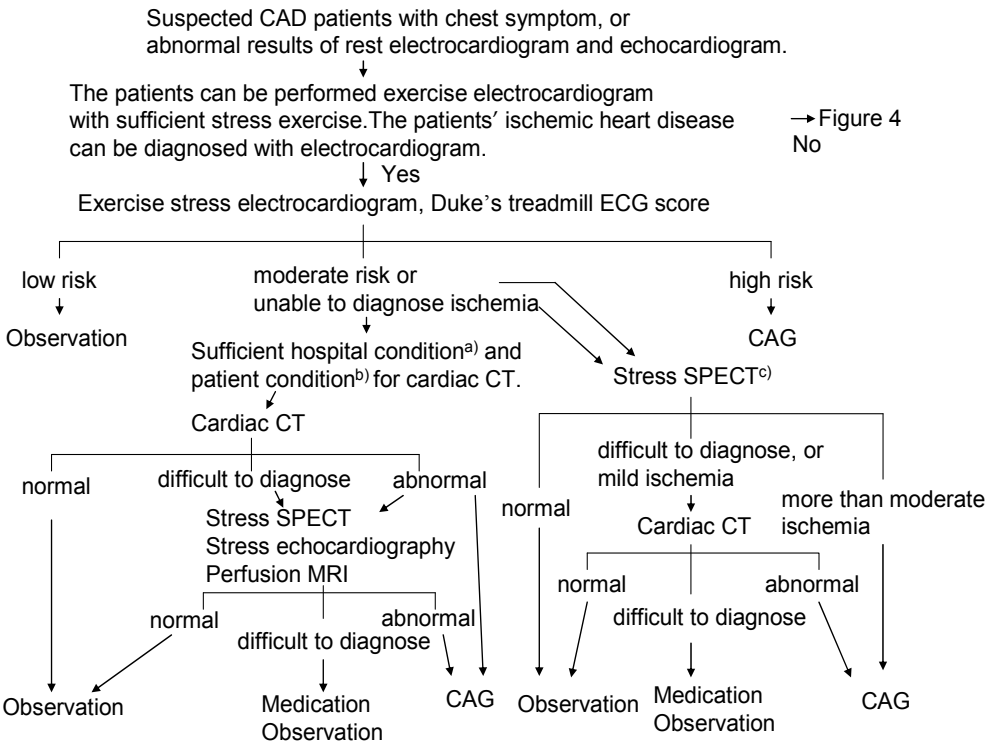

Fig. 3. Algorithm to diagnose stable angina pectoris for the patients who are able to exercise (revised Japanese Circulation Society Guideline 2009, Circulation Journal Vol 73, Suppl. Ⅲ,1019-1089, 2009).

The Duke's treadmill score (Bruce protocol) is calculated as follows:
- duration of exercise in minutes - (5 x the maximal ST segment deviation
- during or after exercise, in millimeters) – (4 x the treadmill angina index).

- The angina index has a value of 0::if the patient had nonlimiting angina,
- 1: if exercise angina occurred, and 2: if angina was the reason the patient stopped exercising. The score had a range from -25 (highest risk) to +15 (lowest risk).

The outpatients had treadmill scores indicating low risk (≥ +5) and their four-year survival rate was 99% (average annual mortality rate, 0.25%). The outpatients had scores indicating high risk (< -10) and their four-year survival rate was 79% (average annual mortality rate, 5.0%)[23].

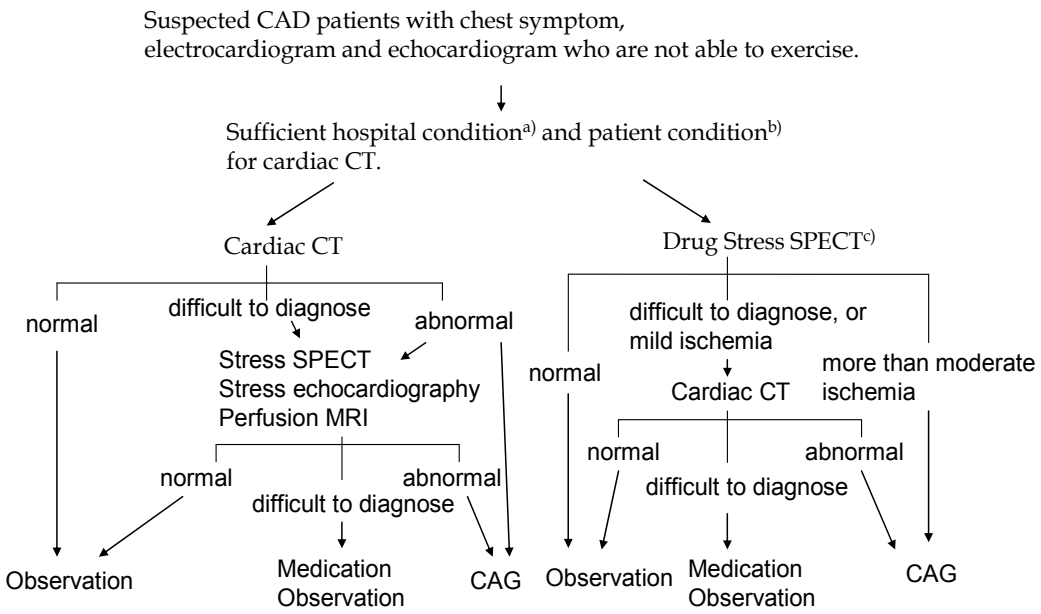

Fig. 4. Algorithm to diagnose stable angina pectoris for the patients who are not able to exercise (revised Japanese Circulation Society Guideline 2009,Circulation Journal Vol 73, Suppl. Ⅲ,1019-1089, 2009).

a. Hospital condition for cardiac CT
   1. The staffs have sufficient experience on cardiac CT.
   2. The hospital equips with cardiac CT which is better than 64 slice.
   3. The staffs are able to show good images and work the proper reporting system.
   4. The staffs understood the feature of each CAG and cardiac CT.
   5. The staffs try to make the protocol for lowering the radiation exposure.

b. Patient condition for cardiac CT
   1. The staffs are careful of the risk of radiation exposure in women which is less than 50 years old.
   2. It is difficult to diagnose in the patients with many calcified coronary arteries, for example, the patients with hemodialysis, and the elderly patients.
   3. Serum creatinin is less than 2.0 mg/dL.
   4. The eGFR is more than 60 mL/min/1.73m^2.
   5. Diabetes mellitus nephropathy patients, including to microalbuminuria are not recommended
   6. The contrast alergy patients are not recommended.
   7. Asthma patients are not recommended.

c. Stress SPECT
   1. The stress test is recommend exercise stress, rather than drug stress.
   2. The diagnosis is recommended 17 or 20 segment method to diagnose the area and degree of ischemia.
   3. The staff confirm whether the patients have contraindication of the stress drug.
   4. Echocardiogram and perfusion MRI can be alternative stress test depending on the facilities.
   5. Less than 50% count of maximum count in the defect area is more than moderate ischemia.
   6. The count between 50% and 70% count of maximum count in the defect area is mild ischemia.
   7. More than 70% count of maximum count is normal myocardium.

2.4 Visualization of the coronary artery lumen in cardiac CT

Cardiac CT can be applied for visualization of the coronary artery lumen after intravenous injection of a contrast agent. The administration of beta blockers before the cardiac CT scan and the use of sublingual nitroglycerin can achieve coronary vasodilation and maximize image quality. Studies for the diagnosis of coronary artery stenoses using 64 slice CT scanning with invasive coronary angiography report sensitivities and specificities of 87-99% and 86-97%, respectively, and importantly, a negative predictive value of 98-100% [24-27].
Cardiac CT could be an efficient initial triage tool in patients with acute chest pain with low to intermediate risk, because of the high sensitivity and negative predictive value. In the symptomatic population, there is lack of study that shows an improved prognostic power of cardiac CT over other modalities including coronary artery calcium scores and carotid intima media thickness [28]. The clinical use of cardiac CT to detect plaque for purposes of risk stratification asymptomatic individuals has not recommended yet, although the clinical use of risk stratification in asymptomatic high risk individuals has been reported repeatedly [29-31].
Patency and occlusion of bypass grafts can be established with sensitivity and specificity of nearly 100% in cardiac CT, because of the large size and limited mobility of these structures [14,32,33]. But, the limitations are the detection of stenoses at the site of anastomosis to the coronary artery and in the peripheral run-off coronary artery. Metallic clips and severe coronary calcium lead to reduced sensitivity and specificity in post CABG patients. Before re-operative coronary surgery, cardiac CT can be used to define the relationship of sternal wires to cardiac and graft structures for the purpose of planning surgical reentry techniques.
Cardiac CT with volume rendering images is useful to patients with known or suspected congenital coronary artery anomalies. Cardiac CT can classify the origin and the complex course of anomalous coronary arteries [34,35].
Although artifacts caused by metal and partial volume effects limit the evaluation of in-stent restenosis with 64-slice CT, high accuracy, approximately 90%, can be obtained in stents 3mm or greater in diameter [36,37].

3. Conclusion

Cardiac CT is a rapidly developing and advancing technology. The increase of radiation will reduce artifact, improve coronary artery visualization, although an excess of radiation exposure leads to cancer. Cardiac CT is useful in the non-invasive diagnosis of coronary

artery disease for the stable and acute chest pain patients, especially who cannot be sufficiently evaluated by electrocardiography, echocardiography, scintigraphy or cardiac MRI.

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8

Simultaneous Assessment Beyond Coronary Stenosis by Multislice Computed Tomography

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1. Introduction

Whereas in the past computed tomography (CT) of the coronary arteries could detect only calcifications, recently multislice computed tomography (MSCT) has been already accepted as an efficient noninvasive tool for the detection of coronary artery stenosis, providing good sensitivity, specificity, and very high negative predictive value. However, it must be recognized that these good results are obtained when technically inadequate scans or patients with rapid heart rates, arrhythmia, or severe calcification were excluded. Therefore, for patients who already have a clinical indication for coronary angiography by MSCT in a real world, it would be a clinically important advantage if the same MSCT data could be used to gain additional information about the coronary artery disease (CAD). It has been reported that MSCT enables the analysis of the coronary plaques, left ventricular (LV) function, left atrial volume, myocardial enhancement, aortic valve, and the thoracic aorta, besides the assessment of the coronary stenosis. In this chapter, we focus on the additional information obtained simultaneously from the same data for coronary angiography by MSCT.

2. Assessment of myocardial contrast enhancement concomitantly with an assessment of coronary stenosis

MSCT has reached a spatial and temporal resolution that is high enough to visualize not only coronary arteries but also infracted and non-infarcted myocardium (Paul et al., 2003). Nevertheless, coronary arteries with severely calcified plaques can still be difficult to evaluate by MSCT angiography (Leschka et al., 2005) because blooming artifacts and beam hardening effects can lead to misinterpretation of the luminal area.

Here, we analyzed first-pass myocardial enhancement by 64-MSCT, evaluated its use for detecting significant stenosis and compared the results with quantitative coronary angiography (QCA) (Yoshida et al., 2009). In our study, myocardial contrast enhancement is quantified by deriving the signal density (SD) from the Hounsfied units (HU) values of the CT images. Data from 70 patients with single-vessel disease who underwent 64-MSCT followed by catheter-based coronary angiography were analyzed. After an evaluation of coronary stenosis, early- and late-diastolic (at 60% and 90% of the RR interval, respectively)

short-axis images at 3 levels of the LV (basal, mid and apical), were analyzed. The SD in segments of the LV wall supplied by coronaries with and without stenosis was assessed. In this study, the SD of the LV cavity and remote myocardium was used as a reference for quantifying impaired myocardium. A region of interest of approximately 50 mm^2 was drawn over each myocardial segments and LV cavity. A standardized SD was then calculated from the SD of the myocardium and the SD of the LV cavity according to the following formula:

$$\text{Standardized SD} = \frac{\text{the SD of the myocardium}}{\text{the SD of the LV cavity}} \times 100\%$$

The reduction in the percent SD between the myocardium surrounding a stenotic lesion and non-impaired myocardium away from a stenotic lesion was then defined as:

$$\%SD = \frac{\text{standardized SD stenosis} - \text{standardized SD no} - \text{stenosis}}{\text{standardized SD no} - \text{stenosis}} \text{x}\, 100\%$$

In total, 48 patients had 70 atherosclerotic lesions (83 segments) detected by QCA: 35 segments were categorized as a calcified plaque, and the other 48 segments were categorized as a non-calcified plaque. Forty-six segments were found to have more than 50% coronary stenosis by QCA. Diagnosis of significant stenosis by MSCT coronary angiography (segment-based analysis) had a sensitivity, specificity and accuracy for segments without calcified lesions of 92%, 100% and 99.7%, respectively, and 95.2%, 50%, and 77.1%, respectively, for calcified lesions. The standardized SD and %SD values in patients with a significant stenosis (more than 50% stenosis as determined by QCA) were significantly lower than those in patients without a stenosis. A similar trend was observed in patients with more than 75% stenosis. These values did not change during the early to late diastolic phase of the cardiac cycle. Taking into account the myocardial enhancement by calculating the decrease of the %SD, a sensitivity, specificity and accuracy of diagnosis of significant stenosis could be improved to 95.2%, 85.7% and 91.4%, respectively, for segments with calcified lesions. The analysis of the %SD reduction improved the diagnostic performance of MSCT for stenosis of coronaries with calcified plaques. The present study is a clinically essential investigation of first-pass myocardial enhancement by MSCT. Because the same image data that were used for MSCT coronary angiography were also used for the first-pass myocardial enhancement analysis, the patient was neither subjected to additional radiation exposure nor additional administration of contrast agent.

Impaired myocardial blood flow and clinically unrecognized myocardial injuries could lead to reduced enhancement on MSCT images of the myocardium. First, the intra-myocardial coronary capillaries make up more than 90% of the microcirculation of the myocardium and the cross-connections serve to homogenize the myocardial pressure and flow distribution (Kassab et al., 1994, 1999). In myocardium supplied by healthy coronary arteries, increased capillary outlet pressure produces pressure dispersion and local flow reversal (Mittal et al., 2005) during systole, whereas during diastole, the rapidly decreasing capillary outlet pressure promotes the washout of contrast agent. In contrast, stenotic epicardial coronary stenosis fails to supply optimal coronary blood flow (Gould et al., 1974) to the intra-myocardial coronary capillary inlets and as a result, enhancement of CT images of the myocardium appears to be reduced. Impaired myocardial blood flow resulting from more

than 75% stenosis has already been visualized by myocardial contrast echocardiography with power Doppler imaging (Masugata et al., 2000). However, unrecognized myocardial injuries are also a possible cause for reduced myocardial enhancement, as has been shown by other studies in which myocardial injuries in patients with a clinical suspicion of CAD, but without known myocardial infarction, were detected by cardiac magnetic resonance imaging. We have shown that myocardial enhancement might be represented in the resting condition by the combination of reduced myocardial blood flow and unrecognized myocardial injuries. The advantage of MSCT imaging is that epicardial coronary stenosis can be analyzed concomitantly with an assessment of myocardial enhancement. It is important to note that an analysis of the degree of coronary stenosis and risk areas of the myocardium can be performed without additional radiation exposure or contrast injections. The introduction of myocardial assessment by first-pass scan protocols into clinical practice is easy without additional stress and cost, because of post-hoc analysis.

3. Assessment of the atherosis and sclerosis in the aorta

Atherosclerosis consists of two pathological processes: atherosis characterized by morphologic atheromatous lesions appearing in the intima, and sclerosis characterized by an increase in stiffness of the vessel walls. Aortic stiffness is known to increase with age (Tomochika et al., 1999), but it is also correlated with various diseases such as CAD (Herrington et al., 2003) and hypertension (Laurent et al., 2003), or hypercholesterolemia (Tomochika et al., 1999). So far, atherosis and sclerosis of the descending thoracic aorta (DTA) could be observed and analyzed during transesophageal echocardiography performed for other indications than CAD (Tomochika et al., 1999; Sugioka et al., 2002). Typical indications are cardiac embolism, valvular disease and atrial fibrillation, and transesophageal echocardiography is not performed for assessing aortic atherosclerosis in patients with CAD. A patient study with the latter aim would pose ethical problems, in particular since transesophageal echocardiography causes some discomfort.

In contrast, for patients who already have an indication for coronary angiography by MSCT, which is non-invasive, it would be a clinically important advantage if the same MSCT data could be used to gain additional information about the CAD through the analysis of the aorta at multiple locations. A time-resolved, electrocardiographic (ECG)-gated CT technique to derive the aortic distensibility from cyclic cross-sectional area changes has already been validated in a phantom set-up with porcine aortic specimens (Ganten et al., 2005). Ganten et al reported the negative correlation between abdominal aortic distensibility and aging with the use of this method albeit 4- or 16-slice CT (Ganten et al., 2007). The distensibility however depends on the blood pressure, whereas the stiffness β is considered to be independent of the blood pressure (Hayashi et al., 1980; Hirai et al., 1989).

Recently, we quantified atherosis and sclerosis of DTA and analyzed differences between patients with and without CAD using MSCT (Okuyama et al., 2008). The population contained 89 patients who underwent ECG-gated MSCT: 40 patients who were suspected of CAD by MSCT underwent invasive coronary angiography, and had documented significant stenoses (CAD group), 49 patients were found without significant stenoses (control group). We quantified atheromatous lesions and stiffness β of DTA. First, twenty cross-sectional images of DTA were reconstructed every 5% (0-95%) of the RR interval, and the largest and

smallest luminal areas were traced at 3 levels of the DTA avoiding sites of severe atheromatous lesion: at the pulmonary artery bifurcation (proximal DTA), below the heart (distal DTA), and in between (middle DTA). The maximum systolic (D_{max}) and minimum diastolic (D_{min}) lumen diameters were calculated from those areas with the assumption that the cross section was circular (diameter=2× [area/ π]$^{1/2}$) (Nakatani et al., 1995). The aortic stiffness β was then calculated according to the formula
β=ln (BP_{sys}/ BP_{dia})/ ([D_{max}–D_{min}]/ D_{min}), where ln was the natural logarithm, BP_{sys} was the systolic blood pressure, and BP_{dia} was the diastolic blood pressure. Next, we assessed the grade of aortic atherosis for each patient. We divided the DTA into 3 segments of 5 cm length (2.5 cm proximal and 2.5 cm distal to each level described above), then continuously assessed the severity of atherosis in the transverse and longitudinal images of each area. We classified the aortic atheromatous lesions into 4 categories and scored each segment according to the severity of atherosis from 0 through 3, as described previously (Tomochika et al., 1999; Dávila-Román et al., 1994). An atheromatous score 0 indicated a normal aortic wall, score 1 indicated mild atherosis (intimal thickening <3.0 mm, without intimal irregularities), score 2 indicated moderate atherosis (intimal thickening ≥3.0 mm, with intimal irregularities) and score 3 indicated severe atherosis (significantly raised plaques, calcified plaques or raised plaques with ulcer formation) .
In our study, the atheromatous score and stiffness β in the CAD group were significantly higher than those in controls. Multivariate analysis revealed that the average atheromatous score was an independent factor associated with CAD ($p<0.005$). Receiver-operating characteristic analyses were carried out on the average, maximum, and minimum atheromatous score and stiffness β for identifying patients with CAD. The areas under the curves for the average, maximum, and minimum atheromatous score were 0.82 (95% CI, 0.73 to 0.91), 0.82 (95% CI, 0.73 to 0.91) and 0.75 (95% CI, 0.65 to 0.85), respectively. Regarding the average, maximum, and minimum stiffness β, the areas under the curves were 0.75 (95% CI, 0.65 to 0.86), 0.74 (95% CI, 0.64 to 0.85) and 0.75 (95% CI, 0.65 to 0.85), respectively. A cut-off value ≥1.33 of the average atheromatous score (total atheromatous score ≥4) had a sensitivity of 73%, a specificity of 84%, a positive predictive value of 78%, and a negative predictive value of 79% for detection of CAD. In contrast, regarding the average stiffness β, a cut-off value ≥14 had a sensitivity of 63%, a specificity of 65%, a positive predictive value of 60%, and a negative predictive value of 68%. Moreover, the combination of the average atheromatous score (≥1.33) and stiffness β (≥14) had a sensitivity of 48%, a specificity of 92%, a positive predictive value of 83%, and a negative predictive value of 68% for detection of CAD. In cases with image qualities unsatisfactory for interpretation of coronary stenoses, the additional assessment of atherosclerosis of DTA will be useful for identifying patients with CAD.

4. Assessment of aortic valve area

In industrialized countries the most frequent cause of aortic stenosis (AS) is degenerative changes of valve leaflets, such as the congenital bicuspid aortic valve (AV) and atherosclerotic valves. These changes frequently occur in elderly patients, who are also at risk for CAD. Severe AS (an AV area (AVA) of less than 1.0 cm^2) accompanied by CAD presents problems for surgical decision-making in whether AV replacement should be performed with coronary artery bypass graft surgery.

Transthoracic echocardiography is an important and non-invasive method for assessing the significance of cardiac murmurs, which are derived from diseased heart valves. For diagnosing and evaluating the AVA by 2-dimensional echocardiography in patients with AS, the planimetric method without disturbed visualizations and the Doppler approach using an adequate angle of ultrasound beam are highly sensitive (Caidahl et al., 1998; Okura et al., 1997). The high spatial and temporal resolution of MSCT might allow for detailed images of the AV orifice and measurements of the AVA.

We investigated whether the AVA in patients with AS assessed by MSCT corresponds to that by echocardiographic assessment and to evaluate simultaneously the clinical accuracy in detecting CAD with MSCT (Tanaka et al., 2007). The AVA of 29 consecutive AS patients with transthoracic echocardiography and MSCT were analyzed. The AVA was estimated by means of the continuity equation method in 2-dimensional echocardiography. Regarding AVA measurement method by MSCT, initially, one data set was reconstructed with the reconstruction window starting at 10% of the cardiac cycle. If motion artifacts were present in the AV, image reconstruction was repeated with the reconstruction window offset 2% toward the beginning and end of the systole until images without motion artifacts were obtained or until 20 data sets had been created, in which case the data set with the fewest motion artifacts was used for further evaluation for each coronary artery separately. The mid-systolic image data set, which was extracted with reference to the R wave of recorded ECG trace, was used to assess the AV orifice. The AV orifice images were reconstructed with a slice thickness of 2.0 mm in 1.5 mm intervals using the 2-step double-oblique method. The long axis of the transaxial image was obtained at mid-systole, and the next image was oblique at the line connecting the center of the ascending aorta and the AV orifice. Short-axis views of the aortic root were reconstructed as a first step. Further procedures were performed by inclining the short-axis image as a second step. AVA represented as planar images contained AV commissures. Finally, the calculations of the AVA were performed by planimetry. Concomitantly, the severty of the coronary artery stenosis was assessed by MSCT.

In our study, regression analysis showed that the AVA in patients with AS determined by MSCT correlated well with those determined by 2- dimensional echocardiography ($r=0.96$, $p<0.001$). Significant coronary stenosis of more than 50% diameter reduction was present in 48% of the study population. The results of the present study are demonstrated by modern non-invasive technique of MSCT as follows: 1) the morphological appearance of AV orifice was clearly visualized; 2) the measurement of the AVA was accurately estimated to be the same as transthoracic echocardiography assessment; and 3) a moderate incidence of significant coronary stenosis was determined. Current guidelines recommend a diagnostic coronary angiography before surgery in symptomatic patients with AS. Analysis of the severity of AVA concomitant with CAD is important for patients with AS. Therefore, assessment for AS and CAD by MSCT is ideal for realization of these purposes. MSCT can investigate not only the degree of CAD and AS, but also the anatomical assessments of a calcified ascending aorta and patency of graft arteries, such as bilateral internal mammary arteries.

5. Assessment of coronary plaque

Although CAD is the leading cause of death in the individuals with coronary risk factors, the majority of these individuals do not actually develop coronary symptoms before the

onset of acute myocardial infarction (AMI) or sudden death. In fact, AMI or sudden death frequently occurs as the first symptom of coronary diseases. Therefore, screening of the patients with unstable plaque is important for prevention of the onset of AMI or sudden cardiac death. Intracoronary thrombus, immediate result of plaque rupture plays key roles in the onset of acute coronary syndromes (ACS) (van der Wal et al., 1994; Moreno et al., 1994). Ruptured plaques are histopathologically characterized by large lipid core and large plaque volumes that are covered by the thin fibrous cap often inflamed with macrophages and T lymphocytes infiltration. Moreover, in recent intravascular ultrasound (IVUS) studies, the term "arterial remodeling" was defined as a change in vessel size, using the ratio of the external elastic membrane area (vessel area) at the culprit lesion site to that at the minimally diseased reference site within the same vessel segment. Most of their studies demonstrate that the vessels are positively remodeled at the site of plaque rupture in patients with ACS (Schoenhagen et al., 2000; Ehara et al., 2004). Various diagnostic techniques have identified the imaging characteristics of vulnerable plaques and proposed that these characteristics may allow recognition of coronary lesions likely to result in ACS.

Recently, MSCT has reached a spatial and temporal resolution high enough for assessment not only of coronary artery stenosis but also coronary atherosclerotic plaques. For the first time, Schroeder et al demonstrated that the IVUS-based coronary plaque configuration can be accurately identified by MSCT (Schroeder et al., 2001). They reported that IVUS-based soft plaques showed a mean density of 14±26 HU, intermediated plaques of 91±21 HU, and calcified plaques of 419±194 HU. Similarly, Motoyama et al showed that the corresponding values were 11±12 HU, 78±21 HU, and 516±198 HU (Motoyama et al., 2007). Regarding arterial remodeling determined by MSCT, on the other hand, Achenbach et al reported that cross-sectional vessel areas and remodeling indices measured by 16-slice CT scanner correlated closely to IVUS ($r = 0.88$ and $r = 0.91$, respectively) (Achenbach et al., 2004). More recently, Leber et al provided comparative data of vessel areas, plaque areas, and lumen areas determined by IVUS and 64-slice CT, and reported that the correlation coefficients for these measurements were $r = 0.88$, $r = 0.73$, and $r = 0.81$, respectively (Leber et al., 2005). Based on these promising studies, the prospective MSCT study was designed by Motoyama et al (Motoyama et al., 2009) to evaluate the role of CT plaque characterization for predicting acute coronary events in 1059 subjects with established or suspected CAD who were followed up for an average of 27 months. They reported that of the 45 patients showing plaque with both positive remodeling and low-attenuation plaques (<30 HU), ACS developed in 10 (22.2%), compared with 1 (3.7%) of the 27 patients with plaques displaying either feature. In only 4 (0.5%) of the 820 patients with neither positive remodeling nor low-attenuation plaques did ACS develop. None of the 167 patients with normal angiograms had acute coronary events. ACS was independently predicted by positive remodeling and/ or low-attenuation plaques. They concluded that the patients demonstrating positively remodeled coronary segments with low-attenuation plaques in CT angiography were at a higher risk of ACS developing over time when compared with patients having lesions without these characteristics. We also demonstrated that in patients with IVUS-based plaque rupture, the prevalence of an ulcer-like enhancement space, a ring-like sign was higher than those in patients without IVUS-based plaque rupture (Tanaka et al., 2008). Thus, MSCT might provide a useful and promising tool for the non-invasive detection of vulnerable plaques and vulnerable patients.

6. Conclusion

The diagnostic accuracy of MSCT in the detection of coronary artery stenoses has been reported by many investigators, with a very high specificity and very high negative predictive values. However, most previous studies did not consist of consecutive patients and most studies excluded patient images with image qualities unsatisfactory for interpretation. MSCT scans of the coronary arteries are still associated with a relatively high radiation dose and administration of contrast agent, it is therefore important to obtain as much relevant information as possible from the scan. The simultaneous assessment beyond angiography, which is often possible even in cases with unsatisfactory coronary image quality, could offer a way to increase the diagnostic usefulness of MSCT for CAD patients.

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9

Assessment of Coronary Artery Bypass Graft (CABG) Patency and Graft Disease Using Multidetector Computed Tomography (MDCT)

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1. Introduction

Coronary artery bypass graft (CABG) surgery is the standard care in the treatment of advanced coronary artery disease. Notwithstanding the clear benefits of bypass grafting, recurrent chest pain after myocardial revascularization surgery is a common postoperative presentation and the long-term clinical outcome after myocardial revascularization surgery is largely dependent on graft patency and the progression of coronary artery disease. Therefore, assessment of the status of the grafts and graft disease after CABG surgery is an important issue in cardiology. Although conventional coronary angiography is still standard method for assessment of the status of naïve and recipient vessels after CABG surgery, it is an invasive and costly procedure that is not risk-free. Recently, multidetector row computed tomography (MDCT) with retrospective electrocardiographic (ECG) gating has gained rapid acceptance as a diagnostic cardiac imaging modality, allowing assessment of coronary bypass graft patency with high spatial resolution. Initial assessment of bypass grafts was done with single-slice scanners and electron-beam CT. Subsequently, the addition of electrocardiographic ECG gating and the improved capabilities available with 4- or 16-slice MDCT scanners for rapid scanning of the area of interest led to promising results in the imaging of bypass grafts (Marano et al., 2005; Ueyama et al., 1999). Recently, the introduction of 64-slice MDCT permitted improved temporal resolution (94 to 200 msec) and spatial resolution (upto submillimeter) and reduction of both cardiac and respiratory motion, leading to improved assessment of graft stenosis and occlusion (Frazier et al., 2005; Lee et al., 2010). Moreover, 3-dimensional (3D) image processing and advanced volumetric visualization techniques now allow radiologists and cardiologists to evaluate coronary grafts in multiple planes using various projections. With the capability of acquiring 3D data volumes along with its tomographic nature, it shares many of the advantages of intravascular ultrasound and thus has the potential to enhance the practice of percutaneous

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coronary intervention (PCI) in the catheterization laboratory by providing data which was difficult to obtain by invasive coronary angiography (Dikkers et al., 2007; Vembar et al., 2003). Recent studies using 64-slice MDCT have reported sensitivity and specificity values of 95% to 100% and 93% to 100%, respectively, for graft occlusion and high-grade stenosis with > 50% luminal narrowing (Table 1). Since naïve coronary arteries and coronary grafts are small vessels, 2 to 4 mm in diameter, and are characterized by both complex anatomy and continuous movements, high spatial and temporal resolutions are mandatory to visualize these vessels at MDCT. MDCT scanners characterized by submillimeter spatial resolution and a temporal resolution of 94 to 200 ms are now available and are increasingly used for cardiac imaging with promising results.

Authors	MDCT	No. of Patients	No. of Grafts	Sensitivity (%)	Specificity (%)
Achenbach, et al., 1997	Electron-beam	25	56	100	100
Engelmann, et al., 1997	Spiral	49	134	92	97
Marano, et al., 2004	4-MDCT	57	122	89	95
Ropers, et al., 2001	4-MDCT	65	182	92	95
Anders, et al., 2006	16-MDCT	32	94	100	98
Schlosser, et al., 2004	16-MDCT	48	131	96	95
Dikkers, et al., 2007	64-MDCT	34	69	100	99
Jabara, et al., 2007	64-MDCT	50	147	95	100
Nazeri, et al., 2009	64-MDCT	89	287	98	97
Ropers, et al., 2006	64-MDCT	50	138	100	94
Tochii, et al., 2010	64-MDCT	19	90	100	93

High-grade stenosis is defined as 50-99% stenosis

Table 1. Results of studies of the use of MDCT to evaluate occlusion and high-grade stenosis of grafts

2. Imaging acquisition

2.1 Image protocol

There are a variety of protocols for image acquisition in the evaluation of patients after CABG surgery. In many respects, the protocol is similar to that for coronary CT angiography (CTA). One important difference is that the scan should be extended superiorly to include the origins of the internal mammary arteries. Scanning is performed with the patient in the supine position, during breath-hold. After placement of the leads for ECG recording on the chest wall and a check of the heart rate, a noncontrast CT scan image is acquired through the entire thorax in order to define the volume of the subsequent CT angiography and to detect associated or unsuspected findings. Hence, MDCT angiography is performed during ECG recording, from the subclavian arteries to the cardiac base; in patients with venous grafts, a smaller scanning volume starting from the lower third of the ascending aorta is usually sufficient. On the contrary, when a right gastroepiploic artery (RGEA) has been used, the scanning volume should include the upper abdomen. Cardiac CTA technique requires rapid injection of nonionic, iodinated, low-osmolar intravenous

contrast. A bolus of 100 to 120 mL nonionic contrast material (high iodine concentration is recommended) is administered intravenously using an automatic injector at a flow rate of 3 to 4 mL/s. A region of interest was placed in the descending aorta by using a preset threshold of 150 HU; a 10-second delay followed before scanning was begun to ensure filling of the distal vessels with contrast material. Since the left internal mammary artery (LIMA) is the most frequently used graft to the anterior cardiac wall, a right arm venous access is preferable in order to avoid streak artifacts from the left subclavian vein that may hamper a complete evaluation of LIMA course and takeoff. Axial images are reconstructed in the mid-to-late diastolic phase, using a fraction (percentage; relative delay) of the R-R interval of the cardiac cycle. Images are acquired with a heart rate < 70 beats per minute, if possible, and with breath-holding during mid-inspiration to prevent substantial inflow of unopacified blood into the right atrium, which may result in heterogeneity of contrast. Low heart rates (< 65 beats/min for 16-slice MDCT or < 70 beats/min for 64-slice MDCT) are recommended to obtain high-quality CT scans, and in the absence of contraindications (heart failure, systolic BP < 100 mm Hg, atrioventricular blockade greater than grade I, and referred adverse reaction), beta-blockers can be administered before CT acquisition (Frazier et al., 2005; Marano et al., 2005). Oral or intravenous beta-adrenergic blocking medications, specifically metoprolol (Lopressor; Novartis Pharmaceuticals Corp., East Hanover, NJ), are administered prior to scanning to prevent heart rate variability and tachycardia. Retrospective ECG-gated CTA is essential for optimal image acquisition and reconstruction of evenly spaced phases of the cardiac cycle. The images are acquired in a limited field of view with axial images centered on the heart. Using 60% to 80% of the R-R interval, with 0.6-0.75 mm thick images reconstructed in 0.4-0.5 mm increments, axial source images, three-dimensional (3D) volume-rendered images, and multiplanar reformatted (MPR) images are generated. Both 3D volume-rendering and MPR images are used to assess the bypass grafts, proximal and/or distal graft anastomoses, and the cardiac anatomy. In particular, curved multiplanar images with centerlines through the bypass grafts and native coronary arteries are obtained. To correctly assess graft patency and/or the presence of significant stenosis and occlusion, a thorough knowledge of CABG anatomy and its configuration on CTA is important for radiologists and cardiologists. There are 2 types of bypass grafts, arterial and venous. Venous grafts are generally larger in caliber than arterial grafts, and for this reason, jointly to the absence of surgical clips along their course, venous grafts are usually better assessable by noninvasive imaging techniques. In order of frequency of use, graft arteries include the internal mammary arteries (IMAs), radial arteries (RAs), right gastroepiploic artery (RGEA), and inferior epigastric artery (IEA). Although arterial grafts have better long-term outcomes, venous grafts, specifically saphenous vein grafts (SVGs), are more readily available. CTA following CABG surgery is done by first assessing the morphology and size of the ascending aorta and the origin of the in situ vessel such as the IMA. Then, graft patency is assessed for homogeneous, contrast-enhanced graft lumen and for regular shape and border of the graft wall. The graft is usually divided into 3 different segments: the origin or proximal anastomosis of the graft, the body of the graft, and the single (or sequential) distal anastomosis. During the CTA evaluation of bypass grafts, the proximal anastomosis is usually better visualized than the distal anastomosis. In cases in which the distal anastomosis is not well evaluated, the bypass graft is usually considered patent as long as contrast is evident within the graft lumen.

2.2 Image noise

The advantages of MDCT are the relatively rapid imaging time and high spatial resolution attributable to the multi-row detector system. Numerous studies dealing with MDCT coronary bypass angiography have reported cardiac and respiratory motion artifacts as the most significant limitations in the reliable assessment of graft patency and stenosis of recipient vessels. It is well known that heart rate greatly influences image quality and stenosis detection. The introduction of 64-slice MDCT scanners, with faster gantry rotation times and shorter breath-hold times, improved diagnostic image quality by reducing cardiac and respiratory motion artifacts. However, optimum performance was observed primarily in patients with heart rates below 70 beats per minute. Even with improved spatial and temporal resolution with 64-slice technology, routine administration of β-blockers is still required. If graft segment image quality is suboptimal due to motion artifacts, a potential remedy is to obtain additional image reconstructions in smaller increments throughout the cardiac cycle. The other limitations of MDCT are the presence of calcification and metal clip artifacts, which make assessment of graft patency difficult, and accurate evaluation of the degree of stenosis impossible. Nevertheless, the thinner slices of 64-slice MDCT give increased temporal resolution, and 3-dimentional reconstructions show consistent detail in every plane. Moreover, bypass grafts are characterized by minor calcification compared to naive vessels, allowing more accurate analysis in most cases. Coronary calcifications and metal clip artifacts still remain a challenging issue with 64-slice cardiac CT despite improvements with the use of sharper image filters, e.g. the B46 Kernel (Siemens Medical Solutions) (Seifarth et al., 2005). The another important limitation is the high radiation dose required for 64-slice MDCT, although electrocardiogram-dependent dose modulation can reduce this by 30%–50%. The minimization of radiation exposure as well as optimization of the diagnostic accuracy in calcified vessels remain the chief goals for future MDCT advances.

3. Type of arterial or vein graft

3.1 Saphenous Vein Graft (SVG)

The SVG was first successfully used in a CABG operation by Sabiston in 1962. Both the benefits and limitations of SVG have been well documented in the literature (Bourassa et al., 1985; Campeau et al., 1983). Saphenous veins are fairly simple to access and harvest from the lower extremities, and they are more versatile and widely available than arterial grafts. In addition, during the intra- and perioperative period, saphenous veins are resistant to spasm versus their arterial counterparts. However, the use of SVG is limited by distortion from varicose and sclerotic disease as well as a higher occurrence of intimal hyperplasia and atherosclerotic changes after exposure to systemic blood pressure, resulting in lower patency rates. Graft occlusion can also occur due to vascular damage during harvesting of the saphenous vein. In a large study, the SVG patency was 88% perioperatively, 81% at 1 year, 75% at 5 years, and 50% at greater than or equal to 15 years (Fitzgibbon et al., 1996). The graft attrition rate between 1 and 6 years after CABG surgery is 1% to 2% per year, and between 6 and 10 years is 4% per year. The great saphenous vein is the vein routinely used for CABG surgery. The proximal anastomosis of the venous graft with the ascending aorta is usually performed cranial to the origin of coronary arteries and as distal as the proximal portion of the aortic arch. The SVG can be sutured directly to the anterior portion of the ascending aorta or attached with an anastomotic device, allowing faster, sutureless

attachment. The device, called the Symmetry Bypass System aortic connector (St Jude Medical, St Paul, Minn), alters the common appearance of the bypass graft by requiring the aortic connector to be anastomosed perpendicularly to the aorta (Mack et al., 2003; Poston et al., 2004). Recent reports have documented the development of significant stenosis and occlusion in 13.7%-15.5% of vein grafts attached with the aortic connector (Carrel et al., 2003; Wiklund et al., 2002). In order to support the course of the aortovenous anastomosis, the left-sided SVG is connected to the left side of the aorta, stabilizing the graft on top of the main pulmonary artery. A right-sided SVG is attached either to the lower aspect or right side of the ascending aorta, allowing the graft to traverse the right arterio-ventricular groove. SVGs tend to appear as large contrast-filled vessels (Fig.1).

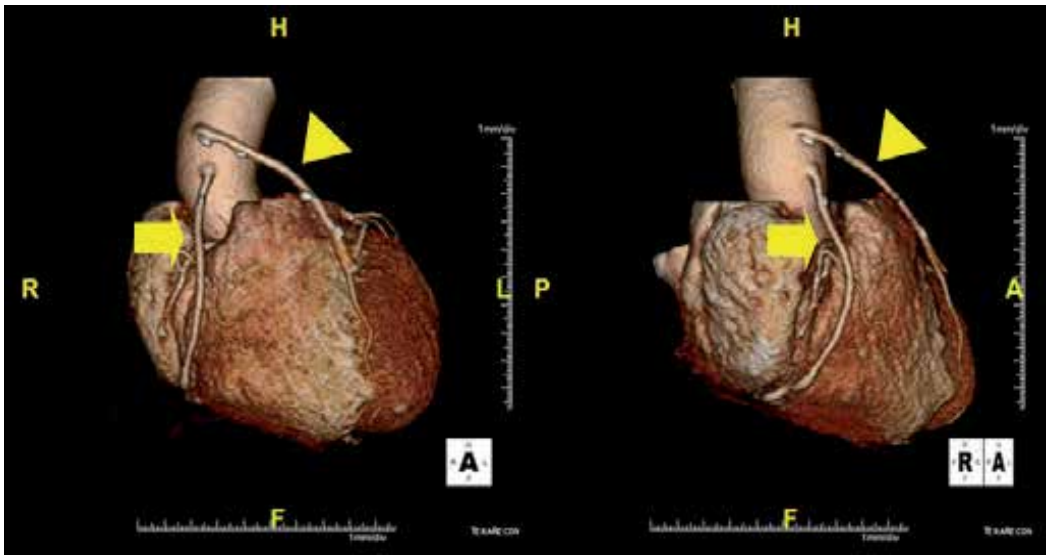

Fig. 1. Saphenous vein grafts. Three-dimensional volume-rendered images show the typical appearance of right (arrow) and left (arrowhead) saphenous vein grafts (SVGs) sutured to the anterior aorta. The left SVG is attached to the mid-portion of left anterior descending (LAD) artery and the right SVG is attached to the distal-portion of right coronary artery (RCA).

An SVG to the right side is attached to the distal right coronary artery (RCA), posterior descending artery (PDA), or distal LAD artery. The distal anastomosis may lie on the phrenic wall of the heart. An SVG to the left side is attached distally to the LAD artery, diagonal artery, left circumflex (LCx) artery, or the obtuse marginal (OM) arteries, by traversing anteriorly and superiorly to the right ventricle outflow tract (RVOT) or main pulmonary artery (Fig. 2, 3, 4).

SVG may present a horizontal or slightly oblique course on axial images, especially when the distal anastomosis is placed on the LCx or a diagonal branch to supply the left cardiac wall. In these cases, the graft can be recognized in the fatty tissue of mediastinum, posterior to the sternum and anterior to the RVOT. On occasion, the distal SVG is anastomosed sequentially to greater than or equal to 2 coronary vessels or in the same vessel, using side-to-side and end-to-side anastomoses. The naive vessel distal to the anastamotic site should be assessed and is recognized by its position and smaller caliber compared with the SVG

(Fig. 3, 4). Typically, venous grafts are larger than arterial grafts and are not accompanied by surgical clips along their course. Sometimes a circumferential clip can be identified at the site of proximal anastomosis with the ascending aorta (Fig.1).

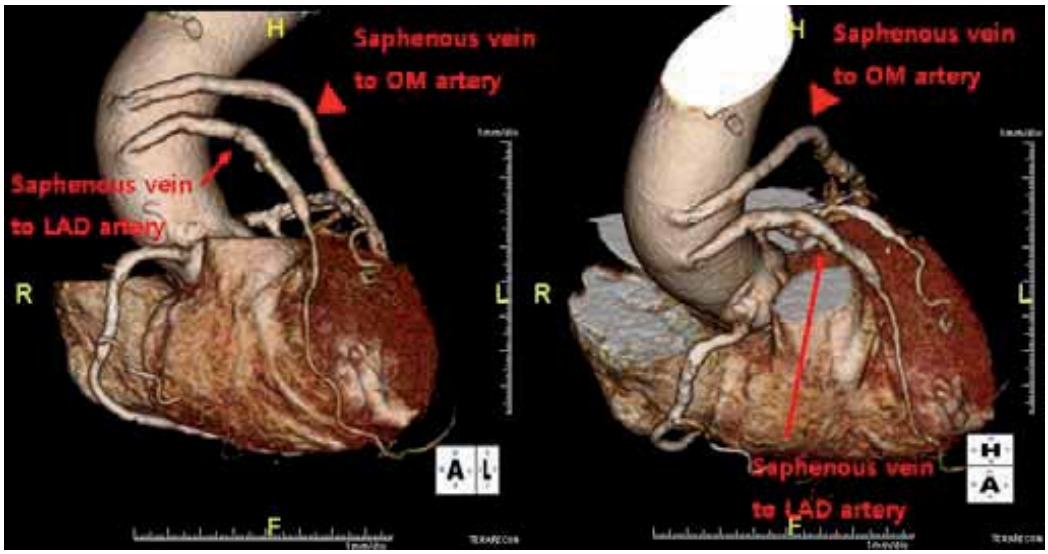

Fig. 2. Saphenous vein grafts. Three-dimensional volume-rendered images show the typical appearance of right (arrow) and left (arrowhead) saphenous vein grafts (SVGs) sutured to the anterior aorta. The right SVG is attached to the mid-portion of left anterior descending (LAD) artery and the left SVG is attached to the obtuse marginal (OM) artery

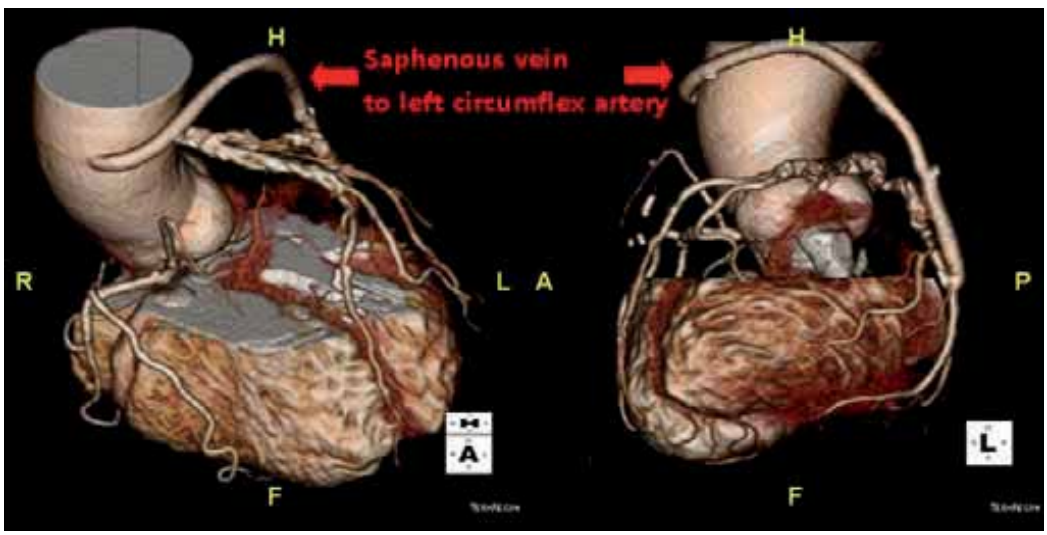

Fig. 3. Saphenous vein graft. Three-dimensional volume-rendered images show the left saphenous vein graft (SVG) with its anastomosis with the left circumflex (LCx) artery.

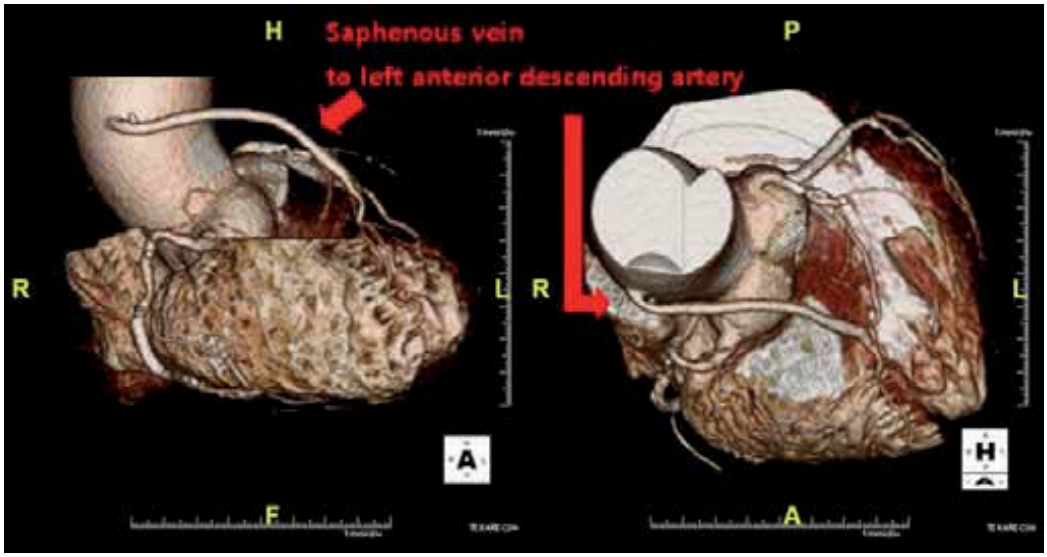

Fig. 4. Saphenous vein graft. Three-dimensional volume-rendered images show the left saphenous vein graft (SVG), which is attached to the mid-portion of left anterior descending (LAD) artery.

3.2 Internal Mammary Artery (IMA)

The internal mammary artery (IMA) is characterized by unique resistance to atherosclerosis and extremely high long-term patency rates compared with the saphenous vein. The IMA has a nonfenestrated internal elastic lamina without vaso vasorum inside the vessel wall, which tends to protect against cellular migration and intimal hyperplasia. Moreover, the medial layer of IMA is thin and poor of muscle cells with poor vasoreactivity. In addition, the endothelium produces vasodilator (nitric oxide) and platelet inhibitor (prostacyclin). Glycosaminoglycan and lipid compositions of IMA result in being less atherogenetic in comparison with venous grafts. Therefore, use of the IMA decreases all postoperative cardiac events and mortality, and is associated with a long-term patency rate well >90% at 10 years (Loop et al., 1986; Motwani & Topol, 1998).

3.2.1 Left IMA

The Left IMA (LIMA) is the vessel of choice for the surgical revascularization of the left anterior descending (LAD) artery for its biological and anatomical characteristiscs, being the conduit more proximal to the LAD artery and the easiest to harvest both in median sternotomy and mini-thoracotomy. Due to anatomical proximity to the LAD artery and favorable patency rates, the left IMA (LIMA) is most commonly used as an in situ graft to revascularize the LAD or diagonal artery, supplying the anterior or anterolateral cardiac wall. The LIMA extends from its origin at the subclavian artery and courses through the anterior mediastinum along the right ventricle outflow tract (RVOT) after being separated surgically from its original position in the left parasternal Region (Fig. 5).

Infrequently, sequential distal anastomoses, with side-to-side and end-to-side anastomoses to the diagonal and LAD arteries, respectively, or involving separate sections of the LAD artery, are performed. On axial images, the LIMA is no longer visible in its usual site, on the

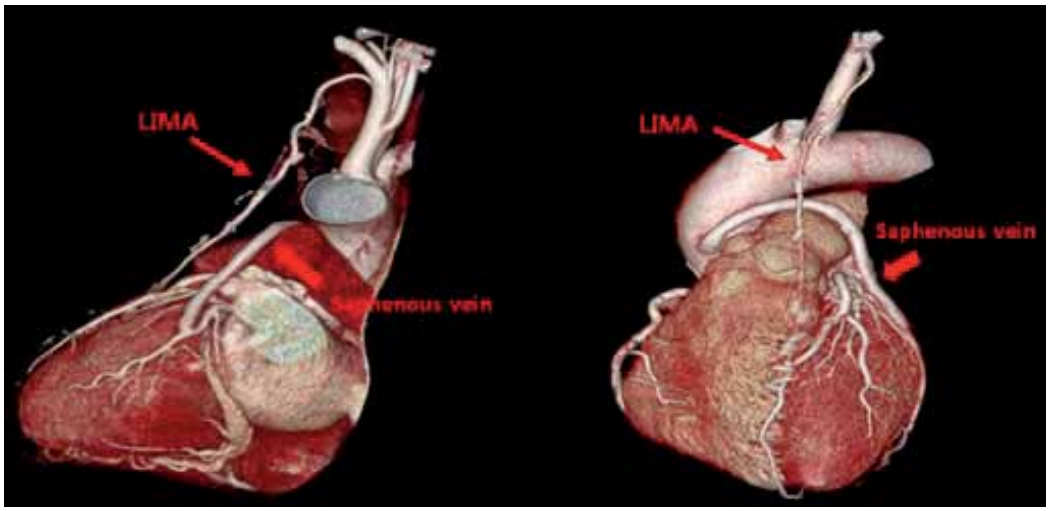

Fig. 5. Left internal mammary artery (IMA) graft. Three-dimensional volume-rendered images show the left IMA graft from its origin at the left subclavian artery to its anastomosis with the left anterior descending (LAD) artery. There is also a left saphenous vein graft (SVG), which is attached to the obtuse marginal (OM) artery. Note the smaller diameter of the arterial graft compared with that of the venous graft.

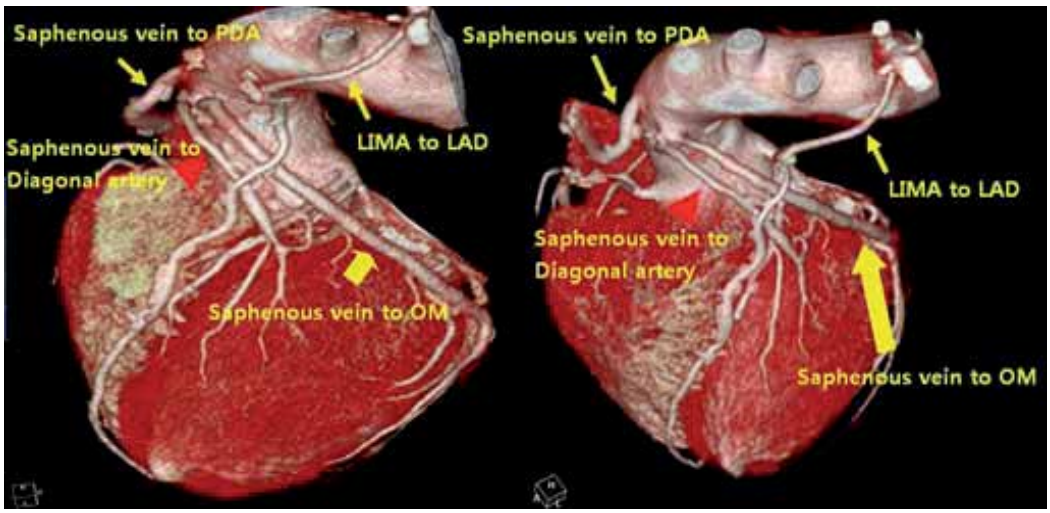

Fig. 6. Left internal mammary artery (IMA) graft. Three-dimensional volume-rendered images show the left IMA graft from its origin at the left subclavian artery to its anastomosis with the left anterior descending (LAD) artery. There is also a right saphenous vein graft (SVG) sutured to the anterior aorta with its anastomosis with the posterior descending artery (PDA). The left saphenous vein grafts (SVG) are attached to diagonal artery and the obtuse marginal (OM) artery.

left side of the sternum, but courses as a small vessel in the anterior mediastinum along the right ventricle outflow tract (RVOT). Although in most cases LIMA grafts show a single distal anastomosis to the left anterior descending artery (LAD) or a diagonal branch, multiple sequential anastomoses to both the LAD and diagonal branches are sometimes performed. Surgical clips are routinely used to occlude collaterals and to avoid arterial bleeding and can be seen either adjacent to the graft or at the original site of the LIMA. As with other grafts, on CTA, the distal anastamosis is typically most difficult to visualize. Surgical clips are used routinely to occlude branch vessels of the IMA, and metallic artifact may limit assessment in some instances (Fig. 6).

3.2.2 Right IMA

The right IMA (RIMA) is used less frequently than the LIMA. The RIMA may be used in a variety of ways. As an in situ graft, The RIMA remains attached to the right subclavian artery proximally and anastomoses with the target coronary artery distally. However, it is more commonly used as "free" graft from the ascending aorta to the RCA or from the LIMA to the left circumflex artery (LCx) or obtuse marginal (OM) branches. In cases in which both in situ IMAs are necessary for revascularization of the left heart, either the RIMA is connected to the LCx artery or OM branches by extension through the transverse sinus of the pericardium and the LIMA is attached to the LAD artery or the RIMA is attached to the LAD artery and the LIMA is anastomosed to the LCx artery or other side branches (OM or diagnonal branches). Otherwise, the RIMA can be removed from the right subclavian artery and used as a composite or free graft. As a segment of a composite graft to perform an arterial "T" or "Y" graft, the RIMA is anastomosed proximally to LIMA, allowing total arterial revascularization instead of using a venous graft with LIMA. As a free graft, a RIMA is anastomosed to the anterior ascending aorta and used in the same way as an SVG. The CTA appearance of the RIMA is similar to that of the LIMA. As already described for LIMA grafts, surgical clips are used to occlude collaterals. Studies have shown that total arterial myocardial revascularzation has the advantages of decreased recurrent angina and superior patency rates at 1 year when compared with those of conventional coronary artery bypass surgery in which a LIMA graft is coupled with an SVG (Muneretto et al., 2003).

3.3 Radial Artery (RA)

The first use of the radial artery (RA) as arterial conduit for coronary revascularization has been de-scribed by Carpentier et al in 1971 (Carpentier et al., 1973). As a muscular artery from the forearm, the RA has a prominent medial layer and elevated vasoreactivity, which results in a lower patency rate than that of IMA grafts (Possati et al., 2003). The RA is usually harvested from the nondominant arm and is used as a third arterial graft, either as a free or composite graft or to avoid using a venous graft in case of unavailability of IMA grafts. The RA is often grafted to supply the left cardiac wall (LCx, OM). On CTA, the caliber of the RA is similar to the IMA, but it typically is visualized coursing from the ascending aorta to the naïve coronary artery (Fig. 7). In the early postoperative period, the RA may be reduced in caliber and may be difficult to identify because of vasospasm. In addition, because the RA is a muscular artery, the number of surgical clips used to close collaterals along the graft is usually higher than with IMA. This may represent a limit for noninvasive assessment of RA grafts with MDCT because of artifacts from surgical clips limiting a full CTA evaluation of an RA graft.

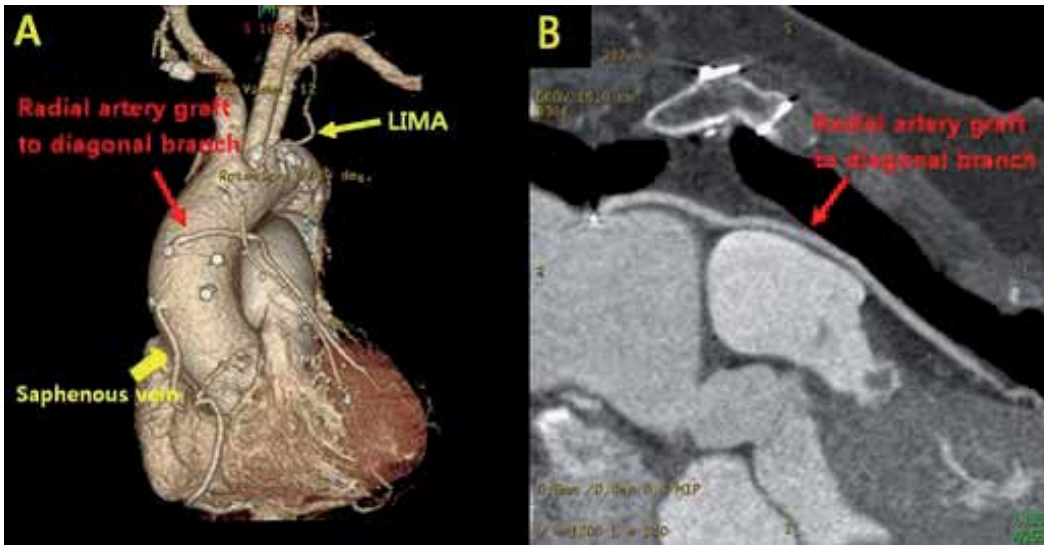

Fig. 7. Radial artery (RA) graft. (A) Three-dimensional volume-rendered image shows radial artery graft sutured to the anterior aorta with its anastomosis with diagonal artery. There are also left internal mammary artery (LIMA) graft from its origin at the left subclavian artery to its anastomosis with the left anterior descending (LAD) artery and right saphenous vein graft (SVG), which is attached to the distal right coronary artery (RCA). Note the diameter of the RA is similar to the IMA, but it typically is visualized coursing from the ascending aorta to the diagonal artery. (B) Curved multiplanar reformation image shows patent RA graft within the anterior mediastinum. The full extent of the graft is seen from the ascending aorta to diagonal artery.

3.4 Right Gastroepiploic Artery (RGEA) and Inferior Epigastric Artery (IEA)

The use of right gastroepiploic and inferior epigastric arteries in CABG procedures has been limited because of the need to extend the median sternotomy to expose the abdominal cavity (Buche et al., 1992; Manapat et al., 1994; Pym et al., 1987). Although the use of these arteries increases surgical time and technical difficulty of the surgery, these arteries can be used as a free graft to perform total arterial revascularization. The use of the RGEA was first described by Pym et al in June 1984 (Pym et al., 1987). Although it has been originally used in reoperation, in the absence of other suitable conduits, RGEA is now used as secondary, tertiary, or quaternary arterial conduit to provide all-arterial revascularization. The biological characteristics of RGEA are similar to IMA, but unclear benefits for third or fourth arterial grafts, the increment of surgery time, and the involvement of an additional body cavity are the main drawbacks limiting the widespread use of this conduit. Occasionally, the RGEA is used to supply the inferior cardiac wall and is anastomosed as an in situ graft to the posterior descending artery (PDA). In these cases, the mobilized artery is seen coursing anterior to the liver and through the diaphragm to reach the site of anastomosis. Small clips can be identified at the original site of the RGEA, near the small curvature of stomach. These instances require that the surgical history be conveyed to the radiologist so the CTA protocol can be modified to include the upper abdomen, because the gastroepiploic artery is freed to course anteriorly to the liver and through the diaphragm to reach the target vessel. The inferior epigastric artery (IEA) is an arterial branch of the abdominal wall, arising from

the external iliac artery and coursing inside the abdominal rectus muscle. Similar to the radial artery (RA), the IEA has a predominant muscular structure, while the limited length of the vessel with an adequate caliber is a constraint to using this vessel only as a lateral branch of a multiple arterial graft.

4. Planning percutaneous intervention (PCI) or repeat surgery of bypass graft

As medical and surgical treatments for coronary artery disease have improved, patients are living longer. Consequently, PCI or second CABG operations have become more common. Sometimes, because the proximal anastomosis of the bypass grafts with the aorta is occluded or severe stenotic, visualization of bypass grafts is difficult or impossible with invasive coronary angiography, and if it is possible, the radiation dose must be increased for visualization of the grafts and naive vessels. MDCT is emerging as a useful tool of mapping the course of bypass grafts before PCI (Dikkers et al., 2007; Hecht & Roubin, 2007; Song et al., 2010). Three-dimentional volume-rendered images with computer software delineate relationships between the aorta, naïve arteries, and bypass grafts. Figure 8 (Song et al., 2010) is an example of successful PCI for occluded coronary artery bypass grafts with the aid of MDCT. A 68-year-old man was admitted to our hospital with severe exertional chest pain of 2 months duration. He had been diagnosed with unstable angina 2 years earlier. At that time, coronary angiography had revealed severe 3-vessel disease with diffuse 50% stenosis from the distal portion of the left main coronary artery to the left anterior descending coronary artery (LAD), diffuse 90% stenosis of the left circumflex artery, and total occlusion of the obtuse marginal (OM) branches and proximal right coronary artery. Subsequently, CABG had been performed using left internal mammary artery to the LAD, saphenous vein for aorta-diagonal-OM1-OM2 grafts, and free right internal mammary artery to the posterior descending coronary artery. In invasive coronary angiography, the left internal mammary artery-to-LAD graft was patent, but no aortic ostium could be seen despite repeated attempts with various catheters and aortography. Because conventional angiography failed to visualize the grafts, we examined the feasibility of a PCI using 64-slice MDCT. The aortic graft anastomosis was totally occluded, but there was no vessel wall calcification. All grafted vessels could be identified. Based on these findings, a successful second-stage PCI was performed. Injury to a preexisting left IMA graft at sternal reentry is a well-recognized risk in second CABG surgery (Fullerton et al., 1994; Gillinov et al., 1999). MDCT is emerging as a useful means of mapping the course of a left IMA graft before repeat surgery (Gilkeson et al., 2003; Ohtsuka et al., 2000). Three-dimensional volume-rendered images delineate relationships between the sternum, ribs, and bypass grafts, thereby minimizing the risk of injury to the graft vessel during surgical reentry. Understanding the sternal proximity of preexisting bypass grafts, as well as normal structures including the aorta, pulmonary artery, and naïve coronary arteries, allows the surgeon to plan an appropriate surgical approach.

5. Complication

5.1 Graft failure

Bypass graft failures are classified either as early or late following CABG surgery. During the early phase, usually within 1 month after CABG surgery, the most common cause of graft failure is thrombosis from platelet dysfunction at the site of focal endothelial damage

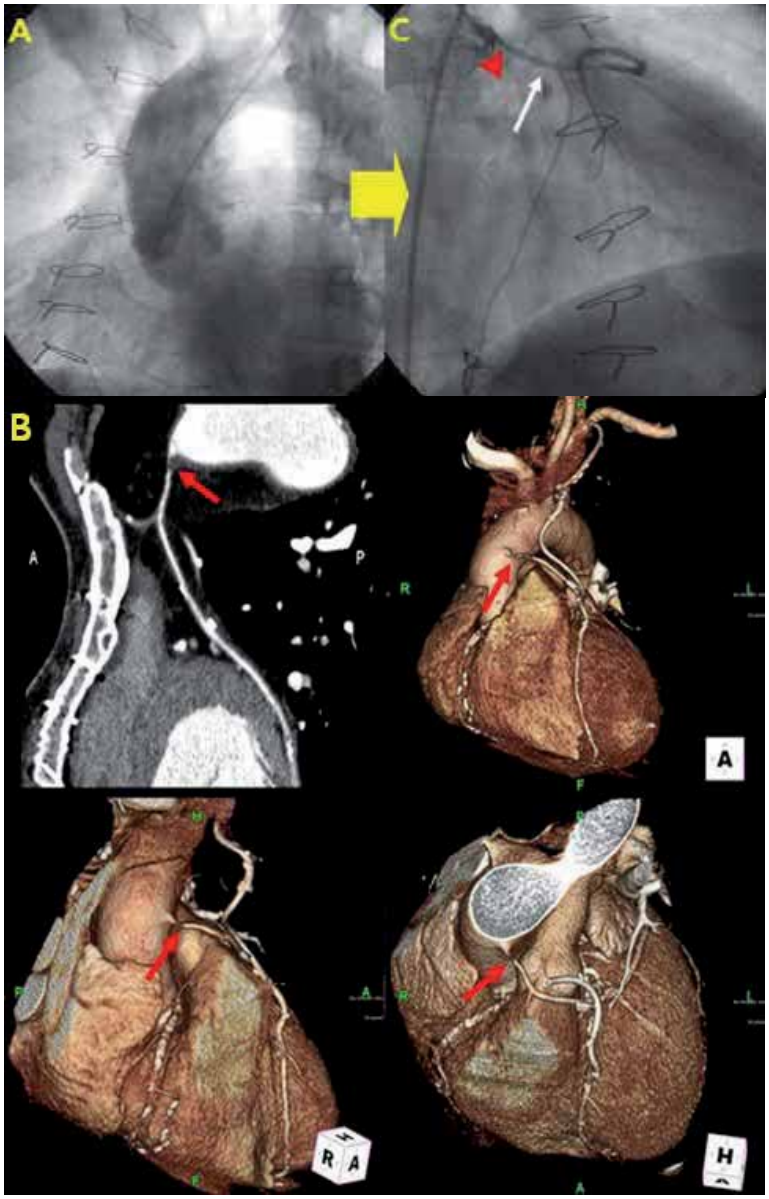

Fig. 8. Coronary artery graft dilatation aided by multidector computed tomography. (A) In invasive coronary angiography, aortic ostium of saphenous vein graft (SVG) could not be seen despite repeated attempts with various catheters and aortography. (B) Curved multiplanar reformation image and three-dimensional volume-rendered image showed the aortic graft anastomosis of saphenous vein graft (SVG) was totally occluded. There was also a patent left internal mammary artery (LIMA) graft from its origin at the left subclavian artery to its anastomosis with the left anterior descending (LAD) artery. (C) Based on these 64-MDCT findings, a second-stage percutaneous coronary intervention (PCI) was performed successfully. Note that 64-MDCT is a useful tool for visualizing bypass graft in a selected group of patients with failed angiography for graft assessment.

during surgical harvesting and anastomosis. Graft closure from thrombosis at 1 month is a recognized complication in 10-15% of cases (Fitzgibbon et al., 1996). Perioperative venous graft failure after off-pump CABG procedures is chiefly determined by the two factors of graft endothelial damage and patient hypercoagulability. Early bypass graft failure can also be due to a malpositioned graft (Ricci et al., 2000). If the graft is too long, it may twist or kink. Technical factors associated with use of an aortic connector may predispose venous

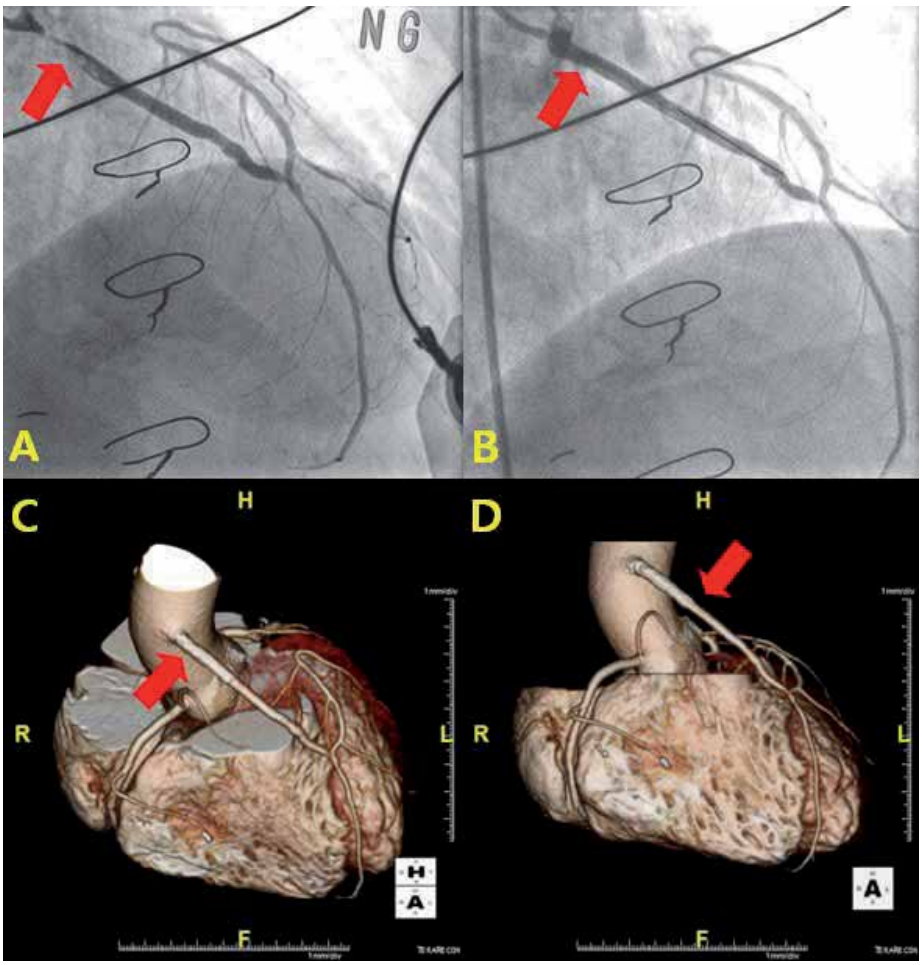

Fig. 9. Acute occlusion of saphenous vein graft (SVG). (A) Invasive coronary angiography showed the proximal-portion of saphenous vein graft (SVG) was severely occluded with thrombi. (B) After successful percutaneous coronary intervention (PCI), invasive coronary angiography showed the proximal-portion of saphenous vein graft (SVG) with stenting was patent. (C, D) At 6-month follow-up, three-dimensional volume-rendered image showed the proximal-portion of saphenous vein graft (SVG) with stenting was still patent. Note that the findings of invasive coronary angiography is similar to those of three-dimensional volume-rendered images of 64-MDCT. The figures also demonstrate that 64-MDCT has a useful role to follow-up of patients who have undergone percutaneous intervention for bypass graft stenosis and occlusion.

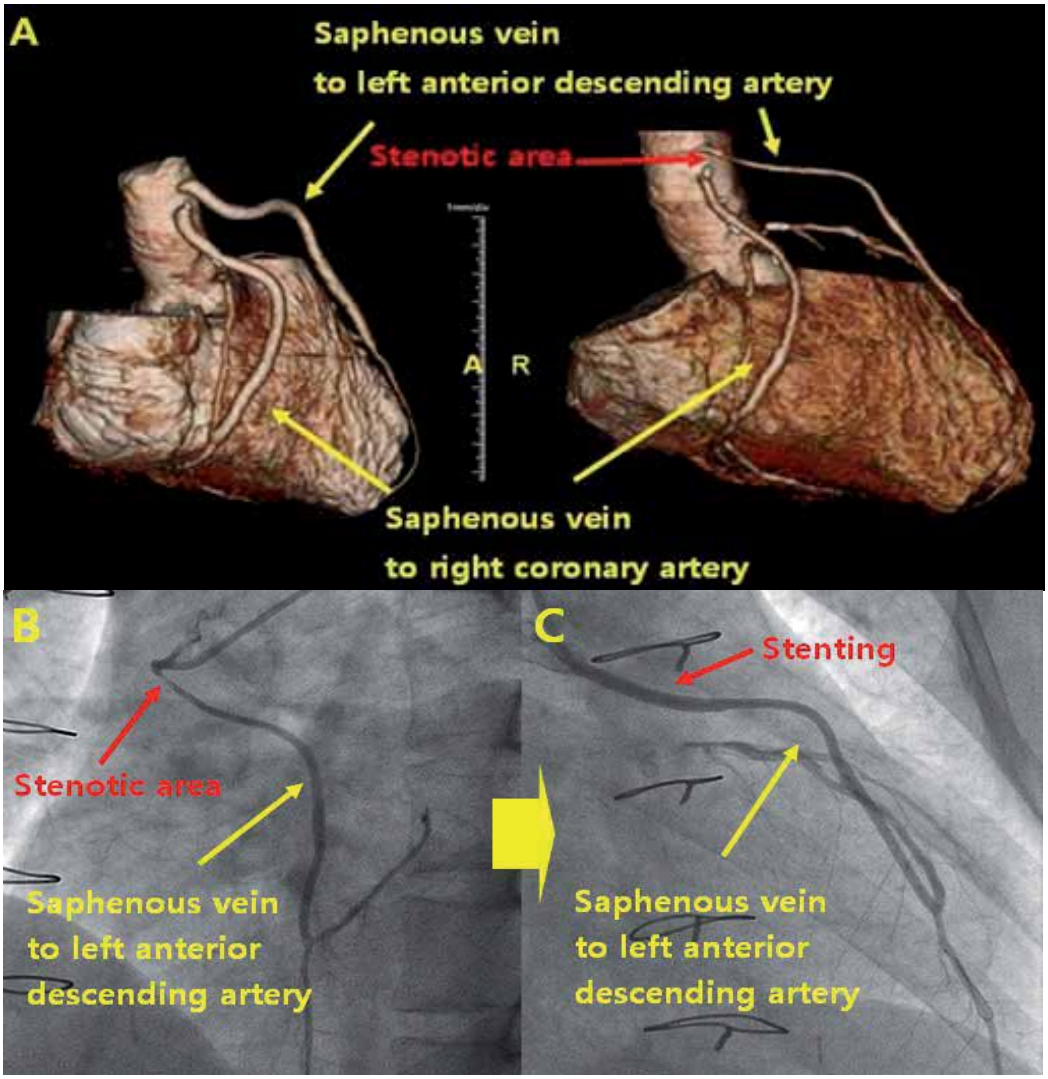

Fig. 10. Chronic occlusion of left saphenous vein graft (SVG). (A, left panel) Immediate after CABG surgery, three-dimensional volume-rendered image showed that left saphenous vein graft (SVG) was patent with its anastomsis to the mid-portion of left anterior descending (LAD) artery. There was a patent right saphenous vein graft (SVG), which was attached to the distal-portion of right coronary artery (RCA). (A, right panel) At 8 months after CABG surgery, three-dimensional volume-rendered image showed severe stenosis at proximal-portion of left saphenous vein graft (SVG), which was attached to the mid-portion of left anterior descending (LAD) artery. Right saphenous vein graft (SVG) was still patent despite the graft had diffuse mild narrowing from proximal anastomosis site to distal anastomosis site. (B) Invasive coronary angiography showed the proximal-portion of left saphenous vein graft (SVG) was severely occluded. (C) Percutaneous coronary intervention was performed successfully. Note that the findings of invasive coronary angiography is similar to those of three-dimensional volume-rendered images of 64-MDCT.

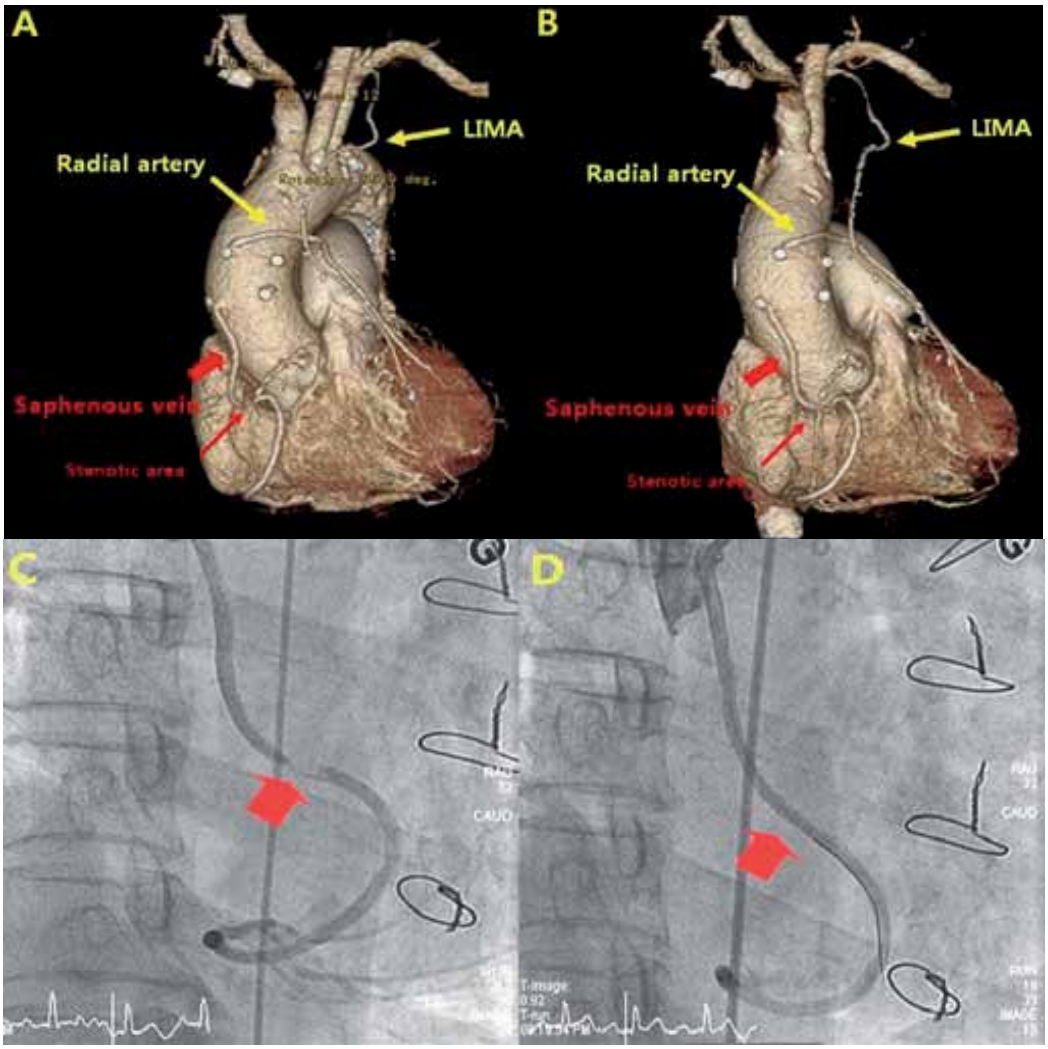

Fig. 11. Chronic occlusion of right saphenous vein graft (SVG). (A, B) Three-dimensional volume-rendered image showed severe stenosis at mid-portion of right saphenous vein (SVG) graft, which is attached to the distal right coronary artery artery (RCA). Radial artery (RA) and left internal mammary artery (LIMA) grafts were patent. (C) Invasive coronary angiography showed the mid-portion of right saphenous vein (SVG) graft was severely occluded. (D) Percutaneous coronary intervention was performed successfully. Note that the findings of invasive coronary angiography is similar to those of three-dimensional volume-rendered images of 64-MDCT.

grafts to kinking (Traverse et al., 2003). Late-phase venous graft failure is due primarily to progressive changes related to systemic blood pressure exposure. One month after surgery, the venous graft starts to undergo neointimal hyperplasia. Although this process does not produce significant stenosis, it is the foundation for later development of graft atheroma. Beyond 1 year, atherosclerosis is the dominant process, resulting in graft stenosis and occlusion. On the other hand, arterial grafts, specifically IMA graft, are resistant to atheroma

development. Late IMA graft failure is more commonly due to progression of atherosclerotic disease in the native coronary artery distal to the graft anastomosis. CTA can delineate multiple findings associated with graft stenosis and occlusion. Calcified and noncalcified atherosclerotic plaque is readily identified, and the calculation of the extent of graft narrowing is straightforward. Occlusion can be determined by non-visualization of a vessel which is known to have been used for surgical grafting. In many instances, the most proximal part of an occluded aortocoronary graft fills with contrast, creating a small out-pouching from the ascending aorta, allowing a diagnosis. Acute or chronic graft occlusion can sometimes be differentiated by the diameter of the bypass graft. In chronic occlusion, the diameter is usually reduced from scarring, as compared with acute occlusion in which the diameter is usually enlarged (Fig. 9, 10, 11).

5.2 Graft vasospasm

Radial artery (RA) grafts are susceptible to vasospasm because the RA is a muscular artery with elevated vasoreactivity. The appearance is similar to fixed graft stenosis, although the luminal narrowing is more extensive in length. Nevertheless, the administration of intraoperative alpha-adrenergic antagonist solution or posteroperative calcium channel blockers can overcome many cases of graft vasospasm postoperatively (Locker et al., 2002; Myers & Fremes, 2003).

5.3 Graft aneurysm

There are 2 types of bypass graft aneurysms: true aneurysms and pseudoaneurysms (Dubois & Vandervoort, 2001; Mohara et al., 1998). True aneuryms are usually found 5 to 7 years after CABG surgery and are related to atherosclerotic disease. On the other hand, pseudoaneuryms more commonly occur within 6 months after surgery, although they may also arise several years later. Pseudoaneurysms arise at either proximal or distal anastomotic sites. Pseudoaneurysm cases that are found earlier may be related to infection or tension at the anastomotic site, resulting in suture rupture. In late-onset pseudoaneurysms, similar to true aneurysms, atherosclerotic changes likely played a role. Currently, there is no clear guideline for surgery. Nevertheless, size >2 cm has been a cause for concern (Memon et al., 2003). Graft aneurysms may lead to various complications, including compression and mass effect on adjacent structures, thrombosis and embolization of the bypass graft leading to an acute coronary event, formation of fistula to the right atrium and ventricle, sudden rupture leading to hemothorax, hemopericardium, or death.

5.4 Pericardial and pleural effusions

Approximately 22%-85% of patients have postoperative pericardial effusions after CABG surgery (Meurin et al., 2004; Pepi et al., 1994). Although pericardial effusions are common, only 0.8%-6% of patients progress to cardiac temponade (Katara et al., 2003). Risk factors include postoperative coagulation abnormality or use of anticoagulation agents that are often related to the use of cardiopulmonary bypass. Nearly all significant pericardial effusions are diagnosed within 5 days postoperatively, peak in 10 days, and resolve within a month (Kuvin et al., 2002). Postoperative pleural effusions are even more numerous after surgery, a prevalence of 89% within 7 days after surgery (Hurlbut et al., 1990; Vargas et al., 1994). These pleural effusions are usually unilateral, small, left-sided, and without clinical

significance. Only 1%-4% of CABG surgery patients proceed to develop clinically significant effusions that require thoracentesis (Peng et al., 1992).

5.5 Sternal infection

The sternal infection is an important complication of the CABG surgery, with a prevalence of 1% to 20% (Roy, 1998). Three different compartments may be affected: the presternal (cellulitis, sinus tracts, and abscess), sternal (osteomyelitis, and dehiscence), or retrosternal (mediastinitis, hematoma, and abscess) compartments (Li & Fishman, 2003). Risk factors include diabetes mellitus, obesity, current cigarette smoking, and steroid therapy. Surgical risk factors include complexity of surgery, type of bone saw used, type of sternal closure, length of surgical time, blood transfusions, and early reexploration to control hemorrhage. The CTA is important in revealing the extent and depth of infection, which, in turn, will help guide treatment planning. Usually, the preservation of mediastinal fat planes in CTA excludes surgical intervention. On the other hand, obliteration of mediastinal fat planes and diffuse soft tissue infiltration without or with gas collection, or low-density fluid collections within the mediastinum, are concerning for sternal infection. Recently published studies reported a 1-year mortality rate of approximately 22% (Loop et al., 1986; Sarr et al., 1984).

5.6 Pulmonary embolism

Clinical diagnosis of deep vein thrombosis and pulmonary embolism may be especially challenging because postoperative atelectasis, pleural effusion, or fluid overload may all contribute to the development of chest pain and dyspnea after CABG surgery. A recent report regarding pulmonary embolism in the post-CABG surgery population showed an overall prevalence of 23% for deep vein thrombosis by 1 week after surgery, with less than 2% of these cases identified clinically (Shammas, 2000).

5.7 Incidental findings

Although the intent of CTA after CABG surgery is to assess bypass graft patency and surgical complications, incidental findings are also frequently detected. In a recent study, 13.1% of patients in the immediate postoperative period had unsuspected noncardiac findings, including pulmonary embolism, pulmonary nodules, pneumonia, mucous plugging, and pneumothorax. (Mueller et al., 2007) Therefore, radiologists need to be aware of clinically significant findings with possible life-threatening consequences.

6. Conclusions

In recent years, MDCT with retrospective ECG gating has gained rapid acceptance as a diagnostic cardiac imaging modality, allowing assessment of coronary bypass graft patency with high spatial resolution. This tool could play an important role in patients with recurrence of chest pain or with unclear stress test results after myocardial revascularization surgery. Furthermore, the newest technological development of the CT scanner could strengthen the role of MDCT before percutaneous intervention of occluded or stenotic grafts, allowing the consensual assessment of occluded or stenotic bypass grafts which are difficult or impossible to be visualized with invasive coronary angiography. Therefore, it is crucial that cardiologists and radiologists understand CABG anatomy with knowledge of the type and number of bypass grafts used during myocardial revascularization surgery.

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Detection Myocardial Bridging Using Non-Invasive Technique

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1. Introduction

Myocardial bridging(MB) is considered as a congenital condition. Usually, coronary artery runs through epicardially. Myocardial bridging occurs when a segment of a coronary artery or its major branch travels intramurally through the myocardium. The myocardium overlying the intramural segment of epicardial coronary artery is called a myocardial bridging, and the artery coursing within the myocardium is called a "tunneled artery". It may dip into the myocardium for varying lengths. The mid portion of left anterior coronary artery has been reported as the most frequent site of myocardial bridges. This phenomenon was first described by Grainicianu in the early 1920s. In 1960, Portmann and Iwig first reported the radiological appearance of transient occlusion in a segment of the left anterior descending coronary artery during systole. A large discrepancy exists between pathological series, in which the incidence has varied from 15% to 85%, and angiographic series, in which it is reported as being between 0.51% and 2.5%.

The clinical significance of MB is controversial. Myocardial bridging has been shown linking to clinical complications that include ischemia, acute coronary syndrome, coronary spasm, arrhythmia, and sudden death, although in the vast majority of cases, myocardial bridging remains clinically silent.

Coronary angiography remains the current gold standard for diagnosing MB. Lower prevalence of myocardial bridging on coronary angiography may partly due to thin bridging. In addition, coronary angiography is an invasive technique with complications and risks. Until now, intravascular ultrasound (IVUS) is the most accurate method to diagnose MB. Intracoronary Doppler ultrasound (ICD) has also been used in the diagnosis of bridging. However, they are all invasive and expensive and not routinely used in clinical settings. Therefore, the need for a non-invasive technique for detection of bridging has emerged. While multi-detector computed tomography (MDCT) angiography is faster and more adequate, it has the ability to assess the course and the anatomic relationships of the coronary arteries. With the advent of high-resolution magnetic resonance imaging and shorter scan time, it will have a bright future for the reason of no contrast and radiation.

In this paper, we will discuss the non-invasive method to detect myocardial bridging in comparison to invasive technique.

2. Prevalence

The prevalence varies substantially among different studies. It was higher at autopsy studies than conventional coronary angiographical studies. The incidence of myocardial bridging among postmortem studies had been reported from 5% to 86%[1-11]. However, the prevalence of myocardial bridging among patients with conventional coronary angiography varied from 0.5% to 33%[12-27]. The discrepancy may be partly due to that the compression during systole is little and lack of provocation with nitroglycerin at the time of angiography. In a large cohort study of Chinese patients, myocardial bridging is up to 16.1% after intracoronary nitroglycerin [27]. It was reported that the incidence of myocardial bridging may be as high as 40% in patients with coronary angiography when positive inotropic medication but not nitroglycerin is used as prevocational agent[23]. Another reason may be partly due to that myocardial bridging does not always induce dynamic compression at conventional angiography. In that case, it is difficult to unmask the myocardial bridging at conventional angiography [28].

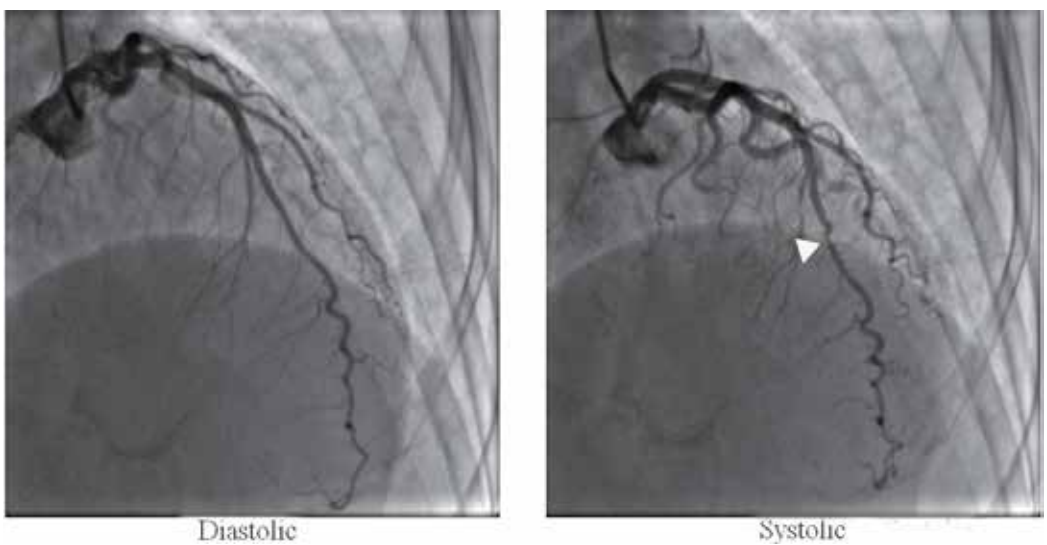

Fig. 1. One case of myocardial bridging on conventional angiography. Compression of coronary artery during systolic occurs at the middle segment of left anterior descending artery(white arrow) and relaxation at diastolic phase.

It was reported that myocardial bridging is higher in patients with heart transplant recipients. In 33% of 64 heart transplant patients, myocardial bridging is detected. The higher incidence might be related increased stiffness and hypertrophy of myocardium[20]. At the circumstance of myocardial hypertrophy, vigorous contraction facilities the detection of myocardial bridging. A higher prevalence of myocardial bridging had also been reported in patients with hypertrophy. Multiple sites of myocardial bridging may be occurred in patients with hypertrophic obstructive cardiomyopathy[29].

Most of myocardial bridging is occurred at the site of LAD. However, myocardial bridging of right coronary and left circumflex is reported not only at postmortem studies but also conventional angiographic studies[30]. The incidence of myocardial bridging at the site of LAD shows no difference at different age and sex[12].

3. Classification of myocardial bridging

At postmortem studies, Ferreira et al distinguished between two types of bridging: (1) superficial bridges (75% of cases) crossing the artery perpendicularly or at an acute angle toward the apex, and (2) muscle bundles arising from the right ventricular apical trabeculae (25% of cases) that cross the LAD transversely, obliquely, or helically before terminating in the interventricular septum[8].

Superficial or deep myocardial bridges form in utero at the time of cardiac development. In most individuals they do not cause symptoms but particularly in those with deep myocardial bridges, the anatomical relation of the myocardial fibers can distort the artery that can be identified angiographically. The possibility of myocardial bridges should be borne in mind in individuals with ischemia but no evidence of coronary atherosclerosis.

Based on conventional angiography, myocardial bridging is classified as three groups according to the percentage of systolic compression of left anterior descending coronary artery. Systolic compression less than 50% was classified as group I. Systolic compression between 50% and 70% represented group II. Patients with systolic compression≥70% represented group III. [12].

Schwarz proposed a new MB classification for symptomatic patients with MB. Three types of myocardial bridging were divided. Type A indicates patients with MB with no objective signs of ischemia. MB was detected occasionally. Type B indicates patients with MB with objective signs of ischemia. Type C indicates patients with or without objective signs of ischemia and altered intracoronary hemodynamics (by QCA/CFR/intracoronary Doppler). The classification is of clinical importance since 5-year follow-up showed that type B responded well to beta-blockers or calcium channel antagonists [31].

Based on computed topography angiography(CTA), MB can be classified as three types. Type I is myocardial bridging with partial encasement as LAD being within the interventricular gorge and in direct contact with left ventricular myocardium. Type II is myocardial bridging with full encasement as LAD being surrounded by myocardium but

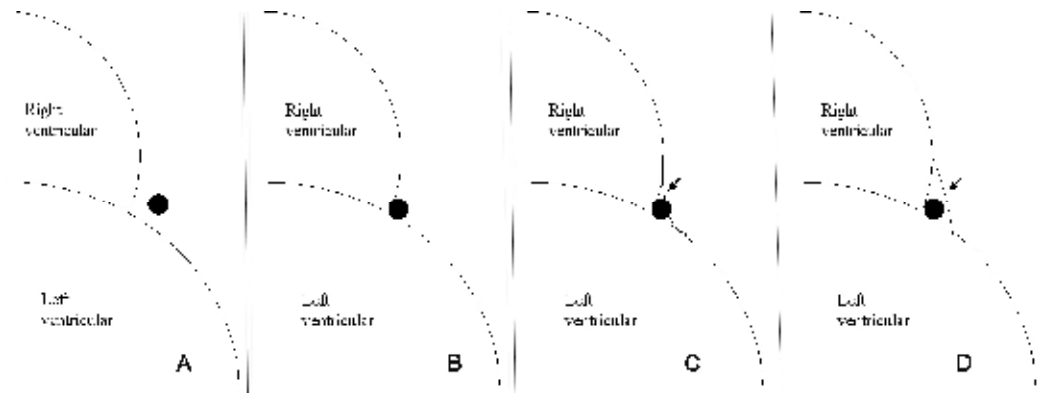

Fig. 2. Schematic illustration of classification of MB at MDCT. At normal condition, coronary runs through the ventricular septal without contacting with myocardium(A). When the artery is closely contacting with septal myocardium without myocardium overlying it, it is classified as partial encasement(B). If there is myocardium overlying the artery, it is called full encasement(C and D, arrow indicates myocardium overlying coronary artery). The full encasement is further classified as overlying myocardium without measurable (less than 0.7mm, C) and measurable type (D) [28].

without measurable overlying myocardium. Type III is myocardial bridging with full encasement as LAD being surrounded by myocardium but with measurable overlying myocardium (>0.7mm) [28](Figure 2).

4. Modern view of MB

Usually, dynamic compression of myocardial bridging is caused by myocardium overlying tunneled artery. During systolic phase, contraction of myocardium induces stenosis of coronary artery, while coronary artery turns to normal because of the relaxation of myocardium during diastolic phase. However, recent study by Kim et al raised some new features on MB. They demonstrated that systolic compression does not always occur in segments with overlying muscle. Dynamic compression affects only one third of patients with overlying myocardium. The length of dynamic compression was longer than that of tunneled segment. It suggested that coronary artery entrapped within the interventricular gorge is the mechanism of dynamic compression [28].

5. Mechanisms for atherosclerosis and ischemia

Myocardial bridging is usually considered as a benign condition since coronary flow is maximal during diastole but not systole. However, frame by frame analysis of myocardial bridging at intrvascular ultrasound (IVUS), which half moon phenomenon is seen as new characteristic at IVUS, showed delayed relaxation during early diastole [32]. At the same time, studies with intracoronary Doppler in patients with myocardial bridging showed that a peak of high flow velocity was detected in early diastole [33-34]. The reason for this hemodynamic disturbance may be related to the pressure gradient between the proximal and distal segment of myocardial bridging. The lower shear stress may contribute to atherosclerosis at proximal segment of myocardial bridging, whereas higher shear stress may protect it from atherosclerosis at the tunneled segment of myocardial bridging, which is of interest to study [35].

Also, those high pressure gradients ultimately increase local wall tension and subsequent endothelial dysfunction and atherosclerosis formation at the proximal segment. The extent of atherosclerosis is higher in the proximal segment of myocardial bridging than the tunneled segment is attributed to the higher expression of eNOS, ET-1 and ACE [36]. Recent study showed that properties of the MB enhance the development of atherosclerosis in the LAD proximal to the MB, resulting in MI[37]. Studies by MDCT showed that length and thickness of MB as well as MB location are associated with the formation of culprit lesions of LAD proximal to MB in MI. In myocardial infarction (MI) patients with culprit lesions in the LAD proximal to MB, MB length, MB thickness, and index of the length multiplied by thickness of MB were significantly greater than non-culprit group. The distance from the orifice of the left main trunk to MB entrance was anatomical significantly shorter [38]. It further supports the notion that anatomatical properties of MB are associated with atherosclerosis at proximal segment of MB.

It demonstrated that coronary atherosclerosis is more pronounced and extended up toward to the coronary ostium. It suggests that myocardial bridging enhances the predisposition to coronary atherosclerosis and myocardial infarction. This novel anatomic risk factor for coronary atherosclerosis and myocardial infarction changes previous opinion that myocardial bridging is considered as a benign condition. Future studies must demonstrate not only the presence of bridging but also the disease stage and the presence of signs of

complicated lesions, which may be an even more pronounced hint of the anatomic risk factor for a acute coronary events.[39]
Ischemia induced by myocardial bridging is related to several reasons. In addition to systolic compression and delayed diastolic relaxation, vasospasm of coronary artery may be another mechanism. The considerable delay in blood flow and reduced distal coronary pressure are presumed to impair coronary vasodilator reserve, which may induce ischemia in patients with myocardial bridging. Another reason for coronary ischemia may be related to coronary spasm, of which is stimulated by endothelial dysfunction of coronary artery. Coronary angina even myocardial infarction may occur in this situation.

6. Detection of myocardial bridging using non-invasive method

Multi-detector computed tomography (MDCT)

Technique

The heart is moving fast during computed tomography imaging. So it requires a higher temporal resolution and higher spatial resolution for visualization of coronary artery as well as myocardial bridging. Despite improvement of imaging acquisition of coronary artery, there is still some gap between coronary CT angiography and invasive coronary angiography. At normal condition, temporal resolution of MDCT((4-MDCT, 250 milliseconds; 16-MDCT, 183–250 milliseconds; 64-MDCT, 165–210 milliseconds) is much more lower than that of invasive coronary angiography(<10 milliseconds). However, with dual source CT, the temporal resolution can be achieved less than 100 milliseconds, which eliminates the need for cardiac control before coronary CT angiography with beta-blockers. When beta-blockers is used before imaging acquisition, it may reduce the systole compression and decrease the detection rate of myocardial bridging. Furthermore, the use of dual source CT may manifest the milking effect, as shown in invasive coronary angiography. [40]

7. Myocardial bridging on MDCT

16-MDCT

Although conventional coronary angiography is considered as golden standard for detecting myocardial bridging, other techniques are also used at clinical, including intravascular ultrasound and Doppler. However, they are all invasive method with much trauma and not cost-effectiveness. With the advance of multi-detector row spiral computed tomography(MDCT) and multiplanar reconstruction, coronary artery disease is possible to be visualized accurately and non-invasively.
At the beginning, myocardial bridging is estimated about 22(3.5%) of the unselected 626 patients with 16-MDCT scanners, 15(2.4%) at the middle segment of LAD, 5(0.8%) at the distal segment of LAD and 2(0.3%) at proximal segment of LAD. The length and depth of myocardial bridging is between 6 and 22 mm (mean, 17mm) and between 1.2 and 3.3 mm (mean, 2.5 mm). [41] In their study, they first examine axial resource and the multiplanar reconstructions for all patients. Myocardial bridging is diagnosed if coronary vessel coursing in the muscle or getting closer to the septum. By means of changing the window width and level, the muscle fibers overlying the coronary and the narrowing of the vessel at this area can be analyzed. Compared with detection of myocardial bridging with coronary angiography, MDCT does not require an experienced eye and deep style myocardial bridge. It is more easily to unveil the mask of bridging even a few muscles overlying the tunneled artery based on constructional 3D volume-rendering images. [41]

In another study with 16-row MDCT of 148 patients with coronary heart disease, 23 patients (15.8%) with myocardial bridging is detected over 1.0 mm in thickness: 21 (87.5%) were located in LAD with a mean thickness and length of 1.8±0.7 and 20.0±8.6 mm. Moreover, although the tunneled segment beneath MB was always free of coronary wall lesions, 79.2% (19/24) of the segments proximal to MB demonstrated coronary wall lesions. Of special significance were three symptomatic MB patients without any atherosclerotic lesion throughout all the coronary arteries. [42]

64-MDCT

In a study, 277 patients studied with 64-slice MDCT for suspected or known coronary atherosclerosis were retrospectively reviewed for myocardial bridging. MB was presented in 82 patients (30%). Bridges were of variable length (<1 cm 58%; 1-2 cm 32%; >2 cm 10%) depth (superficial 69%, intramyocardial 31%) and frequently localized in the mid-distal segment of the left anterior descending artery (95%). Coronary segments proximal to the bridge showed no atherosclerotic disease (33%), positive remodeling (27%), <50% stenosis (20%) or >50% stenosis (20%). In this study, 12 non-calcified, 32 mixed and 17 calcified plaques were identified. The distal segments were significantly less affected ($p<0.0001$). [43]

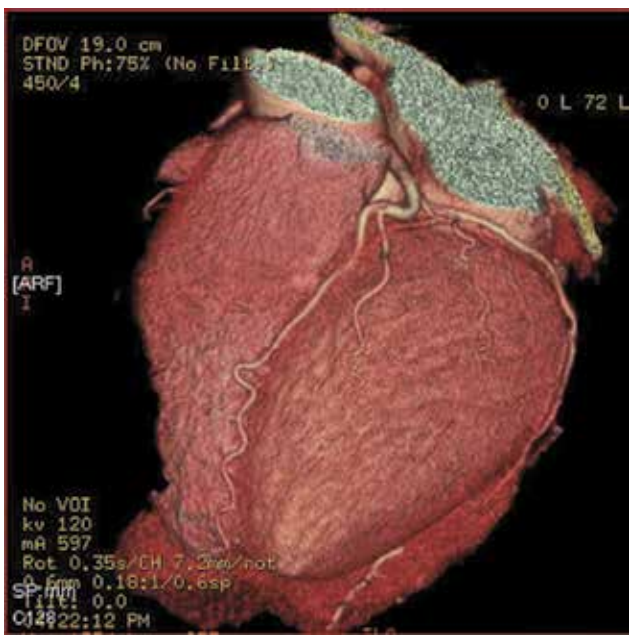

Fig. 3. MB at 64-MDCT. It clearly demonstrates that coronary artery coursing through myocardium at the middle segment of LAD.

128-MDCT

Lazoura et al evaluates the prevalence, length, depth, and location of myocardial bridging of the coronary arteries using 128-MDCT. 875 patients were enrolled in this study. 184 subjects (21%) were found to a single myocardial bridge, including complete bridging in 161 patients (18.4%) and incomplete bridging in 23 patients (2.6%). Most of MB were at the middle segment of LAD(67.9%). The mean length and maximum myocardial thickness overlying

the complete bridging were 20.9 mm (range 8–32mm) and 2.6 mm (range 1.2–5.3 mm), respectively. The mean length of the incomplete bridging was 17 mm (range 9–2.3 mm). [44]

Dual source MDCT

Dual source MDCT has fewer imaging time and better resolution for detecting myocardial bridging and is not influenced by heart rate. The results by Hwang et al showed that 536 patients of the 1,275 patients (42%) were found with MB in this study. Superficial MB was observed in 368 of 557 (66%) cases, and deep MB was seen in 189 of 557 (34%) cases. Superficial MB showed 2 types: complete (128 of 368, 35%) and incomplete (240 of 368, 65%).The mean length of a tunneled segment for superficial MB was 16.4 ± 8.6 mm. The mean length and depth of a tunneled segment for deep MB were 27.6 ±12.8 mm and 3.0 ± 1.4 mm, respectively. The incidence of atherosclerotic plaques in a 2-cm-long segment proximal to MB was 16%[45]. Studies evaluate MB using MDCT are illustrated in table 1

Author (Refer.)	Sample size	Frequency (%)	MDCT	Comment
Takamura et al [38]	228	18.9	16	AMI patients
Ou et al [46]	2530	5.4	DSCT	All patients
Kim et al [47]	607	6.42	64	All patients
La Grutta et al[43]	277	30	64	Suspected or known coronary artery disease
Lazoura et al[44]	875	21	128	All patients
Bayrak et al[48]	990	22.5	64	All patients
Liu et al [49]	3011	5.8	64	Coronary heart disease
De Rosa [50]	235	48.7	16	Atypical chest pain
Jacobs [51]	506	10.4	64	All patients
Atar [52]	165	17	64	All patients
Zeina [53]	300	26	16(226),64(64)	All patients
Yang [54]	900	18.6	64	Suspected coronary artery disease
Kawawa[55]	148	15.8	16	Coronary heart disease
Hwang [45]	1275	46	DSCT	All patients
Chen [56]	114	50	64	APH patients
Koşar et al [57]	700	37	64	Suspected or known coronary artery disease
Chen[58]	276	8.7	16	All patients
Kim [28]	300	58	64	Suspected or known coronary artery disease
Jodocy [59]	221	23	64	All patients
Lubarsky[60]	245	44	64	All patients
Konen[61]	118	30.5	64	All patients

DSCT indicates dual source computed tomography; APH: apical hypertrophic cardiomyopathy

Table 1. Illustration of MB at different MDCT studies

8. Comparison of coronary CT angiography and invasive coronary angiography for detection of myocardial bridging

Several studies have explored the effect of MDCT and invasive coronary angiography for detection of myocardial bridging. Leschka et al studied 100 patients (38 women, 62 men; mean age, 63.8 ±11.6 years) who underwent 64-section MDCT and conventional coronary angiography. They found that the depiction rate of MB is greater with 64-section MDCT coronary angiography than with conventional coronary angiography. MB was detected with MDCT in 26 (26%) of 100 patients and with conventional angiography in 12 patients (12%). In 14 patients in whom MB was found at MDCT but not at conventional angiography, length, depth, and systolic compression were significantly lower than in patients in whom both modalities depicted the anomaly. The degree of systolic compression of MB significantly correlates with tunneled segment depth but not length [62].

In another study with 120 patients who underwent MDCT and coronary angiography, 30 patients were observed with MB. The within-MB diameters on MDCT-CTA and coronary angiography showed a significant correlation during systolic and diastolic phases. In case of MB, segments with sufficient systolic compression (>50%), length of MBs on MDCT and coronary angiography correlated significantly not only at systolic phase but also at diastolic phase [63].

Kim et al in a study with 300 patients who received 64 section MDCT showed that frequency of MB was 58% which partial encasement of 57 patients and full encasement of 117 paxents, while only 40 patients (13.3%; partial encasement in 1 patient and full encasement in 39) demonstrated dynamic compression at conventional angiography. The length of the dynamic compression was considerably longer than the respective tunneled segment in all patients. Total length correlated with the dynamic compression, but depth did not. The higher prevalence of MB on MDCT is considered to the inclusion of partial and full encasement on CTA, the use of short-axis images obtained perpendicular to the long axis of the LAD for all analysis and measurement, the consistently high image quality of CTA with 64-section CT, observation of a single artery (LAD) with a specific purpose and the convenience of their system for reviewers[28].

9. Comparison of myocardial bridging detection by MDCT with intravascular ultrasound

The intravascular ultrasound has also been used to study MB. Instead of systolic compression, “half-moon” phenomenon has been demonstrated. It is specific for the existence of MB.[33,64]Invasive intravascular ultrasound (IVUS) is considered as the most accurate method for detecting MB under current situation. Data from our study showed that comparing with IVUS, the sensitivity of detection by MSCT was 93% and specificity was 100%. Minimal and maximal diameters of MB derived from MSCT were significantly smaller than those from IVUS [65]. MSCT offers a reliable non-invasive method for MB in LAD and atherosclerosis diagnosis with diagnostic accuracy comparable with invasive IVUS.

From studies above, it can be concluded that prevalence of MB on MDCT is relatively high than we have thought to. The specificity and sensitivity on MDCT is higher than that on conventional angiography and is nearly equal to IVUS, which is thought to be golden

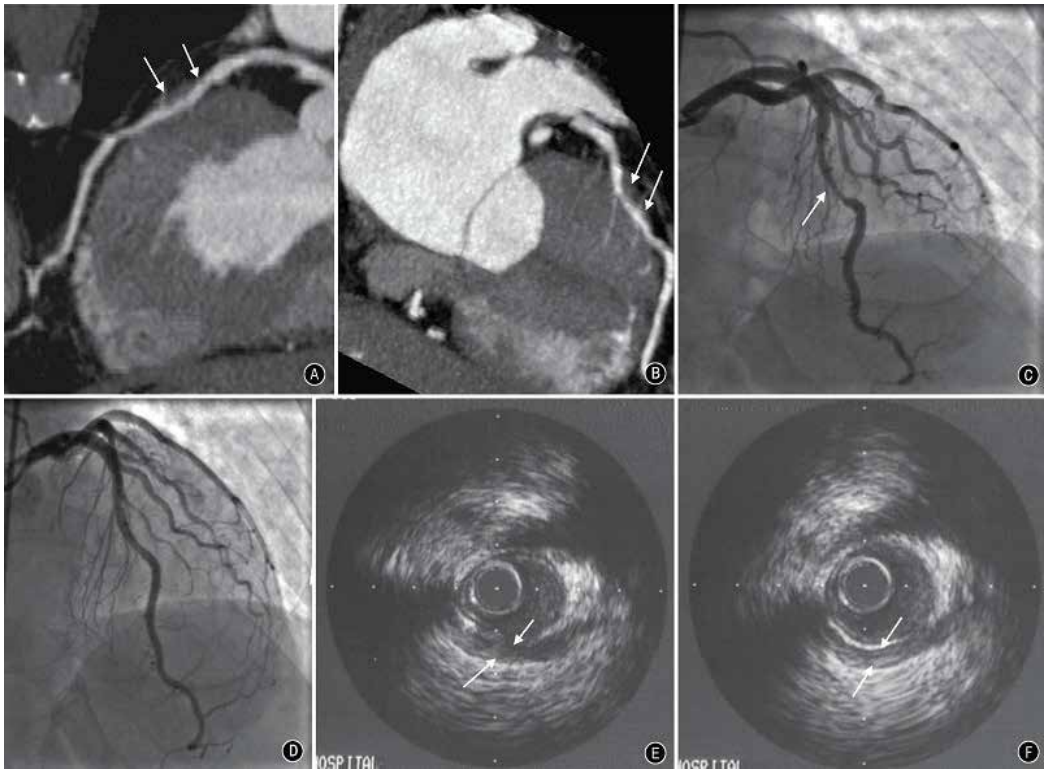

Fig. 4. MDCT,CAG and IVUS images in a patient with MB. MB at the middle segment of LAD is showed in MDCT image (A and B). Myocardium over tunneled artery is clearly demonstrated (arrows). The compression segment is much longer than the tunneled artery which suggests compression occurs not only at the segment with overlying myocardium. Coronary angiography clearly demonstrated the milking effect during systolic phase which is relaxed during diastolic phase (C and D). "Half moon" phenomenon is seen at IVUS both systole (E, arrows) and diastole (F, arrows)[65]. (Reprinted with permission of the author)

standard. The lower rate of MB on coronary angiography may be due to less compression during systole, shorter length of MB, course of the tunnel segment. Not only MB can be assessed on MDCT, but also the atherosclerosis progress at the proximal segment of MB can be evaluated on MDCT during follow up. In the future, MDCT studies will need to evaluate the natural course of coronary atherosclerosis in those with and without myocardial bridging and to demonstrate whether the congenital variant is clearly a anatomic risk factor for myocardial infarction, possibly related to earlier onset and manifestation of coronary artery disease [39].

10. Detection of MB with magnetic resonance imaging (MR)

Substantial progress has been made since first study of visualizing coronary arteries in the late 1980s. [66-67]

The advantage of without contrast and radiation with MR makes it preferable for younger athletes and renal insufficient patients to identify possible coronary artery disease as well as

MB. In addition, calcified lesions at MDCT images show positive diagnosis or cannot be clearly demonstrated for the artifact from calcification in many cases. Recent study by Liu et al showed that detection of calcified lesion of coronary artery by MR is better than that by MDCT [68].

At the beginning, a spoiled gradient-echo sequence at 3-dimensional coronary MR was used for detection of coronary artery disease [69]. Later, steady-state free precession (SSFP) imaging [70-71] was used to gain better image results. The major problems affecting coronary MR imaging are the long time scanning and artifacts induced by motion instability during long time scan. With the improvement in parallel processing, multichannel receiver coils and wide use of 3.0-T MR, it will be a promising method for assessing coronary artery disease as well as MB. However, artifact was much more common with the use of SSFP sequence when 3.0-T MR was applied to evaluate coronary artery. A spoiled gradient echo technique has gained much attention for better image quality and less artifacts.[72] Nowadays, the diagnostic accuracy of the coronary MRA technique to detect a patient with a 50% stenosis demonstrated a sensitivity of 88.7%, a specificity of 82.1%, a positive predictive value of 86.5%, and a negative predictive value of 92%.[73]

Recent study showed that coronary artery with diameter larger than 1.5mm could be clearly assessed with 3.0 T MR, which in previous studies, only coronary artery with diameters larger than 2.0mm be clearly evaluated.[74] In this circumstance, MB at the proximal and middle segment of LAD will be assessed very well.

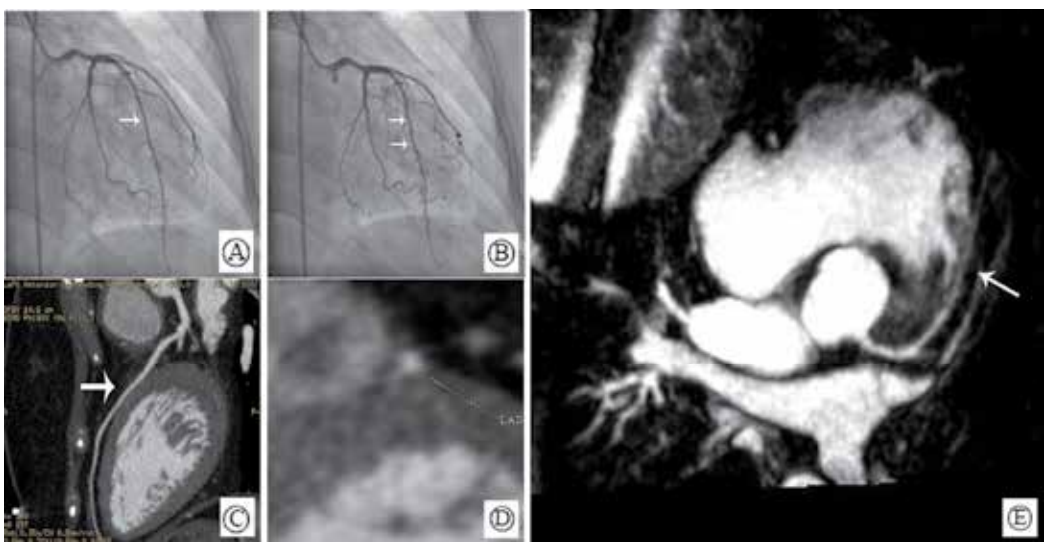

Fig. 5. CAG, MDCT and MR images in a patient with MB. MB at the middle segment of LAD is showed in coronary angiography image. A milking effect is seen during systolic phase(B, arrows) which is relaxed during diastole (A, arrow). Myocardium overlying tunneled artery is not demonstrated at MDCT (C, arrow and D). The compression segment at coronary angiography is longer that tunneled artery at MDCT. MB at the middle segment of LAD and compression of MB are showed at MR image (E, arrow). A soft plaque was demonstrated at proximal segment of LAD at MDCT image (C).

However, until now, there is not a study to evaluate the prevalence of MB at coronary MR. It may be due to about one third of the patients can not complete the coronary MR imaging or the inability to evaluate all the coronary arteries in all patients [73]. In the further, more sensitive technique must be used in clinical for better image quality and shorter time scan. A pilot study should be prepared to assess the prevalence of MB and atherosclerosis progress induced by MB.

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11

When Cardiac Computed Tomography Becomes the Gold Standard Technique to Evaluate Coronary Artery Disease Patients

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1. Introduction

Worldwide the health expenditures as a percentage of each national gross domestic product continue to rise. Cardiovascular diseases as part of the noncommunicable disease group, according to the World Health Organization and the most important scientific associations, are growing due to the aging of the population and the increase of cardiovascular risk factors, due to the epidemiologic as well as to the health transition, especially in the developing countries, which account for the majority of the population in the world (Lopez et al., 2006).

In the last few decades we witnessed a proliferation of diagnostic tests to evaluate cardiac heart diseases and in particular coronary artery disease (CAD): exercise stress test, trans-thoracic echocardiography, stress echocardiography, stress single photon emission computed tomography, myocardial perfusion imaging, magnetic resonance, fractional flow reserve, electron beam computed tomography.

Each diagnostic test, which continuously evolves due to technological improvements, proved to have a high sensibility, specificity and good accuracy in identifying symptomatic as well asymptomatic CAD patients.

All these tests are however unable to give us information about the anatomy of the coronary arteries, which is essential to provide a treatment that goes beyond the medical treatment and in particular when cardiac surgery is needed.

In fact catheter angiography or invasive coronary angiography (ICA), since its introduction in the second half of last century, was the only test able to visualize, in vivo, the coronary tree and to provide images of the coronary artery anatomy upon which both cardiologists and surgeons decide if a patient should be revascularized or medically treated.

With time ICA increased its performance due to the improvement in its software and hardware (quantitative coronary angiography, flat panel digital detectors), and due to the introduction of important tools which can be used routinely like intravascular coronary ultrasound, that for the first time visualized, in vivo, the presence of non calcified plaques and vessel's positive remodeling.

There are other interesting tools that can be associated to ICA, but, for the moment, are the armamentarium of some specialized centers and mainly used for research purposes like elastography, spectroscopy, angioscopy, thermography and optical coherence thermography.

All this data places ICA as the "reference" technique or "gold standard" technique to study the anatomy of coronary arteries.
ICA has been widely employed to validate the results obtained with functional procedures, even though the anatomical findings of ICA are also judged by functional tests (Winchester et al., 2010).
These interdependence of validation shows how a technique, even ICA, cannot be considered the unique "gold standard" technique to study CAD patients. In fact clinicians while studying their patients have to consider more than one question (diagnostic question, prognostic question, therapeutic question) and ICA alone is unable to give an exhaustive answer to all these questions (Mark et al., 2010).
This is the reason why we are in search for technologies to evaluate CAD patients and in particular to study the anatomy of their coronary arteries keeping in mind that these new tests have to be feasible, able to compete with ICA in providing accurate information and, last but not least, economically affordable.

2. Cardiac computed tomography

The introduction of multidetector row systems in the field of computed tomography have made imaging of the heart and in particular that of epicardial coronary arteries feasible. The first and most important force behind the rapid growth of cardiac computed tomography (CCT) imaging in less than a decade is the possibility to obtain information of the anatomy of coronary arteries (Mark et al., 2010).
Since its introduction in the last years of the second millennium, CCT, for the moment, is the only real alternative to ICA able to visualize coronary arteries and most interestingly to obtain this information non-invasively.
Both CCT and ICA visualize only the epicardial vessels, which are also the ones that are usually treated by surgical or percutaneous revascularization procedures.
There are also other factors which may indicate the use of CCT instead of ICA.
There is a significant number of patients who perform ICA (10-20% of the patients) who have normal coronary arteries. Moreover not all patients who have a diseased coronary artery at the ICA should undergo catheter based or surgical based revascularization procedures. For a significant number of patients in whom ICA demonstrates the presence of some degree of CAD it is enough to introduce or change the medical treatment associated with life-style modifications. For this wide group of patients having a non-invasive way to visualize coronary arteries would be preferable to the invasive one, and for them ICA cannot be considered the real "gold standard" technique. On the other side there is an unacceptable high percentage (2%-8%) of patients with acute coronary syndromes who are discharged from the emergency department without a correct diagnosis and who may benefit from a CCT examination (Hoffmann et al., 2006).
There are also other disadvantages in ICA procedures that could be avoided if instead CCT is performed.
The first group of disadvantages is related to the so called major adverse events that occur within the first 24 hours after selective coronary angiography procedures although they have a relatively low incidence, which ranges between 0.2 to 0.3% and is related to the invasiveness of the procedure itself. The catheter during its movement inside the cardiovascular system may cause the rupture of an atherosclerotic plaque and a dislodgment of an embolus to the heart, brain or abdomen causing respectively a

myocardial infarction, stroke or intestinal infarction. All these complications can be resolved, if correctly addressed, but sometimes may cause a severe morbidity and even the death of the patient.

There are also the so called minor complications present in all the hemodinamic laboratories, although their incidence is higher in smaller laboratories and with less skilled operators. The incidence of the minor complications is relatively high, roughly between 1% to 2%. Most of these minor complications are related to problems with the peripheral vessels through which catheters are inserted like dissection of the femoral artery, arteriovenous fistulas, groin and retro-peritoneal haematomas which may lead to severe anaemization, prolonged morbidity and may require surgery to be solved (Bluemke et al., 2008).

There are also other important limits that must be considered while using CCT, that the evolving technologies are trying to overcome. One of these limits, still present with the currently available 64 channel systems, is related to patient's heart rate which must be rhythmic and around or less than 60-64 beats per minute. Patients with atrial fibrillation or with a heart rate that can not be reduced pharmacologically with beta-blockers to the rate of 60-64 beats per minute, for the moment, are not eligible to undergo this kind of examination. The introduction of some new tools like the "tube current modulation" and the "step and shoot" procedures and the 128, 256, 320 channel scanners will offer the possibility to study also patients with higher heart rates and with atrial fibrillation, making it possible to image the entire heart not only, as it is now, in a single breath hold, but in a single heartbeat. Moreover performing CCT in patients with high body mass index or with chronic obstructive pulmonary disease may add some additional technical problems (Mark et al., 2010).

Finally it is worth noting that both ICA and CCT use non-ionic contrast medium to visualize coronary artery lumen. For this reason particular attention must be given in allergic patients who may develop anaphylactoid reactions and especially in patients with a pre-existing renal impairment which is the major risk factor for the development of contrast medium-induced nephropathy. Contrast medium-induced nephropathy is associated with adverse outcomes including higher mortality. For these reasons patients before CCT should be screened by noting their baseline serum creatinine, presence of comorbidities and by ensuring adequate hydration before and after contrast exposure (Mark et al., 2010).

3. Cardiac computed tomography and patients with suspected coronary artery disease

The most interesting indication for CCT to the public and physicians is the possibility to evaluate patients with suspected CAD who highly outnumber those who were already diagnosed to have CAD or those who underwent surgical or percutaneous revascularization procedures. The 64 or more channel CCT systems are currently considered adequate to study symptomatic as well as well asymptomatic patients suspected to have CAD (Taylor et al., 2010).

In patients with suspected CAD, CCT non-invasively provides important information about coronary calcium scoring, left ventricular function and coronary artery anatomy. However while calcium scoring can be obtained also with electron beam computed tomography, left ventricular function can be assessed also with echocardiography, magnetic resonance and myocardial perfusion imaging, coronary artery anatomy, besides ICA, can be evaluated only with CCT.

In a recent meta-analysis CCT compared to ICA had an average sensitivity and specificity of 98% and 91% respectively, to detect/exclude significant stenosis on a patient basis (Abdulla et al., 2007).
The majority of the studies performed with CCT has been done to determine its role in the assessment of patients with suspected CAD by comparing it with the "anatomic" test (ICA) or/and "functional" tests like stress echocardiography, single emission computed tomography, myocardial perfusion imaging or fractional flow reserve.
Till now there are no large scale studies to evaluate the predictive value of the results obtained with CCT. Nevertheless while a negative CCT rules out significant CAD (negative predictive value between 98 and 100% in most studies) a positive CCT test does not have a similar high positive predictive value (93% with a range from 64% to 100%) due to the tendency of this test to overestimate disease severity in small, distal and calcified vessels. This is the reason why CCT is considered an "ideal test" to study patients who have a low or intermediate pre-test probability to have hemodynamically relevant coronary artery stenosis. In those with high pre-test probability to have significant coronary stenosis, the clinical benefit of CCT decreases with the increased probability to have a treatment through surgical or percutaneous revascularization procedures (Achenbach, 2006, Schuijf et al., 2011).
In the recent American Appropriate Use Criteria Task Force for CCT, the use of CCT in the detection of CAD in symptomatic as well as asymptomatic patients with low and intermediate pretest probability to have CAD, was pointed to be appropriate (i.e. the test is acceptable and considered a reasonable approach to study the disease and its expected incremental information, combined with clinical judgment exceeds the expected negative consequences by a sufficiently wide margin) with a quite high appropriateness score (between 7 and 8 out of the highest value of 9; an indication to be appropriate had to have a score from 7-9) (Taylor et al., 2010).

3.1 Cardiac computed tomography and invasive coronary angiography

The published literature in which CCT's ability to evaluate coronary artery anatomy, compared to ICA, in patients suspected to have CAD, although applied in high quality studies in selected subjects and interpreted by experts, has a high diagnostic accuracy, but probably may not reflect its real use in the daily clinical practice. In most of the published papers CCT has an overall high sensitivity and specificity both on a per-patient and on a per vessel basis for the presence of more than 50% obstructive lesions.
Invasive coronary angiography in evaluating coronary arteries is still considered the "gold standard" or "reference standard" technique mainly because in more than 50 years experience it provided an extensive evidence of its value in patient management and because it has, for the moment, the highest spatial (<0.16 mm vs approximately 0.4 mm of CCT) and temporal resolution (33 msec. vs 140 to 200 msec. of the recent cardiac computed systems or 83 msec. of the dual source system). It is however worth noting that ICA visualizes coronary arteries by creating only a limited number of 2D coronary luminograms, whereas CCT gives an unlimited number of 2D reformatted images as well as 3D volume-rendered images from transverse reconstructions that can rule out eccentric lesions that are difficult to be studied by traditional invasive angiography (Mark et al., 2010)
Invasive coronary angiography estimates the coronary artery severity as a percent diameter stenosis by comparing the narrowing of the lumen of the vessel with the adjacent segment,

which arbitrarily is considered normal, while it could have a non-visualized, non calcified, eccentric plaque. This visual procedure to estimate lesions has a high degree of intra and inter-observer variability that can be somehow reduced by a computer assisted interpretation (quantitative coronary angiography).
Cardiac computed tomography on the other hand is, and in the future probably will be able to provide more information not currently available from ICA, and this may be the basis for its more extensive use in patients with suspected CAD. Invasive coronary angiography can visualize the first stages of the atherosclerotic process (vessel's positive remodeling and the presence of non calcified plaques) only if it is performed using expensive and specialized tools like intravascular ultrasound and optical coherence thermography. All this information is already available with CCT and helps to understand the atherosclerotic burden of CAD patients "better" than with ICA.
Although CCT provides images of the plaque both internal and external to the lumen helping in having a more precise evaluation of lumen narrowing, its ability in assessing the real vessel stenosis has still some technical limits. In fact in the presence of calcified lesions, especially in small and distal vessels, CCT tends to overestimate lesion severity because of the "blooming effect", which is related to the X-rays not absorbed by the calcified lesion that during image reconstruction causes the lesion to seem bigger than it actually is (Ropers et al., 2006a).
Another important limit of CCT is related to the fact that less than or equal to 5% of patients have a non-valuable scans due to motion artefacts, because the patient cannot follow breathing commands, involuntary motion of the diaphragm or to arrhythmias occurring during the CCT scan. To study the coronary anatomy of these patients ICA must be performed.

3.2 Cardiac computed tomography and functional tests

Although CCT was introduced to provide information regarding the anatomy of coronary arteries as an alternative procedure to ICA, it is also possible to compare its results with those obtained with functional tests. The utility in performing such comparison is to evaluate if CCT has a similar or superior accuracy than functional tests in the characterization of patients with suspected CAD. In doing so it is however necessary to keep in mind that, as with ICA, functional tests are often not concordant with the results of CCT because they consider different parameters. Cardiac computed tomography allows the detection of atherosclerotic plaques, that may be hemodynamically non significant resulting in a negative functional test, while a positive functional test may be present in a patient with normal epicardial coronary arteries, but with diseased coronary microcirculation able to produce clinically significant ischemia.
When CCT was compared to dobutamine stress echocardiography the positive and negative likelihood ratios for dobutamine stress echocardiography were 4.37 and 0.36 compared with 3.5 and 0.11 for coronary computed tomography respectively (Nixdorff et al., 2008).
Several studies have been performed comparing both CCT and stress myocardial perfusion imaging to ICA. Cardiac computed tomography proved to have a higher sensitivity and specificity than myocardial perfusion imaging (respectively 94% vs 81% and 96% vs 78%; Budoff et al., 2007).
When CCT was compared to fractional flow reserve, which is considered the reference standard for hemodynamically significant obstructive disease, it was found that the sensitivity and specificity of CCT for fractional flow reserve-defined hemodynamically significant lesions were 94% and 40% respectively (Gaemperli et al., 2007).

3.3 Cardiac computed tomography and patients with acute chest pain

The performance of CCT was also tested in specific environments such as the emergency department, comparing it with functional tests as well as combined clinical and marker data. The results obtained from these studies show that a negative CCT exam improves the diagnostic accuracy in ruling out subjects with a low risk to have acute coronary syndromes. Goldstein et al. (Goldstein et al., 2007) found that CCT compared to myocardial perfusion imaging in addition to standard care had a reduced diagnostic time (3.4 h vs 15.0 h; $p<0.001$) and lower costs ($1586 vs $1872; $p<0.001$) while no adverse cardiovascular events occurred in either group after 6 months of follow-up. On the other side according to Hadamitzky et al. finding an obstructive CAD on CCT at the emergency department is associated with an odd ratio of 17.3 to have severe cardiac events (cardiac death, myocardial infarction or unstable angina) while in patients without CAD at the CCT the rate of cardiac events is better predicted than with the Framingham risk score (Hadamitzky et al., 2009).

4. Cardiac computed tomography and anomalous coronary arteries

Invasive coronary angiography provides only a 2D view of the coronary arteries and sometimes fails to clearly visualize the relationship between coronary vessels and the surrounding structures. This issue becomes critical when anomalous coronary arteries must be visualized. Moreover, it is not always easy to selectively engage the anomalous coronary vessel, which may lead to the erroneous assumption that the coronary vessel is occluded. In addition, the declining use of pulmonary artery catheters during the routine invasive angiography procedures has made it more difficult to understand the course of the coronary vessels within the heart and discern the anterior versus the posterior direction of the anomalous vessels.

Congenital coronary artery anomalies are rare and occur in 0.17% of the autopsy cases. The incidence of anomalous origin of the coronary vessels is higher in the population of patients referred for ICA (0.6–1.3%). Although anomalous coronary vessels lack clinical significance in the majority of patients, there are some ''malignant'' anomalies that may cause non-fatal or fatal acute myocardial infarction or sudden death, especially in young athletes without atherosclerotic CAD (Angelini et al, 2002). In older patients, both CAD and coronary vessel anomalies may be present and in these cases it is difficult to clarify the exact mechanism of myocardial ischemia.

In the last few years, several studies showed the usefulness of the non-invasive modalities for the detection of coronary vessel anomalies such as magnetic resonance imaging and especially multidetector computed tomography. Magnetic resonance imaging, as it is free from X-radiations would be preferable to multidetector computed tomography, especially for younger patients in whom an anomalous artery origin is suspected, and in those who have a cardiac anomaly which may be associated with coronary vessel anomalies (i.e. tetralogy of Fallot) or in patients who have to be followed for the presence of coronary artery aneurysms like in the Kawasaki disease (Mark et al, 2010).

Although there are multiple published series of patients who underwent comparison of coronary magnetic resonance angiography with ICA with a quite good accuracy, CCT due to its excellent spatial resolution, which allows an excellent detection of the origin of the coronary vessels and visualizes their course within the heart, is the real alternative to invasive angiography (Cademartiri et al., 2007) and sometimes it can be considered the real "gold standard" technique in studying the anomalous coronary arteries. In fact in the recent

American Appropriate Use Criteria Task Force for CCT, the use of CCT in the "assessment of anomalies of coronary arterial and other thoracic arteriovenous vessels" was pointed to be the most appropriate indication (score 9 out of 9) (Taylor et al., 2010).

5. Cardiac computed tomography and cardiac bypass surgery

Another indication for CCT is to follow up patients who performed coronary artery by-pass grafting. This was indeed the first clinical application of CCT. In fact arterial as well venous by-passes are easier to be studied than epicardial coronary arteries because they are bigger vessels (typically 2 to 4 mm), have lower motion and usually not calcified.

By-passes have been evaluated beginning with the 4 slice CCT with good results (Moore et al., 2005) and now with the latest generation of CCT systems their accuracy is very high (Stein et al., 2008).

There are however several difficulties in the assessment of by-passes related to the presence of artifacts caused by metal clips, especially when they are at the level of the distal anastomotic site where there can be found also calcified lesions and the grafts have a greater motion.

Cardiac computed tomography has a good accuracy in assessing graft occlusion; the sensitivity, specificity and accuracy of CCT for detection of bypass occlusion in studies performed with 16-slices computed tomography was respectively of 97%, 100% and 99% (Martuscelli et al., 2004) and in the most recent meta-analysis on the diagnostic accuracy of the 64-channel CCT the sensibility and specificity in the detection of graft occlusion calculated on a per-graft rather than a per-patient basis were respectively 97% and 100% (Stein et al., 2008).

Cardiac computed tomography's accuracy in assessing graft stenosis is however lower and reduces the overall specificity to 97% while the overall sensitivity remains almost unchanged (98%). The latest generation CCT systems have a positive likelihood ratio above 10 and a negative likelihood ratio of 0.02 which leads to a very high positive predictive value and especially a very high negative predictive value compared with traditional ICA indicating that CCT can rule out or rule in graft disease with a very high accuracy (Stein et al., 2008).

It is however important to consider that assessing the native coronary arteries downstream from the grafts and in the un-grafted segments in patients in whom in the meantime CAD may have progressed, can be very challenging since these vessels tend to be small and sometimes heavily calcified (sensitivity 86%, specificity 76% and accuracy 78%) (Ropers et al., 2006b).

In the recent American Appropriate Use Criteria Task Force for CCT the use of CCT in the "evaluation of graft patency after cardiac surgery" was pointed to be appropriate with a high indication score (8 out of the highest value of 9) (Taylor et al., 2010).

Another issue is related to the area that must be studied during CCT examination. While patients who did not perform a coronary bypass are studied from the level of the carina to the diaphragm, those who have performed a bypass surgery should be studied from the level of the aortic arch and sometimes even from the clavicle in case we want to study the origin of the internal mammary arteries. Nevertheless CCT is preferred by patients because it is considered "non-invasive", even if it needs a greater amount of contrast medium and greater amount of radiation. In fact patients who performed bypass surgery have already experienced a pre-operative ICA, cardiac surgery and sometimes, after the cardiac bypass surgery, a second or third diagnostical and/or interventional ICA.

There are some cases where CCT could be considered the "gold standard" technique to follow-up patients who underwent bypass surgery. These patients are those who underwent a gastroepiploic bypass to the posterior descending coronary artery. It is easier to study the gastroepiploic bypass with CCT rather than performing a challenging ICA, which may need a great amount of contrast medium and a great amount of radiation (Ropers et al., 2006b).
Cardiac computed tomography can be also the "ideal technique" in planning the re-do cardiac bypass surgery patients as it provides the exact relationship between the intra-thoracic organs and the chest wall (spatial relationship between grafts, epicardial coronary arteries, right ventricle and sternum) (Mark et al., 2010).
Another filed in which CCT could be used is that of patients who have to undergo cardiac bypass surgery. Cardiac computed tomography, unlike ICA, does not explore the coronary circulation dynamically and is unable to provide information regarding the direction of the blood inside the vessel and, because of its limited spatial resolution, is unable to detect the presence of homo and/or hetero coronary collateral circulation, which is very important to characterize patients with CAD and is useful in deciding their management. This information however is not always essential for the surgeon. In fact although CCT does not supply the surgeon with images showing the presence and direction of collateral circulation in most of the cases, it clearly indicates if the vessels after the stenosis or occlusion are pervious and could be grafted. Having preoperatively clear images of the diseased vessels for the presence of calcified or soft plaques is also useful for the surgeon to decide where to perform the anastomosis. Moreover, the study of the wall-thickness changes over the cardiac cycle obtained with CCT may guide both surgeons and interventional cardiologists to decide the real necessity for the revascularization and could help to avoid sudden decisions during ICA procedures for patients who may benefit more from cardiac bypass surgery rather than percutaneous interventions (Bamoshmoosh et al., 2008).
Recently some published papers hypnotized that CCT could be judged enough to decide if a patient can be directly sent to perform bypass cardiac surgery without confirming CCT results with traditional ICA. In fact the information obtained with CCT supplies the surgeon with a virtual 3D images showing the diseased vessel and help in pre-planning the length of the required conduit. This can be of great importance especially in off pump and in minimally invasive surgery.
In a recent interesting prospective clinical trial, CCT was compared to conventional coronary angiography to evaluate if CCT alone was adequate for proceeding in cardiac bypass surgery without coronary angiography. In this study 50 patients with proven severe CAD underwent CCT and ICA prior to cardiac bypass surgery. CCT images were compared with those of ICA for the accuracy, sensitivity and specificity in detecting significant stenosis. An excellent correlation was found between ICA and CCT results. The overall sensitivity, specificity, positive and negative predictive values for quantitative assessment of stenosis > 70% by CCT compared to ICA were 98.5 %, 99.1 %, 82.3 % and 99.8%, respectively. The Authors conclude that the improved spatial and temporal resolution of the 64 row scanners provided an excellent correlation of CCT with ICA, and in selected patients, they even recommend the consideration of CCT as a sole criteria for proceeding in cardiac bypass surgery without coronary angiography (Bedi et al., 2008).
These interesting results that indicate a possible future use of CCT not only complementary but also as an alternative to ICA in the flow chart in the evaluation of CAD patients prior to cardiac bypass surgery needs however to be further analyzed in multi-centric interdisciplinary studies.

6. Cardiac computed tomography and prior coronary stenting

The evaluation of coronary stents by CCT is challenging because there are important technical limitations related to the presence of the metal in the stent struts that cannot be completely bypassed by "convolution algorithms". The metal present in the stent absorbs the lower-energy portion of the X-ray beam leaving the not absorbed X-rays to reach the detector realising what is called the "blooming artefact", that causes the stent struts to appear thicker than they actually are and to produce also the "partial volume averaging" which is an artefact that affects the voxels immediately adjacent to the stent struts. During the reconstruction of the images both the "blooming artefact" and the "partial volume averaging" produced by the stent, associated with the eventually "blooming artefact" of the calcified plaque beneath the stent, interfere with the ability to assess the presence of in-stent restenosis and in general to evaluate if and how much the vessel is diseased (Mark et al., 2010)

All these data led the American Heart Association to consider in 2006 statement CCT non advisable to study patients with prior coronary stenting (class III with a level of evidence C) (Budoff et al., 2006).

However stents are not to be considered as equivalent because they have different metal composition, different designs and more importantly different sizes. With the new generation CCT systems the indication changed and stents 3.5 mm or larger are now judged 100% assessable. The ability to evaluate in stent restenosis reduces to 80% in the 3 mm stents and to 33% in the smaller stents (Sheth et al., 2007). Thus in a patient with a known large stent, like those in the left main, CCT could be considered a real alternative to ICA to rule out significant in-stent restenosis. In fact in the recent American Appropriate Use Criteria Task Force for CCT the use of CCT in the "risk assessment in asymptomatic patients with prior left main coronary stent with stent diameter ≥ 3 mm " was pointed to be appropriate with a high indication score (7 out of the highest value of 9) (Taylor et al, 2010).

Moreover as it is pointed by the writing group of the American Appropriate Use Criteria Task Force for CCT the 2010 appropriateness criteria reflect the actual most common clinical scenarios, keeping in mind that from the 2006 appropriateness criteria to those of 2010 some indications shifted up 1 category from either uncertain to appropriate and from inappropriate to uncertain (Taylor et al, 2010). The increasing amount of published papers with the most advanced computed tomography machines indicate the usefulness of CCT in the daily work to rule out or to rule in the presence of in-stent restenosis of CAD patients who may have performed several diagnostical and/or interventional coronary angiography procedures and ask for CCT instead of ICA (Sun & Almutairi, 2010). In the daily experience both patients and their physicians more and more frequently ask for an alternative to ICA at least at the time of the characterization of the CAD and want to have the opportunity to choose off-line the therapeutical decision.

This is the reason why the writing group of the American Appropriate Use Criteria Task Force for CCT do not believe that an uncertain rating must be used as a reason to deny reimbursement for CCT imaging, but consider that in these cases additional documentations have to be presented to justify reimbursement (Taylor et al, 2010).

7. Cardiac computed tomography and radiation dose

Since the introduction of CCT in the clinical practice the dose of radiation delivered to patients during the exams is the most often discussed drawback, because it has been

considered a critical safety issue, especially when besides CCT, ICA and/or stress myocardial perfusion imaging have to be performed. In the commonly used CCT systems the amount of radiation, expressed as units of millisieverts (equivalent to millijoules per kilogram of tissue), absorbed by patients during the test is 2-4 folds that of ICA (Mark et al., 2010). The introduction of improvements in CCT technologies like the "ECG-controlled tube-current modulation" and the ''step and shoot'' protocols, which use a prospective gating and predict when diastole will occur, decrease x-ray tube current during systole leading to a significant reduction of the radiation dose from about 18 millisieverts to less than 4 millisieverts, which is almost equal to that of traditional coronary angiography (Scheffel et al., 2008; Hausleiter et al., 2006). As with ICA patient radiation dose is directly correlated with cardiac tomography equipment, practice and experience of the center where the CCT is performed and patient related factors like patient weight and heart rate.

If it is difficult to ascertain the developing of malignancy as a consequence of a biologic damage due to radiation in adults, the population most likely studied with CCT. Nevertheless as a consequence of a good clinical practice CCT must be performed only when its indication is appropriate (see the 2010 appropriateness criteria of the American Appropriate Use Criteria Task Force for CCT) and if patient's diagnostic question cannot be adequately addressed by other investigations (Taylor et al., 2010).

8. Illustrative case

The patient is a 63-year-old man who was in treatment for arterial hypertension (Amlodipine 10 mg, Ramipril 10 mg and Furosemide 25 mg) and hyperlipidemias (Atorvastatin 10 mg). In 2000 he was operated for an adenoma of the adrenal cortex. In 2004 for a "type A" aortic dissection, in extra-corporeal circulation, he underwent prosthesis implantation (26 mm).

The patient was doing fine till June 2006 when he began to suffer of chest pain associated with dyspnoea. For this reason he performed a maximal treadmill stress test which was positive by electrocardiographic criteria (2.5 mm ST depression in lead V4-V6) and typical effort chest pain.

The patient was sent to perform ICA. The test, which was performed through a femoral approach, was however unable to selectively catheterize the right coronary artery and a severe left main and left anterior descending CAD were suspected. The indication given to the patient by the interventional cardiologist was to undergo bypass surgery.

However, in such a complex patient, the surgeon wanted to have a complete anatomical description of the coronary tree and the aorta to better pre-plan the surgical intervention and asked for a CCT test.

Cardiac computed tomography was performed with 64-slice multidetector computed tomography Brilliance scanner (Philips, USA) by administering 100 ml of iodinated contrast medium (Iomeron 400 mg/dl, Bracco Imaging, Milano, Italia) at a rate of 5 ml/s through a 18 gauge cannula placed in the antecubital vein.

To reduce patient's heart rate, 30 minutes before the test he was given 100 mg of Metoprolol per os. Cardiac computed tomography provided images which were judged of good quality and both right and left coronary arteries were correctly visualized (Fig. 1).

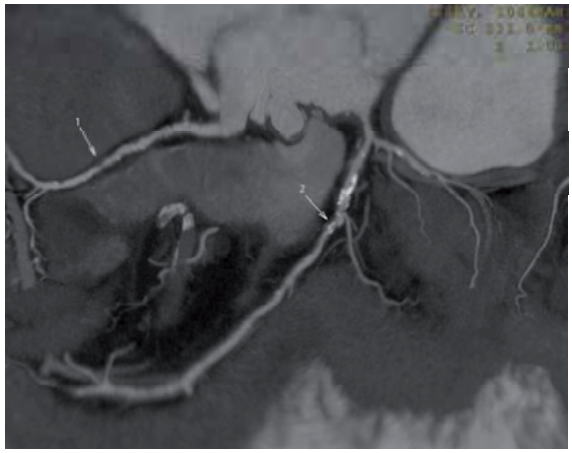

Fig. 1. 2D map in which in the middle segment of the right coronary artery (arrow n°1) there is a non calcified plaque (50% stenosis) and in the middle segment of the left anterior descending coronary artery (arrow n°2) a mixed plaque (50-75% stenosis).

Right coronary artery was the dominant vessel; in the proximal segment there were small, mixed, non critical lesions; in the middle segment there was a non calcified plaque that produced a 50% stenosis; no lesions were seen in the distal segment and in the posterior descending coronary artery (Fig. 2).

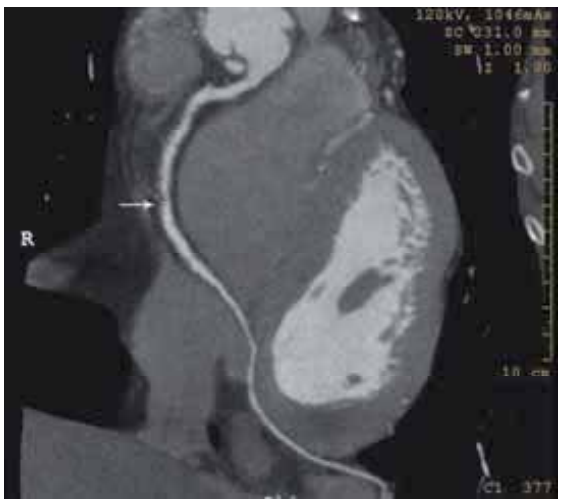

Fig. 2. Multiplanar reconstructed image of the right coronary artery and posterior descending coronary artery: in the middle segment (arrow) there is a non calcified eccentric plaque (50% stenosis).

The left main had a non calcified lesion which caused less than 20% stenosis. The left anterior descending coronary artery had in the proximal segment a non significant lesion followed in the middle segment by a mixed stenosis that caused a critical 50-75% stenosis; no lesions were seen in the distal segment and in the diagonal branches (Fig. 3 A). No significant lesions were seen in the circumflex coronary artery (Fig. 3 B).

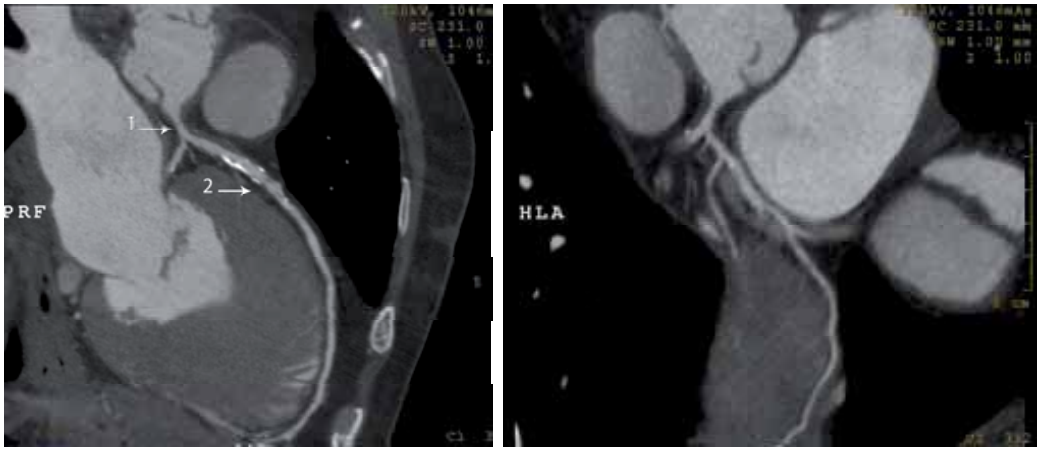

Fig. 3. Multiplanar reconstructed image of the left coronary artery. In A the left main (arrow n°1) has a non calcified lesion (20% stenosis); in the middle segment of left anterior descending coronary artery (arrow n°2) there is a mixed, eccentric lesion (50-75% stenosis). In B there are no significant lesions in the circumflex artery.

However more interestingly CCT showed also a severe aortic dissection due to a rupture of an intimal flap ahead of the ascending aorta prosthesis from where the false lumen was supplied (Fig. 4)

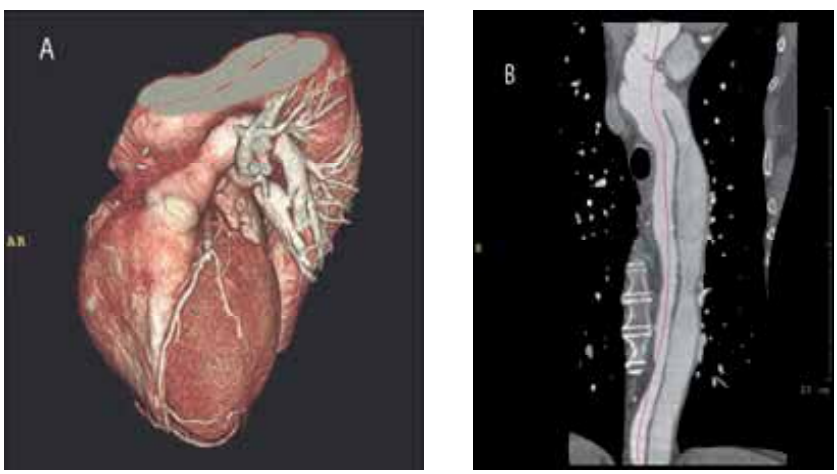

Fig. 4. Volume rendering image of the heart ad aorta (A) and lumen image of the aorta (B) where the aortic dissection with the true and false lumen are visible.

In this case ICA was unable to visualize coronary arteries because the catheters during the exam were engaging the false lumen due to the presence of the aortic dissection. CCT on the other side was very useful as it was the only test that provided the correct anatomical view of the coronary tree. Moreover CCT indicated the presence of a severe aortic dissection in a patient already treated for "type A" aortic dissection.
In this case CCT was also a very useful test for the surgeon to pre-plan the operation. In fact the patient underwent a second extra-corporeal circulation surgery for a radical aortic

replacement employing simultaneous modified Bentall and elephant trunk procedure and a coronary artery bypass surgery for the anterior descending coronary artery using the left internal mammary artery. At the 2 year follow up the patient was doing fine.

9. Conclusion

Invasive coronary angiography is an indispensable test to evaluate coronary arteries and in more than a half century experience it proved to be extremely reliable in the diagnostical as well therapeutical processes of CAD patients. For this reason it is considered the "gold standard" or "reference standard" technique upon which we compare the results of the other cardiac exams. However ICA is not always the most appropriate test with which we can evaluate CAD patients. The introduction in the cardiac arena of CCT, that with very good accuracy investigates coronary arteries, provided us with a complementary and sometimes an alternative test to ICA. In particular settings that can range from quite simple cases (identification of a coronary artery with an anomalous origin) to very complex cases (need to pre-plan a re-do bypass surgery), or that of the patient of the illustrative case it can be even considered the "real gold" standard technique. However to better understand the real usefulness of CCT and correctly allocate it in the flow chart of the evaluation of CAD patients more experience must be gained by routine users, further multi-centric interdisciplinary studies must be performed till the production of approved clinical practice guidelines.

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Physiologic Risk Assessment in Stable Ischemic Heart Disease – Functional Evaluation Versus Coronary Anatomy

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1. Introduction

The prognostic power of angiographic coronary anatomy has been definitively established since the CASS registry (1), where the 12-year survival rate of medically treated patients with no significant coronary lesions was 91%, compared with 74, 59 and 50% for patients with one, two and three vessel disease. Survival rate is known to further decrease in the presence of a left main coronary artery disease, and of a severe proximal left anterior descending stenosis (2).

However, several lines of evidence, obtained with different approaches, demonstrate that a physiologic risk assessment is superior to an angiographically oriented approach in prognostic stratification of patients with stable ischemic heart disease. In particular, in a recent study, Gimelli et al (3), in order to predict patient survival rate, analyzed a group of 676 consecutive in-patients who underwent a complete diagnostic work-up that included gated single photon emission computed tomography (SPECT) and coronary arteriography for known or suspected ischemic heart disease. Patients with acute myocardial infarction (MI), previous coronary artery bypass surgery, overt hyperthyroidism or who were undergoing dialysis treatment were excluded.

To predict patient survival rate, the Authors utilized an angiographic semi-quantitative score, that takes into account the number of stenotic coronary arteries, the location of coronary stenoses (proximal, middle, or distal), and the degree of luminal diameter reduction (using a 50 and a 70% coronary stenosis threshold). As expected, this score was an independent predictor of event-free survival. However, the prognostic impact of this score disappeared once gated SPECT variables were included into the model (3).

The notion that the prognostic impact of functional risk assessment is superior to angiography is not novel. In patients with defined coronary artery disease, exercise variables primarily relating to the functional state are known to provide an incremental prognostic information over coronary anatomy (4). The treadmill score also adds independent prognostic information to that provided by clinical data, coronary arteriography and left ventricular (LV) ejection fraction (5). The predictive power of clinical data is also strengthened by adding the results of dobutamine echocardiography (6); however, the power of the model was increased to a minor degree by the addition of coronary anatomy data. In a group of patients who underwent stress echocardiography with

either dipyridamole or dobutamine, and who also underwent coronary angiography within a year without an intervening procedure, coronary angiography parameters did not add significant predictive power to the model compared with stress echocardiographic variables (7). As to nuclear cardiology, the superiority of myocardial perfusion imaging over coronary angiography in risk stratification of patients with ischemic heart disease is also well known. In 1992, Pollock et al. demonstrated that myocardial perfusion is superior to coronary angiography in risk stratification of ischemic heart disease patients (8); at variance with our study, these authors used Thallium-201 (Tl-201) and static planar imaging. In a series of 316 medically treated patients, Iskandrian et al. (9) showed the independent and incremental prognostic information of exercise SPECT Tl-201 imaging even when catheterization data are available. All these evidences definitively underline the superiority of functional risk stratification over an approach based solely on angiographic coronary anatomy.
These observations can be partly explained by the limitations of coronary angiography. First of all, coronary angiography has a limited sensitivity when compared to necropsy studies (10) and to intravascular ultrasound investigations (11). Furthermore, the identification of significant lesions may be confounded by coronary remodeling and by the extraluminal location of some plaques (12). Finally, coronary angiography does not allow to study coronary microcirculation, increasingly recognized as independent determinant of impaired blood flow, disease progression and adverse prognosis (13).

2. The role of nuclear medicine as functional imaging

The clinical use of radioisotopes in the evaluation of patients with ischemic heart disease is largely based on noninvasive methodology suitable for visualizing myocardial perfusion. Although contrast echocardiography and magnetic resonance imaging have been proposed recently for the same purpose, at present most of the noninvasive tests for assessing myocardial perfusion reside in nuclear cardiology. The most popular radioactive flow tracers are characterized by a rapid myocardial extraction followed by either a sequestration (for Technetium 99m labeled agents – Tc 99m) or a very slow washout (for Tl-201). These features make the tracer uptake proportional to blood flow in each myocardial region, even if, up to now, the quantitation of absolute flow is not possible with these techniques. Despite this limitation, myocardial perfusion can be imaged and underperfusion can be detected as a relative uptake defect compared with the better perfused myocardium. As "significant" coronary stenosis regionally impairs flow reserve, myocardial perfusion imaging has become a common tool for the diagnosis of coronary artery disease and coronary angiography the gold standard for defining its sensitivity and specificity.
The main strength of radionuclide cardiac imaging in patients with coronary artery disease is to provide pathophysiologic and clinical information related to major objectives of assessment which include myocardial perfusion, viability and their relationship with left ventricular function. For these hypothesis, these data have additional value over anatomic information, provided by other imaging techniques, for patient management and outcome.

3. Overview of imaging principles

The two different approaches used to evaluate myocardial perfusion by nuclear imaging are SPECT and positron emission tomography (PET). SPECT gamma-emitting tracers include Tl-201, Tc-99m Sestamibi and Tetrofosmine. PET positron emitting tracers include O-15 water, N-13 ammonia and rubidium-82, a generator produced readily available agent.

As potassium analogue, Tl-201 has high myocardium uptake and has been the most commonly used perfusion tracer during the past years (14). Tl-201 is distributed into the myocardium proportionally to the flow over a wide range of values. However, some limitations have recently reduced its use: the low photon energy is associated with a lower resolution and significant attenuation by overlying tissues; the prolonged physical half-life (73 hours) causes a significant radiation exposure to the patient.

Tc-99m labeled tracers, having a shorter physical half life (6 hours) and better imaging capabilities, replaced Tl-201 in several indications for clinical SPECT (15). Tc-99m labeled tracers are readily available, the images obtained show higher spatial resolution and tissue attenuation is also less significant. Tc-99m Sestamibi and Tetrofosmine, the two most commonly used labeled tracers, distribute into the myocardium proportionally to the flow but their uptake is also dependent on normal mithocondrial function. After myocardial uptake, the retention of Tc-99 m labeled tracers in the myocardium is high so that two separate tracer injections are required to compare stress and resting perfusion.

Up to now, one of the major advantages of PET over SPECT is the possibility to correct myocardial tissue radioactivity for attenuation of the surrounding organs so that, using appropriate models to describe the kinetics of the single tracer, it is possible to quantitate myocardial blood flow (MBF) in absolute terms ($mL \cdot g^{-1} \cdot min^{-1}$) (16-17). O-15 water is considered the gold standard for MBF quantitation because its kinetic in the heart is independent of myocardial metabolism (18). However, the tracer is freely diffusible and has a very short physical half-life (120 sec) so that images have not a high myocardial definition and approaches to correct for radioactivity in the vascular compartment are required for MBF quantitation (19).

N-13 Ammonia is highly extracted and retained by the heart and has a relatively longer physical half-life than O-15 water (9.96 min) resulting in good to excellent images of the myocardium. However, the uptake and retention are dependent on myocardial metabolism (20), which may also cause regional differences in tracer accumulation (21). Rest and stress studies should be separated by 30 to 40 minutes to allow decay of the previously injected dose. There are a number of approaches for MBF quantitation with N-13 ammonia using one, two or three compartments models (22-24) and corrections for lower extraction at higher flows is used from empirical relationships obtained in experimental studies.

Rubidium-82, is a potassium analog like Tl-201, allows good quality myocardial images and is available by a generator. The very short physical half-life (76 sec) allows to perform rest and stress studies with minimal time intervals but also introduces some challenges for the absolute quantitation of MBF (16).

A typical myocardial perfusion imaging exam includes a rest-stress protocol where either a physical (bycicle or treadmill exercise in conjunction with SPECT) or a pharmacological (dipyridamole, adenosine or dobutamine in conjunction with SPECT or PET) stressor is applied. In general SPECT is finalized to the detection of relative regional differences in myocardial perfusion while PET adds the potential of measuring absolute regional MBF and MBF reserve. The development of ECG-gated SPECT and PET has also enabled accurate regional and global cardiac function measurements based on a true tomographic approach. The methods are relatively operator-independent and comprehensive software packages allow the evaluation of the extent and severity of regional LV dysfunction, in hypoperfused but viable myocardium, as well as of global LV ejection fraction, volumes, diastolic function and geometry (25-27).

4. Endothelial dysfunction and myocardial perfusion imaging

Another confirmation that myocardial perfusion imaging remains one of the best predictor of prognosis in patients with ischemic heart disease comes from coronary microvascular alterations in various heart diseases.

A specific alteration of coronary endothelial function has been found in patients with different diseases such as atherosclerosis (28), dilated cardiomyopathy (29) and arterial hypertension (30). However, the relevance of endothelial dysfunction to physiologic flow control has not been directly documented. Accordingly, coronary artery stenosis is still considered the only factor able to affect coronary blood flow regulation in a relevant fashion during daily life. Nevertheless, endothelium is an important factor in the integrated response of hyperemic flow, even to agents affecting vasomotor tone, through direct action on smooth muscle cells. In fact, the lack of the endothelial contribution might limit the effect of endothelium-independent agents on flow.

Several studies have actually documented abnormal flow responses to dipyridamole or atrial pacing in the myocardium supplied by angiographically normal coronary arteries in patients with dilated cardiomyopathy (31) or arterial hypertension (32) and even in patients with single vessel disease on control coronary arteries (33).

Thus far, the link between reduced vasodilator response and endothelial dysfunction has not been tested directly. However, the coincidence of both abnormalities in populations with various heart diseases suggests their possible association in the same patient and thus a potential pathophysiologic link between the two. With regard to myocardial perfusion imaging, the reduction in maximal flow capacity due to microvascular alterations causes a reduction in perfusion differences between territories perfused by angiographically normal and stenotic vessels during vasodilator stress, thus explaining the relative decrease in sensitivity of myocardial perfusion scintigraphy in the detection of single-vessel coronary artery disease (34). On the other side, microvascular dysfunction might produce regional flow abnormalities per se, thus hampering the postulated cause-effect relationship between epicardial stenosis and perfusion defect.

In agreement with this concept, Zeiher et al (35) demonstrated that coronary microvascular endothelial dysfunction was associated with a high incidence of reversible perfusion defects at stress myocardial perfusion imaging despite the absence of coronary stenosis. According to the traditional criteria, the abnormal scan results of these patients are considered to be “false positive”. In contrast, this feature might represent an actual stenosis-independent abnormality in blood flow distribution rather than the effect of technical artifacts such as attenuation or partial-volume effect. This concept seems of great relevance, as monitoring of microvascular function can also be used for assessing the efficacy of therapy. Gould (36) demonstrated that aggressive cholesterol lowering is able to reduce reversible perfusion defects induced by dipyridamole. Guethlin et al (37) showed that statin therapy improves myocardial blood flow response to adenosine independently of stenosis severity in the related vessel. The most striking feature of these findings is that the interventions thought to improve endothelial function actually improved the flow response to endothelial independent stimuli, underlining the relevance of the endothelium in the integrated tuning of vasomotor tone.

5. Functional risk assessment vs. noninvasive coronary angiography

The prognostic value of coronary artery calcium (CAC) has been consistently demonstrated in large series of patients (39-41). Current evidence also suggests that the use of CAC is

independently predictive of outcome over and above traditional cardiac risk factors. As to functional risk assessment, CAC scores are predictive of a higher likelihood of ischemia on PET myocardial perfusion imaging (42). More importantly, patients with and without ischemia on PET perfusion imaging exhibit a stepwise increase in their risk of cardiac events with increasing calcium scores. These findings suggest that imaging approaches that combine quantitative information on the anatomic burden of ischemic heart disease with its physiological consequences offer improved risk stratification over conventional approaches that use myocardial perfusion alone. However, an independent and incremental prognostic value of CAC over clinical, electrocardiographic, laboratory, echocardiographic and angiographic variables has not been demonstrated yet.
A similar consideration applies to computed tomography (CT) of the coronary arteries. Although the presence of obstructive coronary lesions at 64-slice CT angiography was a predictor of an adverse outcome (death, nonfatal myocardial infarction, unstable angina, and coronary revascularization) as compared to the patients with normal coronary arteries (43), an independent and incremental prognostic value of CT angiography over clinical, electrocardiographic, echocardiographic and scintigraphic variables has not been demonstrated. Up to now, two studies have been designed to test the impact of a combined anatomic and functional non-invasive imaging for detection and characterization of ISCHEMIC HEART DISEASE: the EVINCI study in Europe and the SPARC trial in the United States of America. While we are waiting for the results of these two trials, patients outcome in stable ischemic heart disease should be estimated using the evidences so far collected.

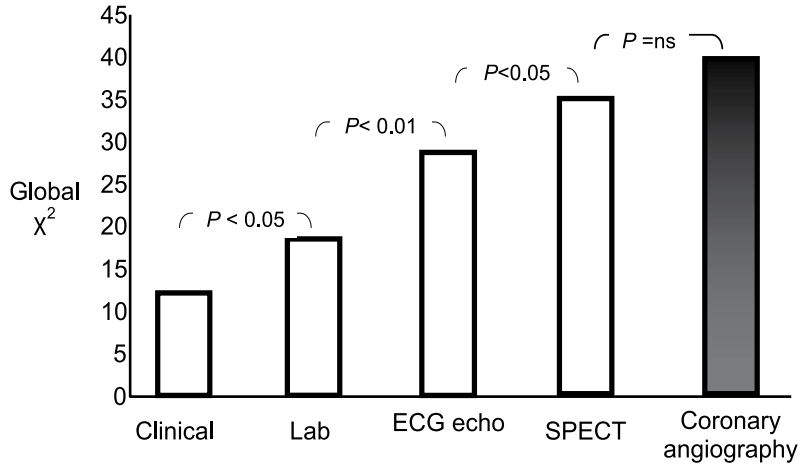
6. Myocardial perfusion imaging and revascularization

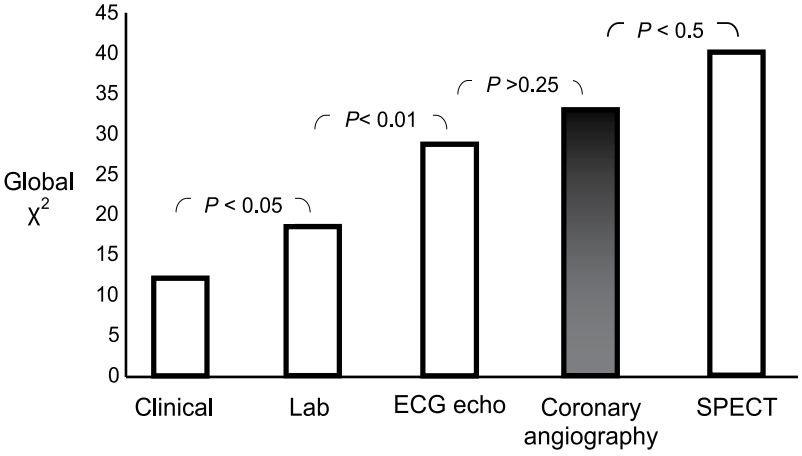
No differences in the composite of death, acute myocardial infarction and stroke was found between patients with stable ischemic heart disease, objective evidence of ischemia and significant coronary stenoses randomized to optimal medical therapy with or without percutaneous coronary intervention in the COURAGE trial (44). The relationship between extent of myocardial ischemia at gated SPECT and coronary revascularization has been explored in a subset of patients enrolled in a nuclear substudy of the COURAGE trial (45). In these patients, the addition of percutaneous coronary intervention to optimal medical therapy resulted in more effective reduction of ischemia than optimal medical therapy alone. This greater reduction in ischemic burden was associated with improvements in angina class and less reliance on nitrate therapy for symptom relief. Moreover, patients with moderate to severe ischemia randomized to percutaneous coronary intervention plus optimal medical therapy more commonly experienced a significant reduction in ischemia at follow-up compared with those receiving optimal medical therapy (78% versus 52%). Thus, the results of the nuclear substudy suggest that gated SPECT could be utilized for the identification of patients who will benefit more from coronary revascularization.

7. The proper diagnostic work-up

In patients with stable ischemic heart disease, myocardial perfusion imaging may be sometimes regarded as an unnecessary, or even redundant investigation. The study of Gimelli and Colleagues shows that myocardial perfusion abnormalities at rest and after stress are still the best predictors of cardiac event-free survival, even when compared with

an extensive diagnostic work-up. Specifically, when gated SPECT data were added to the clinical, laboratory, electrocardiographic and echocardiographic variables, the prognostic stratification significantly improved; however, when coronary angiography was added to gated SPECT, prognostic stratification did not further improve (Figure 1, top panel). On the other hand, if the information provided by gated SPECT was made available after clinical, laboratory, electrocardiographic, echocardiographic and angiographic variables, the prognostic stratification still improved significantly (Figure 1, lower panel). Thus, gated





Modified from Gimelli et al, J Nucl Med 2009;50:546-53

Fig. 1. Incremental prognostic value during the diagnostic work-up. In the upper panel, the information provided by stress/ rest gated SPECT is available after clinical examination, laboratory tests, electrocardiography and echocardiography but before coronary angiography. In the lower panel, the information provided by gated SPECT is available after coronary angiography.

SPECT adds a prognostic information that is greater than that provided by coronary angiography. This observation suggests that the indications for myocardial perfusion imaging in risk stratification of patients with known or suspected ischemic heart disease should be broadened.

In current clinical practice, a functional risk assessment may conflict with a health care delivery policy oriented towards cost saving and direct reperfusion. In this respect, a recent survey of the European Society of Cardiology has shown that non-invasive functional tests are under-utilized, with wide variability between different countries (46), so that several patients without significant ischemic heart disease directly undergo invasive coronary angiography. On the other hand, coronary lesions detected by coronary angiography are often revascularized even without the evidence that either myocardial blood supply or mechanical function is altered (47). This "anatomically oriented" invasive approach may negatively affect patient management, with consequent suboptimal medical treatment, inappropriate revascularizations, additional risks and increased health costs.

8. Conclusions

Several lines of evidence, collected with different approaches, demonstrate that a physiologic risk assessment is superior to an angiographically oriented approach in prognostic stratification of patients with stable ischemic heart disease. On these basis, stress/ rest myocardial perfusion abnormalities should be known – whenever possible - before coronary angiography in order to guide decision making, provided that appropriateness and patient's risk/ benefits ratio are correctly considered.

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13

Clinical Significance of Tetrofosmin Extracardiac Uptake During Myocardial Perfusion Imaging

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1. Introduction

Coronary artery disease (CAD) is a major cause of mortality and morbidity and its management consumes a large proportion of national healthcare budgets (Underwood et al., 2004). It has been estimated that approximately twenty diseases account for over 80% of all the deaths in the world (Satra et al., 2011). Specifically, atherosclerosis which occurs in the coronary arteries as the underlying defect responsible for CAD accounts for nearly half of these deaths. Although CAD treatment protocols are improving, its prevalence has increased. New imaging technologies have added to the immediate costs of investigation but they also have the potential to reduce overall costs, by virtue of their greater diagnostic and prognostic accuracy. This allows a more informed selection of therapy, which in turn can lead to a better clinical outcome (Underwood et al., 2004).

Myocardial perfusion scintigraphy (MPS) was developed in the 1970s and has been used increasingly in clinical cardiology since the 1980s (Underwood et al., 2004). Technical developments that have fuelled this recent increase are single-photon emission tomographic computed imaging (SPECT), pharmacological stress and ECG-gated imaging (Underwood et al., 2004). Nowadays, MPS comprises the only widely available method of assessing myocardial perfusion directly and many previously published reports support its evidence in the diagnosis of myocardial ischaemia and necrosis (Satra et al., 2011). Moreover, the prognostic value of this method for patients' risk stratification has already been extensively reported, with an incremental prognostic value after clinical assessment, exercise electrocardiography and even above coronary angiography (Satra et al., 2011). Thus, MPS is an established imaging technique that is already an integral part of the management of CAD (diagnosis, prognostication, selection for revascularization and assessment of acute coronary syndromes) and is included in a number of professional guidelines (Underwood et al., 2004).

2. SPECT myocardial perfusion imaging agents

MPS involves intravenous injection of small amounts of a radioactive tracer, usually during some form of cardiovascular stress. The three commercially available tracers are thallium-201 thallous chloride (Tl-201), technetium-99m (Tc-99m) 2-methoxy-isobutyl-isonitrile

(MIBI) and technetium-99m 1,2-bis[bis(2-ethoxyethyl) phosphino] ethane (tetrofosmin). These are avidly extracted by cardiac myocytes and hence their initial myocardial distribution reflects a combination of the distribution of myocytes and perfusion. Comparison of images following stress and rest injections of tracer (or following redistribution for thallium) allows myocardial viability and perfusion to be assessed independently (Underwood et al., 2004). Tl-201 is a good tracer of myocardial perfusion and it has been used clinically for more than two decades (Hesse et al., 2005). It is administered intravenously as thallous chloride and the usual activity is 80 MBq. It does, however, have limitations:

i. Relatively long physical half-life: high radiation burden for the patient (80 MBq delivers an effective dose of approximately 18 mSv).
ii. Relatively low injected activity: low signal-to-noise ratio; images can be suboptimal (obese patients) and low count levels impair high-quality ECG-gated SPECT studies.
iii. Relatively low energy emission: low-resolution images and significant attenuation by soft tissue.

Tc-99m compounds do not have these limitations, which has encouraged the development and increasing use of such tracers, even if the physiological properties (somewhat lower fractional myocardial tracer uptake, in particular during high coronary flow values) of both Tc-99m labeled tracers are inferior to those of Tl-201 (Hesse et al., 2005). Two Tc-99m labeled perfusion tracers are currently available commercially: Tc-2-methoxyisobutylisonitrile (sestamibi) and Tc-1,2-bis[bis(2-ethoxyethyl) phosphino] ethane (tetrofosmin). Tc-99m sestamibi is a cationic complex which diffuses passively through the capillary and cell membrane, although less readily than Tl-201, resulting in lower immediate extraction. Within the cell it is localized in the mitochondria, where it is trapped (Li et al., 1990), and retention is based on intact mitochondria, reflecting viable myocytes. Elimination of the radiotracer occurs mostly through the kidneys and the hepatobiliary system. Tetrofosmin comprises an interesting alternative radiotracer for myocardial perfusion scanning, as it combines the exceptional physical properties of Tc-99m with easy and fast preparation. This compound is also cleared rapidly from the blood and its myocardial uptake is rather similar to that of sestamibi (Jain et al., 1993), with approximately 1.2% of the administered dose being taken up by the myocardium. The exact mechanism of uptake is unknown, but it is supposed to be similar to that of sestamibi. Elimination of the radiotracer occurs mostly through the kidneys and the hepatobiliary system, and the hepatic clearance is slightly more rapid than in the case of sestamibi (Münch et al., 1997). For both Tc-99m labeled tracers splanchnic uptake and excretion are markedly higher than for Tl-201, which may occasionally complicate interpretation of the inferior wall perfusion. The tracer molecules taken up by the cardiac myocytes remain within the cells: usually two visits on two different days are necessary to obtain optimal stress and rest images (Anagnostopoulos et al., 2003; Hesse et al., 2005).

After intravenous injection, Tc-99m labeled radiopharmaceuticals are distributed within the myocardium according to myocardial perfusion and viability. Unlike Tl-201, they have little (sestamibi) or almost no redistribution (tetrofosmin) and so separate injections are required for stress and resting studies. The higher energy of Tc-99m generally leads to better quality images (because of less attenuation and scatter). Moreover, the short half-life of Tc-99m permits much higher activities to be administered, giving better counting statistics and thus allowing performance of left ventricular (LV) ECG gating or first-pass imaging, which provides additional functional information. The diagnostic reference level for tomography is

a total of 1000 MBq for a one-day protocol (normally divided as 250 MBq and 750 MBq), or 400 MBq for each study of a two-day protocol. Higher activities can be considered on an individual basis by the practitioner, for instance in obese patients (Anagnostopoulos et al., 2003; Hesse et al., 2005).

3. Clinical evaluation of MPS

Many studies have evaluated the diagnostic accuracy of MPS for the detection of coronary heart disease, but they are of variable size and quality. There is a wide range of values reported for sensitivity and specificity that tend to overlap between different tests, although there have been studies comparing these different protocols in a head-to-head fashion. In the largest single study of 2,560 patients randomized to each of the three tracers and using mainly adenosine stress (the ROBUST study), overall sensitivity in the subset of patients undergoing angiography was 91%, specificity 87% and normalcy rate 89%, with no significant difference between the three tracers (Kapur et al., 2002). However, image quality was superior for the studies acquired with the Tc-99m-based agents, most likely due in part to the lower energy of Tl-201. In contrast, there is generally good agreement between Tc-99m sestamibi and Tc-99m tetrofosmin in identifying myocardial ischaemia (Russell & Zaret, 2006). In one of our previous studies using Tc-99m tetrofosmin as the radiotracer, we have found a good segmental agreement between tetrofosmin scintigram and coronary angiography. The sensitivity, specificity, positive predictive value and negative predictive value ranged between 72.2-91.1%, 80.0-88.2%, 76.4-88.5% and 83.3-85.7% respectively, depending on the obstructed vessel (Georgoulias et al., 1996).
The power of myocardial perfusion imaging (MPI) for predicting future coronary events has been demonstrated in a large number of high-quality studies and in many thousands of patients. It is perhaps the area of nuclear cardiology where the evidence is most strong (Brown, 1991, 1995, 1996). The most important variables that predict the likelihood of future events are the extent and severity of inducible ischaemia (Ladenheim et al., 1986) but other predictors are increased lung uptake of thallium (Gill et al., 1987), stress-induced ventricular dilatation (Weiss et al., 1987) and left ventricular ejection fraction (Gioia et al., 1996; Johnson et al., 1997). In general, markers of left ventricular dysfunction tend to predict cardiac mortality and inducible ischaemia predicts acute coronary syndromes (Hachamovitch et al., 1998; Sharir et al., 2001). MPS has incremental prognostic value even after clinical assessment, exercise electrocardiography and coronary angiography (Iskandrian et al., 1993; Sharir et al., 1999). In other words, patients who appear to be high risk after coronary angiography can be separated into higher and lower risk groups by MPS. In addition, several studies have indicated that a negative SPECT study confers an excellent prognosis with an annual cardiac event rate of <1% for the general population (Geleijnse et al., 1996; Iskander & Iskandrian, 1998; Machecourt et al., 1994; Marie et al., 1995; Schinkel et al., 2005; Shaw et al., 2003). In the setting of a normal myocardial perfusion study in a low-risk patient, it takes 9 years for the risk of a cardiac event to reach 1%, suggesting that, in the absence of new symptoms, a repeat perfusion study may not be needed for 3 to 5 years (Russell & Zaret, 2006). However, this "warranty period" does not appear to be absolute and is affected by clinical and technical factors, including the presence of diabetes or CAD, increasing age and male gender, and the need to perform a pharmacologic stress test rather than an exercise perfusion imaging test, which can increase the annual cardiac event rate in patients with a normal perfusion scan to as high as 1.8% (Hachamovitch et al., 2003). In

these high-risk patients with normal myocardial perfusion studies, it may be prudent to perform repeat perfusion imaging on a more frequent basis.

Because of its prognostic power, MPS can be used as the gatekeeper to coronary angiography. Bateman and colleagues showed that referral to coronary angiography after normal, mild to moderately abnormal and severely abnormal perfusion scans was 3.5%, 9% and 60% respectively (Bateman et al., 1995). Importantly, a policy of selective referral to coronary angiography based upon high-risk findings is defensible, as patients with mild to moderate abnormalities when managed medically have outcomes comparable to those undergoing invasive evaluation and subsequent revascularization (Underwood et al., 2004). Besides, several reports underlie that such a policy can be also cost-effective even if it is more expensive than an alternative test such as the exercise ECG (Underwood et al., 2004). Furthermore, MPS can provide useful information about cardiac risk in patients requiring non-cardiac surgery although these patients are generally at low risk and the predictive value of a normal perfusion study is greater than that of an abnormal study, while the clinical value of MPS using Tc-99m labeled agents (MIBI, tetrofosmin) to assess patients with acute coronary syndrome has been well established (Underwood et al., 2004).

MPS is of proven value to assess patients post revascularization. Information gained from post-intervention myocardial SPECT is important to differentiate patients with angina from those with exo-cardiac chest pain syndromes, to assess peri-intervention myocardial damage/acute vessel closure, to predict-detect restenosis after PCI and graft occlusion/stenosis after CABG surgery, to detect CAD progression in non-revascularized vessels, to assess left ventricular function (gated-SPECT), to evaluate the effects of intervention if required for occupational reasons and to predict patients' long-term prognosis. With respect to detect graft patency, MPS has a 80-96% sensitivity and 61-88% specificity, while regarding restenosis after PCI, sensitivity and specificity range between 74-94% and 67-88%, respectively (Georgoulias et al., 1998, 2008, 2010b). Specifically, using Tc-99m tetrofosmin as the radiotracer, we have reported a sensitivity, specificity, positive and negative predictive value of MPI in detecting restenosis of 81.3%, 88%, 81.3% and 88%, respectively, whereas for the detection of restenosis in specific vessel, the corresponding values were 81.3%, 90%, 76.5% and 89.7%, respectively (Georgoulias et al., 1998, 2008, 2010b).

4. Increased pulmonary uptake during MPI

It is generally supported that increased pulmonary uptake of a myocardial perfusion radiotracer reflects increased pulmonary capillary wedge pressure. This finding may be attributed to either ischaemic or non-ischaemic causes (valvular heart disease, cardiomyopathy, and pulmonary disease).

4.1 Tl-201 pulmonary uptake

A significant number of studies have engaged with the clinical importance of increased thallium (Tl)-201 lung uptake during myocardial perfusion imaging (Gill et al., 1987; Homma et al., 1987; Hurwitz et al., 1992; Kaul et al., 1998; Kurata et al., 1991; Liu et al., 1985; Mahmood et al., 1992). The quantification of lung uptake is mostly estimated using the lung/heart ratio (LHR) of radioactivity (counts/pixel) in two regions of interest (ROIs), one in the lung (usually the left one) and another one over the myocardium of the left ventricle, delineated in the anterior images (Gill et al., 1987; Hurwitz et al., 1992; Kurata et al., 1991;

Mahmood et al., 1992). However, there is a general discrepancy in the reported LHR normal values, depended mainly on the method of calculation. For Tl-201, LHR upper normal limits range in the literature from approximately 0.37 to 0.55 (Georgoulias et al., 2010a). Increased exercise Tl-201 uptake is associated with decreased left ventricular function, the presence of multiple-vessel CAD, and poor patient prognosis (Brown, 1997; Chin et al., 1996; Daou et al., 2000; Gill et al., 1987; Kurata et al., 1991; Morel et al., 1999). Kurata et al. have found that patients with abnormal Tl-201 lung uptake had more extensive multiple-vessel CAD, more severe left ventricular dysfunction, and more perfusion defects than did patients with normal Tl-201 pulmonary activity (Kurata et al., 1991).

Follow-up studies have shown that abnormal lung Tl-201 uptake is in general a significant predictor of subsequent adverse cardiac events for patients undergoing an MPI. Moreover, the Tl-201 LHR prognostic value has been reported in specific subgroups such as in patients treated with thrombolytic therapy during acute myocardial infarction, in patients with unstable angina and non-Q myocardial infarction, in patients after coronary artery bypass grafting, in patients with left ventricular apical aneurysm and in patients with severe post-ischaemic left ventricular dysfunction or after heart transplantation (Dakik et al., 1996; Jain et al., 1997; Krawczynska et al., 1997; Marcassa et al., 2000; Sarda et al., 2001; Wu et al., 2005). Abnormal pulmonary Tl-201 uptake during stress (treadmill or pharmacologic test) imaging has similar clinical significance to that noted during exercise. Thus, high uptake of Tl-201 in the lungs has an important prognostic value (Brown, 1997; Chin et al., 1996; Daou et al., 2000; Gill et al., 1987; Morel et al., 1999). Moreover, published data present an incremental prognostic value of increased Tl-201 pulmonary uptake over clinical, stress testing and other imaging findings, providing clinically useful risk assessment (Marcassa et al., 2000).

4.2 Tc-99m sestamibi pulmonary uptake

Although technetium-based radiopharmaceuticals have been widely used for several years, their limited use in lung uptake assessment has been regarded as a potential drawback. The results of assessing Tc-99m sestamibi pulmonary-to heart ratio of activity on conventional images obtained 30–60 min after stress are ambiguous, while a few studies have suggested that measuring lung uptake of Tc-99m sestamibi on immediate post-stress images may be more valuable (Bacher-Stier et al., 2000; Choy & Leslie, 2001; Giubbini et al., 1995; Hurwitz et al., 1993; Hurwitz et al., 1996; Hurwitz et al., 1998; Hurwitz, 2000; Patel et al., 2004; Romanens et al., 2001; Saha et al., 1994). In addition, the methodology for calculating LHR among investigators varies; for example, a large ROI that enclosed the entire left ventricle or most of the left ventricle respectively and a fixed-size ROI in the left lung (Choy & Leslie, 2001; Patel et al., 2004). Other widely used methods are the following: a transmural segment of the myocardium is outlined containing the area of peak counts and a crescenting ROI placed over the left lung or alternatively, a fixed small rectangular ROI placed over maximal myocardial and left lung activity (Hurwitz et al., 1993; Hurwitz et al., 1996; Hurwitz et al., 1998; Hurwitz, 2000; Romanens et al., 2001). Thus, the reported LHR normal values for Tc-99m sestamibi vary significantly between 0.44 and 0.56, depended on the method of calculation and the time interval between radiotracer's injection and the acquisition of the image for the LHR calculation, although early post-stress calculated values are generally higher than those calculated during the standard acquisition time (Georgoulias et al., 2010a). In addition, conflicting results have been reported about the relation between the values of LHR measured in the delayed images and the presence of extensive myocardial ischaemia

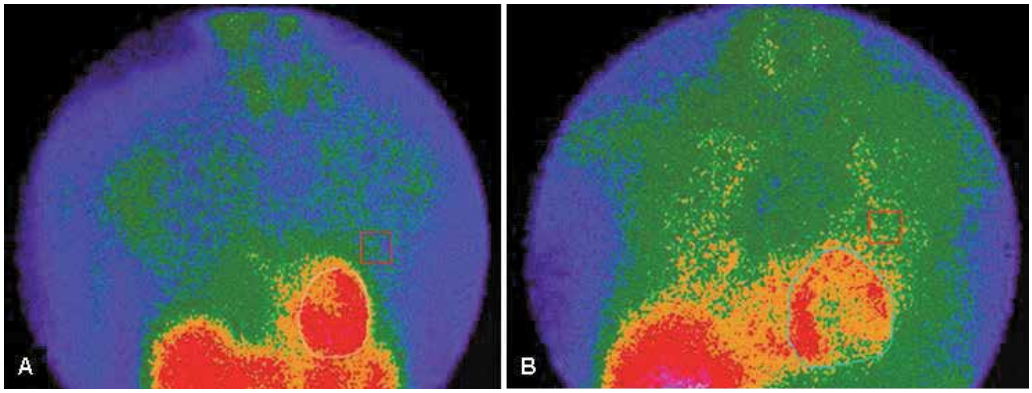
or severe CAD (Bacher-Stier et al., 2000; Choy & Leslie, 2001; Patel et al., 2004; Saha et al., 1994). On the other hand, the clinical value of Tc-99m sestamibi LHR obtained almost immediately after stress (exercise or vasodilatation), has been reported in several studies (Flamen et al., 1995; Münch et al., 1997; Nakajima et al., 1993). They have found that increased pulmonary to myocardial ratio calculated on early post-stress images was associated with severe scintigraphic abnormalities and angiographic findings (mainly three-vessel disease or stenoses in the left mainstem), in concordance to Tl-201 post-stress lung uptake (Hurwitz et al., 1993; Hurwitz et al., 1998; Hurwitz, 2000; Romanens et al., 2001). In addition, Leslie et al. have reported the prognostic value of lung sestamibi uptake in a cohort of 718 patients with known or suspected CAD, who underwent a Tc-99m sestamibi MPI (Leslie et al., 2005).

4.3 Tc-99m tetrofosmin pulmonary uptake

As we have already mentioned Tc-99m tetrofosmin is an interesting alternative radiopharmaceutical for MPS, as it combines the exceptional physical properties of Tc-99m with easy and fast preparation (Georgoulias et al., 1996; Heo et al., 1994; Nakajima et al., 1993; Sridhara et al., 1993). The diagnostic and prognostic value of myocardial perfusion SPECT studies using this radiotracer has already been well established, although a few studies have utilized the usefulness of lung uptake as an ancillary parameter.

We have mentioned above the significant variability of the applied techniques for calculating LHR using Tc-99m labeled tracers. For the quantification of Tc-99m tetrofosmin pulmonary uptake, in our Nuclear Medicine Laboratory we acquire early anterior planar images (1000 kcounts, matrix 256X256), 4–6 min after radiotracer injection at stress (early post-stress images), considering that Tc-99m tetrofosmin has a rapid clearance from the lungs. We then delineate two ROIs: one square, 15X15 pixels, placed over the left mid-lung area at the vicinity of the myocardium (at a distance of at least 3 pixels above the anterolateral wall – 'lung ROI') and the other, manually drown irregular ROI, including the whole myocardial activity of the left ventricle ('myocardial ROI') (Choy & Leslie, 2001; Georgoulias et al., 2006; Patel et al., 2004) (Fig. 1). The pulmonary/heart ratio was determined as the mean counts/pixel in the lung ROI divided by the mean counts/pixel in the myocardial ROI (Choy & Leslie, 2001; Giubbini et al., 1995; Homma et al., 1987; Hurwitz et al., 1992; Hurwitz et al., 1998; Kaul et al., 1998; Kurata et al., 1991; Liu et al., 1985; Patel et al., 2004; Tanigaki et al., 1998; Tsou et al., 2002). Our method for calculating Tc-99m tetrofosmin LHR is similar to that used by Choy & Leslie (Choy & Leslie, 2001) and Patel et al. (Patel et al., 2004). This approach may underestimate the counts from the heart, if severe defects are present, thus potentially overestimating the LHR value. On the other hand, this method avoids problems related to hot spots or other artefacts that could have erroneous effects on LHR. We believe that our method is more representative of pulmonary and heart radioactivity, especially in cases with regional myocardial ischemia.

The mechanism of lung uptake with Tc-99m labeled myocardial perfusion tracers has not been directly established, but it is reasonable to consider that it shares similarities with Tl-201 lung uptake. Our findings (presenting below) that CAD severity, scintigraphic ischaemic abnormalities and clinical-exercise data of myocardial ischaemia have a considerable correlation with stress LHR, support the role of ischaemic left ventricular dysfunction with subsequent pulmonary congestion-increased pulmonary vascular transit time as previously suggested for thallium (although additional factors may be also important) (Choy & Leslie, 2001; Georgoulias et al., 2006).



RCA: right coronary artery; LCX: left circumflex artery; LAD: left anterior descending artery

Fig. 1. (A) Normal early post-stress LHR value (0.402) in a 50-year-old woman without CAD. (B) Elevated early post-stress LHR value (0.693) in a 61-year-old man with 3-vessel disease (RCA 95%, LCX 95%, LAD 80%) (Reprinted from European Journal of Nuclear Medicine and Molecular Imaging, Vol. 37, Georgoulias, P., Tsougos, I., Valotassiou, V., Tzavara, C., Xaplanteris, P., & Demakopoulos, N., Long-term prognostic value of early poststress (99m)Tc-tetrofosmin lung uptake during exercise (SPECT) myocardial perfusion imaging, pp. 789-798, Fig. 1, 2010, with kind permission from Springer Science & Business Media B.V.).

In 2006, we published our results of studying 158 consecutive patients who underwent a stress/rest Tc-99m tetrofosmin myocardial SPECT (rest scans were obtained as gated SPECT) and coronary angiography (Georgoulias et al., 2006). An early post-stress LHR value of 0.500 was defined as the upper normal limit, taking into consideration the early post-stress LHR values calculated in a normal group and using the formula 'mean value ± 2SD'. Patients with a normal early post-stress LHR value generally had a better performance in the treadmill testing than those with an abnormal value, had significantly better myocardial perfusion and function and better angiographic results (Georgoulias et al., 2006). We found a significant correlation ($P < 0.001$) among early post stress LHR, summed stress score (SSS) and the number of stenosed vessels (Georgoulias et al., 2006). The associations between the values of early post-stress LHR and summed difference score (SDS), left ventricular ejection fraction (LVEF) were weaker ($P = 0.031$, $P = 0.017$). The incidence of multi-vessel CAD in the subgroup of patients with increased values of early post-stress LHR, was significantly higher than in the normal group (81% vs. 42%, $P < 0.001$) (Georgoulias et al., 2006). We also reported a significant difference ($P < 0.001$) of the early post stress LHR value between patients with normal coronary arteries or one-vessel disease and patients with multi-vessel disease (Fig. 2). The above-mentioned data could be attributed to the influence of ischaemia on the early post-stress LHR value and are generally analogous with other published data, while other investigators did not find similar results using Tc-99m sestamibi as the radiotracer (Hurwitz et al., 1993; Hurwitz et al., 1998; Saha et al., 1994).

Moreover, early post-stress LHR was an independent predictor of multi-vessel CAD (coefficient 1.85, SD 0.16, $P < 0.001$), with an incremental value for its identification (Georgoulias et al., 2006). Similar results have been presented by Tsou et al., Tanigaki et al. and Okajima et al. who have also reported the incremental value of Tc-99m tetrofosmin

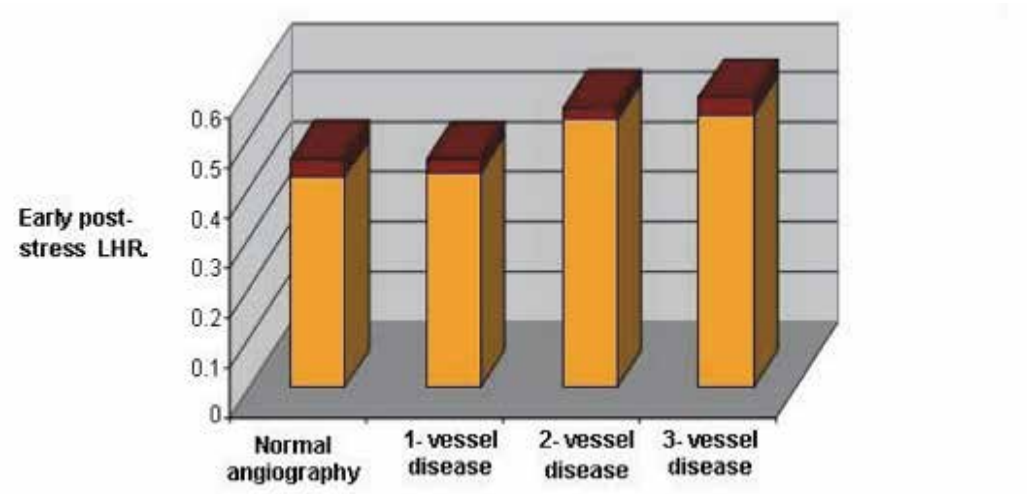


Fig. 2. Mean value (±SD) of early post-stress LHR, in patients with normal angiography, one-vessel disease, two-vessel disease and three-vessel disease (Reprinted from Nuclear Medicine Communications, Vol. 27, Georgoulias, P., Demakopoulos, N., Kontos, A., Xaplanteris, P., Xydis, K., & Fezoylidis, I., Early post-stress pulmonary uptake of 99m Tc tetrofosmin during exercise (SPECT) myocardial perfusion imaging: correlation with haemodynamic, perfusion and function parameters, pp. 119-126, 2006, with kind permission from Wolters Kluwer Health).

LHR, compared to conventional MPI, for the detection of multi-vessel disease (Okajima et al., 2004a; Tanigaki et al., 1998; Tsou et al., 2002). Specifically, the early post-stress LHR added incremental value to clinical, exercise testing, and myocardial perfusion and function data, for the identification of patients with multi-vessel CAD. In detail, if the cut-off point of early post-stress LHR value was set at 0.500, the sensitivity, positive and negative predictive value to detect multi-vessel CAD improved from 87%, 84% and 82% to 94%, 85% and 91%, respectively, while the specificity did not change considerably (78%) (Georgoulias et al., 2006).

On the other hand there are only few published data about the Tc-99m tetrofosmin LHR prognostic value (Casáns Tormo et al., 2001). In a previously published manuscript, we have evaluated the long-term prognostic value of early post-stress Tc-99m tetrofosmin LHR, in a cohort of 276 patients who were investigated with stress/rest Tc-99m tetrofosmin myocardial gated-SPECT (rest studies) and coronary angiography. During a mean follow-up period of 32.4 months (SD=9.6) hard cardiac events (cardiovascular death and non-fatal myocardial infarction) occurred in 28 (10.1%) patients and soft cardiac events (revascularization procedures) in 32 (11.6%) patients (Georgoulias et al., 2010a). Implying multiple Cox regression analysis with stepwise-forward approach, early post-stress LHR was found to be a significant independent predictor for both soft and hard cardiac events. The hazard ratio (for 0.1 unit increase) was 4.41 (95%CI: 1.52 - 12.73, p=0.006) for soft cardiac events and 4.22 (95%CI: 2.07 - 8.62, p<0.001) for hard cardiac events. The other significant prognostic factors were use of β-blockers, SSS and use of nitrates for soft events and exercise duration and SSS for hard cardiac events (Georgoulias et al., 2010a).

The cumulative soft event-free rates for one, two and five years were 100% (SE=0%), 99.2% (SE=0.8%) and 92.8% (SE=3.2%) for patients with early post-stress LHR value less than 0.500 and 98.5% (SE=1.0%), 96.1% (SE=1.7%) and 63.5% (SE=5.8%) for patients with early post-stress LHR value more than 0.500, respectively. Finally, the cumulative hard event-free rates for one, two and five years were 100% (SE=0%), 99.2% (SE=0.8%) and 89.8% (SE=3.7%) for patients with early post-stress LHR value less than 0.500 and 98.5% (SE=1.1%), 92.1% (SE=2.4%) and 75% (SE=5.0%) for patients with early post-stress LHR value more than 0.500, respectively (Fig. 3, 4).

Furthermore, the incremental prognostic value of early post-stress LHR was evaluated by a statistically significant increase in the global chi-square of the Cox proportional-hazard model that included clinical, exercise, angiographic and scintigraphic variables (Valotassiou et al., 2009). Using ROC analysis, the optimal sensitivity and specificity of various early post-stress LHR cut-off values for the prediction of cardiac events, was determined. ROC curve analysis showed that the optimal cut-off of early post-stress LHR for the prediction of soft cardiac events was 0.527 with sensitivity equal to 78.1% and specificity equal to 80.7 (Valotassiou et al., 2009). Similarly, the early post-stress LHR value of 0.530 represented the optimal cut-off for the prediction of hard cardiac events (sensitivity 68% and specificity

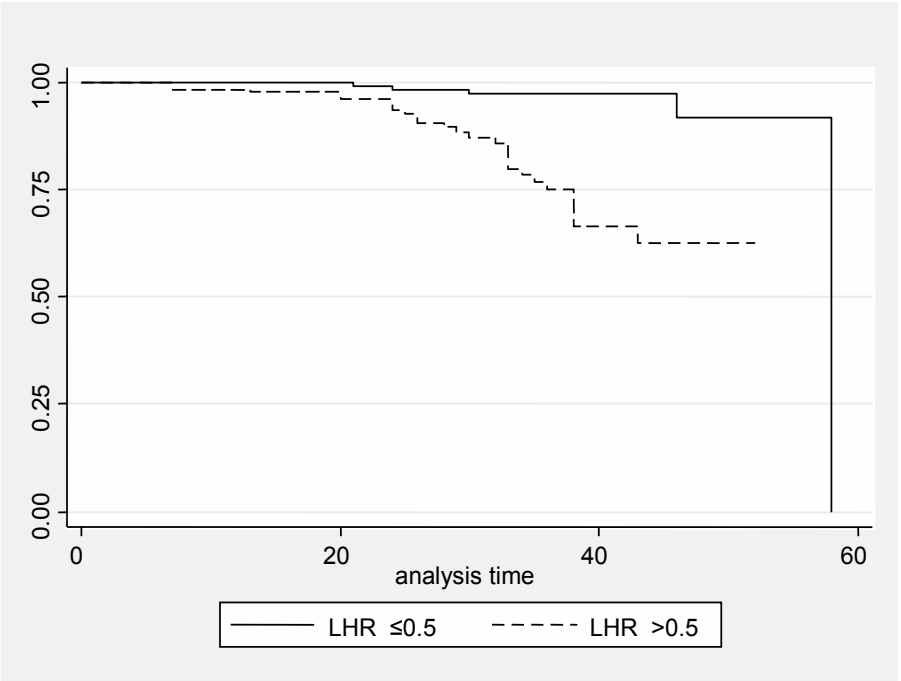


Fig. 3. Kaplan-Meier estimates for soft cardiac events according to early post-stress LHR levels (Reprinted from European Journal of Nuclear Medicine and Molecular Imaging, Vol. 37, Georgoulias, P., Tsougos, I., Valotassiou, V., Tzavara, C., Xaplanteris, P., & Demakopoulos, N., Long-term prognostic value of early poststress (99m)Tc-tetrofosmin lung uptake during exercise (SPECT) myocardial perfusion imaging, pp. 789-798, Fig. 2, 2010, with kind permission from Springer Science & Business Media B.V.).

78.6%). The area under the curve (AUC) was 0.80 (95% CI: 0.71 - 0.88) and 0.76 (95% CI: 0.66-0.86), for soft and hard cardiac events, respectively. The addition of early post-stress LHR in the Cox regression model, included clinical, exercise data and myocardial perfusion SSS, increased significantly the global chi-square for both soft and hard cardiac events ($p<0.001$) declaring the significant incremental value of early post-stress LHR. The adjusted hazard ratios for early post-stress LHR more than 0.53 were 9.44 and 7.51 (95% CI: 3.13 - 28.41, $P<0.001$) for soft and hard cardiac events, respectively (Valotassiou et al., 2009).

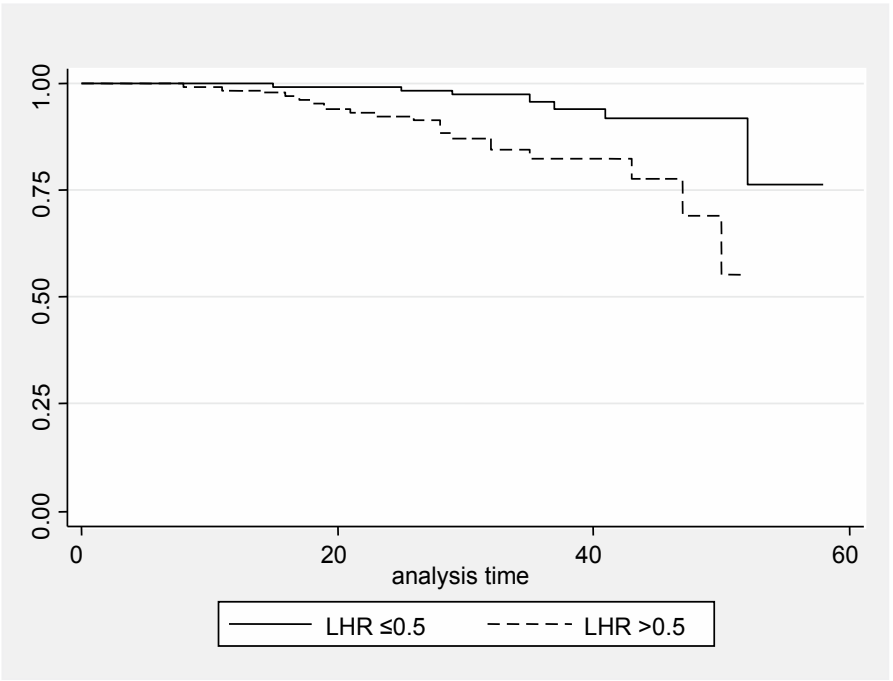


Fig. 4. Kaplan-Meier estimates for hard cardiac events according to early post-stress LHR levels (Reprinted from European Journal of Nuclear Medicine and Molecular Imaging, Vol. 37, Georgoulias, P., Tsougos, I., Valotassiou, V., Tzavara, C., Xaplanteris, P., & Demakopoulos, N., Long-term prognostic value of early poststress (99m)Tc-tetrofosmin lung uptake during exercise (SPECT) myocardial perfusion imaging, pp. 789-798, Fig.3, 2010, with kind permission from Springer Science & Business Media B.V.).

To summarize, early post-stress Tc-99m tetrofosmin LHR appeared to be a useful index of extensive myocardial ischaemia, heart dysfunction and multi-vessel CAD (Georgoulias et al., 2006). Moreover, our results suggest that early post-stress LHR is an independent and powerful predictor for both hard (death or myocardial infarction) and soft cardiac events (revascularization procedures), providing incremental prognostic information to that provided by clinical, exercise testing and scintigraphic data (Georgoulias et al., 2010a; Valotassiou et al., 2009). In addition, an early post-stress LHR value of 0.53 was the optimal-cut off for the prediction of any cardiac event and could be useful in clinical practice, contributing to more accurate patient risk stratification with valuable influence on their therapy (Valotassiou et al., 2009).

5. Incidental pathologic extra-cardiac findings during MPI

Extra-cardiac findings during acquisition of MPS (using Tl-201 or Tc-99m labeled radiotracers) can be visualized in the area being viewed, including mainly the thorax and the upper abdomen, depending on the patient body size and the camera field of view (Kim et al., 2002; Vijayakumar et al., 2005). These extra-cardiac uptake accumulations may be benign or malignant and require further investigation which might be life saving for the patient (Kim et al., 2002; Vijayakumar et al., 2005). After intravenous administration, normal uptake of Tc-99m tetrofosmin is seen in several organs, most commonly localized in the heart, lungs, breasts mainly during lactation and lymph nodes, while in the abdomen significant normal uptake can occur in the liver, gall bladder and bowel (Vijayakumar et al., 2005). Elimination of the radiotracer occurs mostly through the kidneys and the hepatobiliary system (Hesse et al., 2005).

Pathologic uptake of Tc-99m tetrofosmin can occur in benign or malignant tumors and also in infectious or non-infectious diseases (Vijayakumar et al., 2005). The mechanism of uptake of Tc-99m tetrofosmin in non-cardiac lesions is not completely understood, but the size of the lesion, its mitochondrial-rich cellularity and perfusion (factors) play a significant role (Kinuya et al., 2003; Sükan et al., 2004). Over-expression of P-glycoprotein or multi-drug resistance can decrease tumor uptake and are also associated with resistance to cancer treatment. Benign Tc-99m tetrofosmin incidental extra-cardiac uptake in the neck and chest has been reported in thyroid diseases, parathyroid adenomas, benign lymph node hyperplasia, esophagitis, neurofibroma, smoker's lung, lung infections, sarcoidosis and scapular hibernoma (Bestetti et al., 1996; Kannan et al., 2007; Oller et al., 2001; Vijayakumar et al., 2005). Photopenia in the lung bases due to pleural effusions and abnormal right liver configuration caused by elevation of the right hemi-diaphragm has also been reported during Tc-99m tetrofosmin MPS (Shih et al., 2002). In neck and chest malignant diseases, extra-cardiac uptake has been reported in: thyroid cancer, neuroendocrine tumors, mediastinal tumors, lung cancer, breast cancer, esophageal carcinoma, lymphoma, Kaposi's sarcoma, multiple myeloma and in nasopharyngeal cancer (Khairallah et al., 2002; Okajima et al., 2004b; Torreggiani et al., 1999; Vijayakumar et al., 2005). Moreover, Tc-99m tetrofosmin incidental uptake in abdominal abnormalities has been detected during cardiac acquisition, when the area being viewed includes the lower thorax and the upper abdomen, in abnormalities of the liver, gallbladder, kidneys, oesophagus, stomach, bowel and bone marrow (Shih et al., 2002). Hepatocellular carcinoma, melanoma, sarcomas and multiple myeloma have also been described in the abdomen, accumulating Tc-99m tetrofosmin in MPS (Fisher et al., 2000; Hadase et al., 2003; Vijayakumar et al., 2005; Yi & Jacobs, 2004).

Others, reviewing the raw data cine images of 566 patients during Tl-201 dipyridamole Tc-99m tetrofosmin rest-stress MPS, found 234 abnormalities (Shih et al., 2002; Shih et al., 2005) such as: bone marrow visualisation (39.7%), duodenogastric and enterogastric reflux (20.1%), non-visualisation of the gallbladder (13.2%), small-atrophic-scarred, vaguely seen or ectopic kidneys, splenomegaly, liver diseases like hepatomegaly and cirrhosis and breast attenuation causing photopenia in the liver. The authors suggested that Tc-99m tetrofosmin is accumulated in the red bone marrow due to high and/or expanded haematopoetic activity. It is obvious that all these coincidental abdominal abnormalities should alert the referring physician to suggest further investigation. In addition, duodenogastric and enterogastric refluxes, which represent approximately 20% of the abdominal abnormalities, may cause symptoms mimicking angina. Recently, Gratz et al. reported six patients with

unexpected abnormal mediastinal and/or thoracic activities, out of 2155 who underwent Tc-99m tetrofosmin MPI. Subsequently, the patients underwent resection of a thymoma (n=2), nonsmall cell lung cancer (n=1) and breast cancer (n=3) (Gratz et al., 2008).
We have previously published an unusual case of a 60 year-old woman with atypical precardiac symptoms who underwent Tc-99m tetrofosmin stress - rest SPECT imaging (Kotsalou et al., 2008). The MPI gated-study was normal. However, an incidental finding of intense extra-cardiac uptake of the radiotracer in the left paracardiac area was observed (Fig. 5).

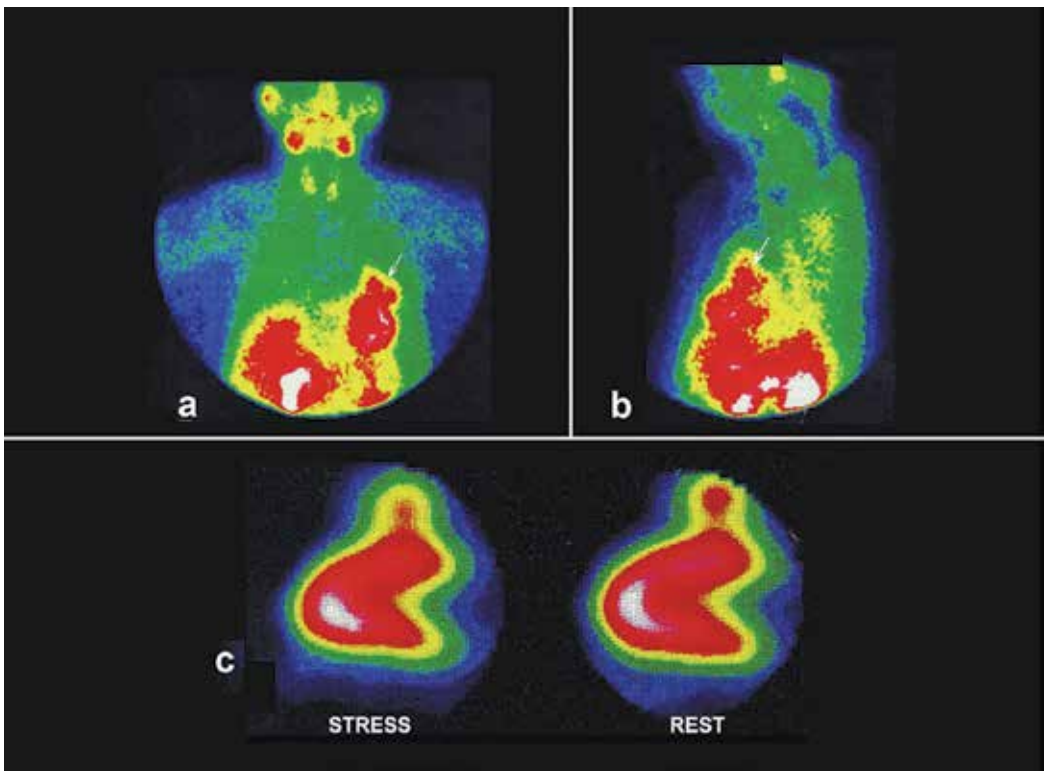


Fig. 5. Anterior (a) and left lateral (b) projections of the thorax, demonstrated intense Tc-99m tetrofosmin uptake in the left paracardiac area (arrows). Myocardial perfusion SPET imaging was normal (c) (Reprinted from Hellenic Journal of Nuclear Medicine, Vol. 11, Kotsalou, I., Georgoulias, P., Fourlis, S., Zoumboulidis, A., Giaslakiotis, K., Androulaki, A., Chronopoulos, P., & Dimakopoulos, N., Incidental pathologic extracardiac uptake of 99mTc-tetrofosmin in myocardial perfusion imaging, pp. 43-45, 2008, with kind permission from the Hellenic Society of Nuclear Medicine).

The computerized tomography (CT) and magnetic resonance imaging (MRI) tests revealed a mass of 6 cm diameter in the left lower anterior mediastinal area (Fig. 6). The patient after a biopsy underwent surgical resection of the mass through medial sternotomy followed by adjuvant radiotherapy of the mediastinum, because of microscopic invasion of the capsule. The histologic-immunochistochemical examination established the diagnosis of a thymoma type AB and stage 2b (Masaoka II2 and TNM Pt2) (Fig. 7).

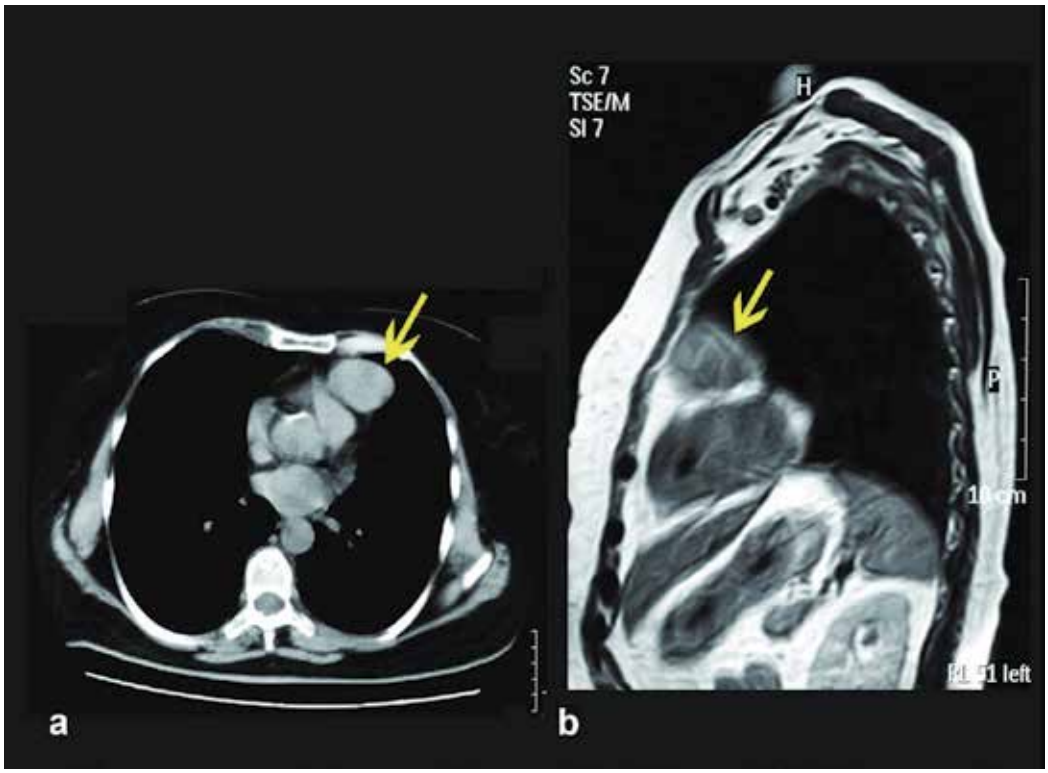


Fig. 6. Computed tomography (a) and magnetic resonance imaging (b) revealed a solid mass located in the left lower anterior mediastinal area, in contact but not infiltrating pericardium, while signs of pressing the left lung were also noticed (arrows). Thymoma was the possible diagnosis (Reprinted from Hellenic Journal of Nuclear Medicine, Vol. 11, Kotsalou, I., Georgoulias, P., Fourlis, S., Zoumboulidis, A., Giaslakiotis, K., Androulaki, A., Chronopoulos, P., & Dimakopoulos, N., Incidental pathologic extracardiac uptake of 99mTc-tetrofosmin in myocardial perfusion imaging, pp. 43-45, 2008, with kind permission from the Hellenic Society of Nuclear Medicine).

Other authors have also reported cases of incidental thymoma detection during MPS (Chadika et al., 2005; Douglas et al., 2000; Rebollo Aguirre et al., 2003; Sciagrà et al., 1998; Vijayakumar et al., 2004; Vijayakumar et al., 2005). Moreover, Douglas et al. reviewed three cases of incidental occult thymoma, detected during dual isotope Tl-201 and Tc-99m tetrofosmin SPECT imaging (Douglas et al., 2000). The authors suggested that solitary extra-cardiac uptake on Tc-99m images without Tl-201 uptake, corresponds to well- differentiated tumors, while the opposite corresponds to poorly-differentiated thymoma (Douglas et al., 2000).

6. Conclusion

In conclusion, early post-stress Tc-99m tetrofosmin LHR has a significant clinical value, as a useful index of extensive myocardial ischaemia, heart dysfunction and multi-vessel CAD. Moreover, early post-stress LHR appears as an independent and powerful predictor,

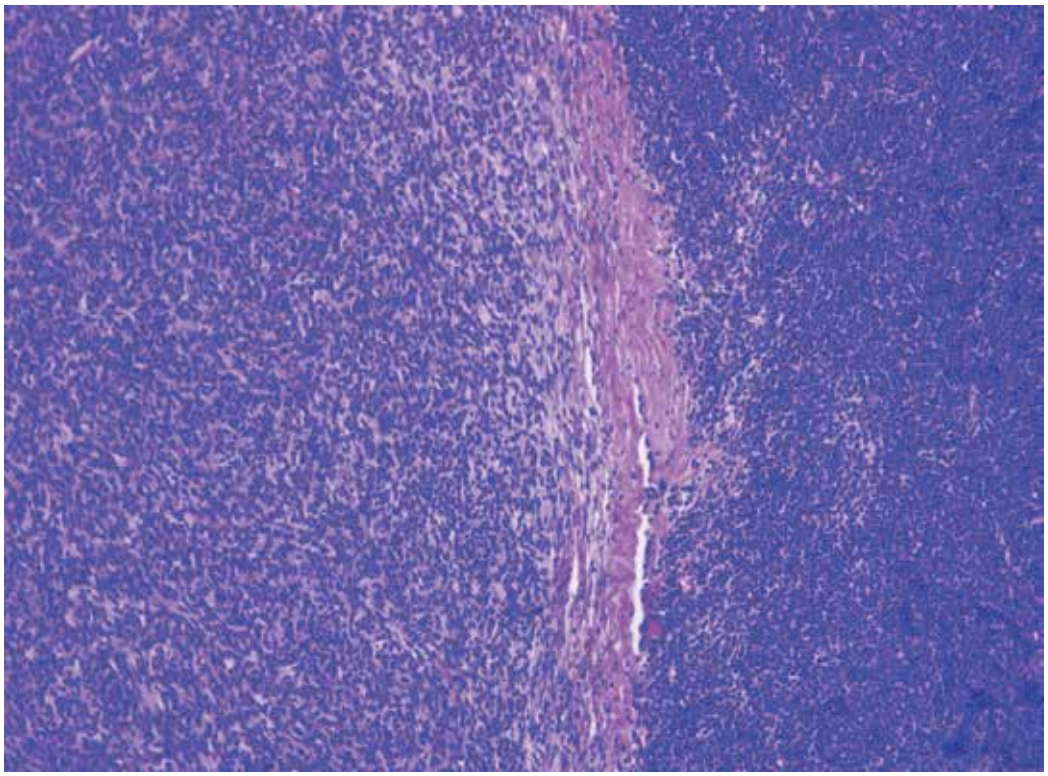


Fig. 7. Histologic and immunochistochemical examination confirmed the diagnosis of a mixed type AB thymoma, stage Masaoka II2 and TNM Pt2 (Reprinted from Hellenic Journal of Nuclear Medicine, Vol. 11, Kotsalou, I., Georgoulias, P., Fourlis, S., Zoumboulidis, A., Giaslakiotis, K., Androulaki, A., Chronopoulos, P., & Dimakopoulos, N., Incidental pathologic extracardiac uptake of 99mTc-tetrofosmin in myocardial perfusion imaging, pp. 43-45, 2008, with kind permission from the Hellenic Society of Nuclear Medicine).

assigning incremental prognostic information to that provided by clinical, exercise testing and scintigraphic data. In addition, an LHR value of 0.53 was the optimal-cut off for the prediction of any cardiac event and could be useful in clinical practice. It seems that the clinical value of routine pulmonary uptake measurements during Tc-99m tetrofosmin myocardial scintigram, in immediate post-stress images, as an ancillary scintigraphic sign, will maximize not only the information it provides for the assessment of the severity of myocardial ischaemia and CAD, but also the prognostic value of the study. In an era where a continuous effort is underway for deriving as many elements as possible from the examinations, the calculation of early post-stress LHR during myocardial perfusion imaging may contribute to a better (more accurate) patient risk stratification with valuable influence on their therapy.

Additionally, careful inspection of projection images should be an integral part of interpreting MPI studies. According to the literature, during Tc-99m tetrofosmin MPI various incidental extra-cardiac neck, chest and abdominal abnormalities have been detected. Even though MPI is performed for cardiac evaluation, Nuclear Medicine physicians should be aware of non-cardiac uptake of the radiotracer which can play an

essential role in early tumor detection resulting in life-saving early therapy. Any extra-cardiac focal uptake of Tc-99m tetrofosmin requires attention of the interpreting physician and has to be mentioned in the report, guiding the referring physician to request further investigation. The identification of these coincidental findings is significant for the early detection of the coexisting pathology and may prove to be essential in saving patient's life.

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14

Myocardial Perfusion Imaging in Diagnosis of Culprit Lesion in Patients Undergoing Elective Percutaneous Coronary Intervention

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1. Introduction

Myocardial perfusion imaging (MPI) was developed in the 1970s and has been used increasingly in clinical cardiology since the 1980s (Underwood et al., 2004). Technical developments that have fuelled this recent increases are single-photon emission computed tomography (SPECT) imaging, pharmacological stress and ECG-gated SPECT imaging. MPI comprises the only widely available method of assessing myocardial perfusion directly and many previously published reports support its evidence in the diagnosis of myocardial ischemia and necrosis. Moreover, the prognostic value of this method for patients' risk stratification has already been extensively reported, with an incremental prognostic value after clinical assessment, exercise electrocardiography and even above coronary angiography. Thus, MPI is an established imaging technique that is already an integral part of the management of coronary artery disease (CAD) (diagnosis, prognostication, selection for revascularization and assessment of acute coronary syndromes) and is included in a number of professional guidelines. (1, 2)

In the past two decades, a great body of literature has established the use of nuclear imaging for risk stratification in patients with known or suspected CAD. Risk stratification is of crucial importance for the practice of contemporary medicine. Extending the paradigm of noninvasive cardiac testing beyond the detection of disease is especially important, may risk assessment permits patients who are identified as being at a high risk for subsequent cardiac events should receive aggressive management, possibly including cardiac catheterization for potential revascularization procedures that may improve their outcome. Conversely, the management focus in patients with low future event rate should be shifted toward risk factor modification and aggressive medical therapy, reserving invasive procedures for

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patients who fail medical management. CAD is disease with a wide spectrum of severity and extent with outcome, such as nonfatal myocardial infarction (MI) or cardiac death being related to the severity of disease. Clinical trials have shown that patients with severe CAD as left main coronary artery disease, especially those with left ventricular dysfunction, can benefit from coronary artery bypass graft surgery (CABG) with significant reduction in their mortality rate (1, 2, 3). Whereas patients with single-vessel or with two-vessel disease (without proximal left anterior descending artery involvement) would have improved symptoms of angina following CABG and percutaneous transluminal coronary angioplasty with or without stent implantation, without any effect on their mortality rate.

Risk assessment based on clinical finding and resting ECG only is limited. Exercise testing can also help, especially when examining the patient's functional capacity. Exercise-induced ECG changes and risk indices also have substantial prognostic value. Unfortunately most patients (55%) with suspected CAD would fall in an intermediate-risk group, necessitating additional risk stratification.

Coronary angiography, considered the "gold standard" for the diagnosis of CAD, often does not provide information about the physiologic significance of atherosclerotic lesions, especially in borderline lesions. More importantly, it does not provide a clear marker of risk of adverse events, especially in patients with moderate disease severity. Andreas Gruentzig said; "*When coronary angiography founded coronary artery disease, I would like to have diagnostic procedure who will give me functional significance that lesion.*"(2, 3, 4, 5)

2. Risk based on nuclear imaging results

Determination culprit lesion

The current definition of culprit lesion; that is zone of ischemia under the coronary stenoses (what degree?) That is not quite good definition. Some autors offer degree of coronary stenoses ≤ 70 %, some ≤ 75%, even < 80-85%) but is not quite wright, because that is not definy two pathophysiologic aspects of ischemia; severity and extent. Ladenhaim et al. have also shown that the magnitude of ischemia (severity and extent) correlates well with cardiac events. Some other autors shown correlation between event rate (death, nofatal IM and revascularization) and extent of ischemia demonstrated by the number of ischemic segments on SPECT scan. Iskander and Iskadrian have also shown that defects reversibility is an important predictor of type of cardiac events, whereas reversible perfusion defects are associated with nonfatal MI. This is very important finding, since a reversible defect on the myocardial perfusion imaging (MPI) by single photon-emission computed tomography (SPECT) is the only available diagnostic tool that can independently predict the risk of nonfatal MI. Therefore, stress perfusion studies should be reported documenting defect severity (mild, moderate, severe), size (small, moderate, large) and reversibility to provide essential risk stratification (2, 4, 5, 6).

The value of MPI comes from the ability to identify and quantify the degree of jeopardized myocardium during stress tests. The size and severity of the perfusion abnormality provide powerful prognostic information and has been shown to directly relate to outcome. SPECT perfusion imaging and determination of culprit lesion is more predicitble of cardiac events than coronary angiography. As SPECT imaging may identify those patients at high risk for subsequent cardiac events, perfusion imaging may be used to help guide further testing and revascularization procedures, and this obviously has important cost-effectiveness ramifications.

The primary objective of those study was to determinate and localizes culprit lesion by knew introduce parameters SRS (*summary reversible score*) and ISRS (*index of summary reversible score*), under the angiographically detected coronary narrowing ≥75% for the least one coronary artery (2, 14).

A welt of literature supports the use of MPI for risk stratification in patients with known or suspected CAD. The ability of SPECT imaging to localize and define the culprit lesion (extent/severity) of disease predicts subsequent cardiac events such as MI or cardiac death. Furthermore, specific applications of these nuclear cardiology techniques, such as post infarction or in patients with unstable angina, have also successfully assessed risk of cardiovascular events. The prognostic applications of perfusion imaging are germane to all health care providers, as these methods may be used to guide subsequent tests and treatments. MPI has significantly impact on patient management decisions and the cost-effective utilization of health care.

The rapid rate of technical advances and improved operator expertise has enabled this technique to gain more widespread application. Despite the large number of PTCA performed yearly, preprocedure documentation of myocardial ischemia is uncommon, occurring in only 29% of patients. Despite the obvious value of nuclear cardiology to detect, localize, and define the extent of ischemia, this procedure appears underutilized before performance of percutaneous intervention. It is unclear whether this reflects an under utilization of noninvasive methods to objectively justify the performance of PTCA or whether the addition of such techniques is considered superfluous.

Myocardial perfusion imaging provides information on the extent and location of myocardial ischemia. The assessment of jeopardized myocardium may be performed and provides a measure of the relative value of PTCA in terms of the amount of jeopardized myocardium. The location of the stenosis may dictate the area at risk: extent and severity of perfusion defects were significantly smaller in patients with proximal compared with distal coronary artery occlusions (2, 14).

Before revascularization is performed, myocardial perfusion imaging may assist in management decisions by demonstrating the presence of myocardial ischemia, viability and delineating the severity and extent of coronary artery disease. The significance of equivocal lesions may be determined and culprit vessel may be successfully defined by SPECT imaging before angioplasty.

The aim of this study was to determinate and localizes culprit lesion by myocardial perfusion imaging (MPI), under the angiographically detected coronary narrowing ≥75% for the least one coronary artery. One hundred thirty-two (132) patients with known coronary artery disease (CAD) were studied. In all of them angiographically detected significant coronary narrowing (≥75% luminal stenosis for the least one coronary artery). Al patients submitted MPI ^{99m}Tc-MIBI, with pharmacologic adenosine stress protocol with concomitant low level bicycle exercise 50W **(AdenoEX)**. We were measured relative uptake ^{99m}Tc-MIBI for each myocardial segment using short-axis myocardial tomogram study. A 5-point scoring system was used to assess difference between uptake degree in stress and rest studies for the same segments, and we were created two index; Sum reversibility score (SRS), Index of sum reversibility score (ISRS). **Results:** A total of 396 vascular territories (2244 segments) were analyzed before elective percutaneous coronary intervention (ePCI). Overall sensivity, specificity, and accuracy using SS were 90.2%, 87.5%, 89.4%, with positive predictive value 94, 1%. Overall sensitivity, specificity, and accuracy using ISRS were 94.4%,

90.6%, 93, 2%, with positive predictive value 95, 7%. Conclusion: MPI with two created index SRS and ISRS significantly improves sensitivity, specificity, and accuracy for determination culprit lesion in patients undergoing PCI. **Conclusion:** AdenoEX MPI significantly improves sensitivity, specificity, and accuracy for determination and localization culprit lesion in patients undergoing ePCI.

3. Coronary artery disease – exercise and pharmacologic stress test

Coronary artery disease (CAD) is still single greatest cause of death of men and women. In the USA more than 9 million patients are referred yearly for diagnostic cardiac stress test with radionuclide imaging. Although an increasing number of patients undergo pharmacologic stress because they are unable to perform adequate physical stress, such as maximal workload, duration, hemodynamic response, exercise-induced symptoms, and electrocardiography (ECG) changes, provide invaluable additional information for assessing a patients condition not available with pharmacologic stress. Pharmacologic stress testing accounts for approximately 48% of stress myocardial perfusion studies done to detect CAD in the US. Exercise stress test is preferred for patients who can exercise and achieve adequate exercise endpoints. Pharmacologic stress testing is reserved for patients who have exercise limitations. It is estimated that at least 25% of patients and 50% of hospitalized patients cannot perform maximal exercise. Pharmacologic stress can be done with vasodilator agents (adenosine, dipyridamole, adenosine triphosphate, or selective adenosine A2a receptor agonist) or with inotropic and chronotropic agents (dobutamine or arbutamine). Patients with left bundle branch block (LBBB) or electronically paced rhythms may have anteroseptal perfusion defects with exercise or dobutamine perfusion imaging unrelated to stenosis of the left anterior descending artery and hence vasodilator stress testing is recommended in these patients (6, 7, 8, 9).

Adenosine and dipyridamole stress have been used in combination with exercise especially in patients with limited exercise capacity. The reported benefits of the combination protocols include improvement in ischemia detection and image quality, and reduction in side effects. The addition of exercise to vasodilator stress might partly overcome the roll-off phenomenon observed with vasodilators alone resulting in more radiotracer extraction and better estimation of CAD.

Simultaneous exercise in conjunction with adenosine stress was safe and significantly reduced adenosine side effects by 30%-40% and enhanced image quality. These benefits were similar with sub maximal or maximal exercise (6-9, 10)

Similar to adenosine, patients who underwent dipyridamole-exercise had fewer noncardiac side effects, more ischemic ECG changes, higher HR and systolic BP, and better image qualities with increased heart to liver and heart to gut count ratios.

One of the most powerful uses of MPI is the evaluation of the risk for future events in patients with suspected or known CAD. Over the years, MPI has evolved as an essential tool in the evaluation and assessment of patient prior to coronary revascularization. It has a dual role. Prior to coronary angiography, MPI is extremely useful in documenting ischemia and determining the functional impact of single or multiple lesions identified subsequently. MPI remains the test of choice for identifying the lesion responsible for the ischemic symptoms, or so called culprit lesion. That is extremely useful for further management decisions with respect to percutaneous interventions. In compare, the absence of reversible ischemia in

patients with known CAD is an excellent prognostic marker and predicts a low annual event rate. The current definition of culprit lesion that is zone of ischemia under the coronary stenoses is not quite right, because that is not defined two pathophysiologic aspects of ischemia; severity and extent. The primary objective of those study was to determinate and localizes culprit lesion by knew introduce parameters SRS (*summary reversible score*) and ISRS (*index of summary reversible score*), under the angiographically detected coronary narrowing ≥75% for the least one coronary artery (2, 14).

4. Methods

One hundred thirty-two (132) patients with known CAD were studied. In all of them angiographically detected significant coronary narrowing (≥75% luminal stenosis for the least one coronary artery). All patients were submitted to 2 iv injections of ^{99m}Tc-MIBI, one in a peak pharmacologic stress test with concomitant low level exercise stress test (50W) **AdenoEx** protocol; we administered adenosine (in the dose of 140 μg/kg/min) in combination with supine bicycle exercise low level 50W. We started infusion at the end of the 1st minute bicycle exercise, and finished in the 5th minute. Bicycle exercise was continuing one minute more until 6th minute. Radiopharmaceutical ^{99m}Tc-MIBI was administrated during the infusion at the end of 2nd minute. Imaging started 15 minutes after iv. ^{99m}Tc-MIBI 740 MBq and the other 370 MBq at rest 3 hour after in the rest study.
Images of the heart were taken; 15 min after injections for the stress studies, and 30 minutes after injections for the rest study, using an Orbiter Siemens gamma camera, which was fitted with a low energy, all purpose collimator, and connected with a dedicated computer system. Briefly, 32 projections were obtained over a semicircular 180^0 arch wich extended from the anterior 0^0 to the left posterior position 180^0. In each patient, we were using Stirner quantification program (Euro menu) modificated and standardized myocardial segmentation and nomenclature for topographic imaging of the heart analyzed SPECT. Quantification regional ^{99m}Tc-MIBI uptake was performed using short-axis myocardial tomography that was divided on 16 segments + apex for each study (2, 11).
The left anterior descedenting artery (LAD) vascular territory including; basal anterior, basal anteroseptal, mid anterior, mid anteroseptal, apical anterior, apical septal, and apex; Left circumflex artery (LCx); basal inferolateral, basal anterolateral, mid inferolateral, mid anterolateral, apical lateral; Right coronary artery (RCA); basal inferoseptal, basal inferior, mid inferoseptal, mid inferior, apical inferior.
We were measured relative uptake, in area individual coronary artery vascular territory, from each segment and compare with the segment with the best uptake, and we founded in the **AdenoEx study;** Normal relative uptake (> 85%); Probably normal (75%-85%); Equivocal (65%-75%); Probably abnormal (50%-65%); Abnormal (< 50%). **Rest study;** Normal relative uptake (> 90%); probably normal (80%-90%); equivocal (70%-80%); probably abnormal (55%-75%); abnormal (< 55%). Different between relative uptake each segment we were scoring with a 5-point scoring system to ass's difference between uptake degree in stress and rest studies for the same segments (1= normal, 2= mild ischemia, 3= moderate ischemia, 4= reversibility, 5= severe reversibility).
We were introduced two knew index score to determinate culprit lesion. **Summary reversible score (SRS)** ≥ 3 in the territory of stenoses coronary artery was determinate culprit lesion. At least two segments with score **5 (index of summary reversible score-ISRS)** in the territory of stenoses coronary artery was determinate culprit lesion.

Results: A total of 396 vascular territories (2244 segments) were analyzed before elective percutaneous coronary intervention (ePCI). Overall sensitivity, specificity, and accuracy using SS were 90.2%, 87.5%, 89.4%, with positive predictive value 94,1%. Overall sensitivity, specificity, and accuracy using ISRS were 94.4%, 90.6%, 93, 2%, with positive predictive value 95, 7%. Conclusion: MPI with two created index SRS and ISRS significantly improves sensitivity, specificity, and accuracy for determination culprit lesion in patients undergoing PCI.

5. State of the art and future directions

Since the introduction of myocardial perfusion imaging (MPI) into clinical medicine in late 1970s, this technique has undergone considerable expansion and evolution. Initially, myocardial perfusion imaging was introduced as a noninvasive diagnostic tool in determining the presence or absence of coronary artery disease (CAD).

The ability to distinguish patients at low risk from those at high risk for future cardiac events has become an essential adjunct for clinicians in the management of patients of CAD. The power of myocardial perfusion imaging (MPI) for predicting future coronary events has been demonstrated in a large number of high-quality studies and in many thousands of patients. It is perhaps the area of nuclear cardiology where the evidence is most strong. The most important variables that predict the likelihood of future events are the extent and severity of inducible ischemia. In general, markers of left ventricular dysfunction tend to predict cardiac mortality and inducible ischemia predicts acute coronary syndromes. MPI has incremental prognostic value even after clinical assessment, exercise electrocardiography and coronary angiography. In other words, patients who appear to be high risk after coronary angiography can be separated into higher and lower risk groups by MPS. In addition, several studies have indicated that a negative SPECT study confers an excellent prognosis with an annual cardiac event rate of <1% for the general population. In the setting of a normal myocardial perfusion study in a low-risk patient, it takes 9 years for the risk of a cardiac event to reach 1%, suggesting that, in the absence of new symptoms, a repeat perfusion study may not be needed for 3 to 5 years (13, 14, 15). However, this "warranty period" does not appear to be absolute and is affected by clinical and technical factors, including the presence of diabetes or CAD, increasing age and male gender, and the need to perform a pharmacologic stress test rather than an exercise perfusion imaging test, which can increase the annual cardiac event rate in patients with a normal perfusion scan to as high as 1.8%. In these high-risk patients with normal myocardial perfusion studies, it may be prudent to perform repeat perfusion imaging on a more frequent basis.

Because of its prognostic power, MPI can be used as the gatekeeper to coronary angiography. Bateman and colleagues showed that referral to coronary angiography after normal, mild to moderately abnormal and severely abnormal perfusion scans was 3.5%, 9% and 60% respectively. Importantly, a policy of selective referral to coronary angiography based upon high-risk findings is defensible, as patients with mild to moderate abnormalities when managed medically have outcomes comparable to those undergoing invasive evaluation and subsequent revascularization. Besides, several reports underlie that such a policy can be also cost-effective even if it is more expensive than an alternative test such as the exercise ECG. Furthermore, MPS can provide useful information about cardiac risk in patients requiring non-cardiac surgery although these patients are generally at low risk and

the predictive value of a normal perfusion study is greater than that of an abnormal study, while the clinical value of MPS to assess patients with acute coronary syndrome has been well established.

MPS is of proven value to assess patients post revascularization. Information gained from post-intervention myocardial SPECT is important to differentiate patients with angina from those with echo-cardiac chest pain syndromes, to assess peri-intervention myocardial damage/acute vessel closure, to predict-detect restenosis after PCI and graft occlusion/stenosis after CABG surgery, to detect CAD progression in non-revascularized vessels, to evaluate the effects of intervention if required for occupational reasons and to predict patients' long-term prognosis (16, 17).

The assessment of patients' prognosis is central to the clinical management of patients with CAD. Patients with CAD can be characterized along a continuum of risk for cardiac events. When the risk of cardiac events low, cardiologists generally employ conservative medical management. Conversely, when the risk of cardiac events is high, aggressive patient management, such as the performance of coronary bypass surgery or coronary angioplasty, tends to be favored. Between these extremes of risk, are a large number of patients who have an intermediate risk of cardiac events, which can be arbitrary and roughly defined as a likelihood of from 2% to 5% of major cardiac events over the ensuing year. Decision making to such patients is challenging, since the indication for conservative versus aggressive treatment is most uncertain in this group. Thus, the clinical often desires additional prognostic information about such patient to better define the likelihood of cardiac events. It is this group of patients in whom radionuclide stress testing finds its greatest prognostic benefit. The prognostic utility of radionuclide stress tests derives from their ability to quantify the magnitude of jeopardized myocardium during exercise or during pharmacologic stress testing with dipyridamole or adenosine. Specifically, MPI measures two indices of ischemia: ischemic extent and ischemic severity. Ischemic extent indices reflect the area of myocardial mass that became during stress. Ischemic severity indices correlate roughly with the number of stenosed coronary arteries. The number of reversible myocardial perfusions defects seen by MPI SPECT, constitutes a typical variable of ischemic extent. By contrast, ischemic severities indices reflect the magnitude of inducible ischemia within a given myocardial region. For instance, the severity of a perfusion defects reflects the severity of subtending coronary stenoses. Variables of ischemia extent and severity that can be assessed with stress myocardial perfusion SPECT are shown in the Table 1.

Included in this list are two variables that may be assessed by obtaining an early anterior planar scintigram before SPECT imaging: a) the post-stress lung uptake of isotope, and b) the transient post-stress ischemic dilatation of the left ventricle.

Because of its clinical importance, information about the extent and severity of jeopardized myocardium should be incorporated into the routine reporting of radionuclide stress test results. Conventional practice is to divide the short axis of the left ventricle into the three regions: apical, mid-ventricular and basal. The apex is assessed from the vertical long axis slices. Our approach is to assess the reduction in regional uptake of isotope in each of the 17 myocardial segments. On a five-point scale, as follows: 1 = none, 2 = mild, 3 = moderate, 4 = severe and 5 = complete reduction in regional uptake. Comparison of the stress and rest scores provides the physician with a quantitative estimation of the degree of reversibility of each myocardial defect. From the location of defects, it can be estimated which coronary vessels are the most likely culprit lesions for the induction of myocardial ischemia (2, 10, 16, 17, 18).

Predictor	Ischemia Extent	Ischemia Severity
- Number and/or location of reversible defections	++++	0
- Magnitude of defects	0	++++
- Delayed redistribution	+	++++
- Lung uptake of isotope[a]	+++	+
- Transient LV dilatation[a]	++++	++++
- Magnitude of WMA[1]	0	++++
- Number/location of WMA[1]	++++	0
- Change in LVEF stress[1]	+++	++

[a]Best assessed by obtaining a 5-minute and 4-hour anterior planar scintigram before the initiation of SPECT imaging.
[1]Obtained from concomitant rest-exercise first-pass radionuclide ventriculography when employing ^{99m}Tc-agents.
LV left ventricle; WMA wall-motion abnormality; EF ejection fraction

Table 1. Predictors of stress-induced ischemia extent and severity using SPECT and adjunctive scintigrams.

The relationship between the magnitude of inducible myocardial ischemia and the likelihood of cardiac events is not linear. Previous investigation has shown that the magnitude of ischemia has an exponential relationship to the occurrence of subsequent cardiac events. Patients who demonstrate only mild ischemia at a peak stress have only a small, relatively flat increase in the likelihood of cardiac events as compared to patients who manifest no scintigraphic evidence of inducible ischemia. But, once ischemia progressed to a moderate magnitude, the likelihood of cardiac events begins to increase sharply. Ladenheim and colleagues performed a 1-year follow-up of 1.689 patients without prior myocardial infarction (MI) who underwent exercises planar MPI for diagnostic or prognostic purposes. The frequency of hard cardiac events (MI/cardiac death) and late (> 60 days) bypass surgery after testing were recorded as cardiac events. Ischemic extent and ischemic severity were exponentially related to the cardiac event rate (2, 17, 18).
Based on the published prognostic literature, four points may be derived that can serve as general rules of thumb for the utilization of scintigraphic testing in clinical practice:

6. Use of radionuclide stress testing in patient-management decision

- Risk assessment in patients with a high likelihood of CAD
- Selection of therapy in patients with angiographically documented CAD;
- Selection between medical therapy versus revascularization.
- Identification of **culprit** lesions prior to coronary intervention.
- Evaluation of borderline coronary artery stenosis.
- Risk stratification of post-MI patients
- Predischarge exercise testing.
- Predischarge pharmacological stress testing.
- Evaluation of patients following thrombolysis.
- Predischarge evaluation of patients with unstable angina

- Risk stratification of the elderly
- Risk stratification of patients with congestive heart failure and/or left ventricular dysfunction
- Evaluation of patients following treatment modalities for CAD
- Percutaneous coronary intervention
- Coronary artery by-pass surgery
- Medical therapy
- Risk stratification of patients prior to elective noncardiac surgery

Substitution of pharmacological stress testing for risk stratification. In general, the performance of myocardial perfusion scintigraphy with exercise as opposed to pharmacological stress is preferable for prognosis purposes. Important prognostic variables associated with exercise ECG testing include exercise capacity, exercise-inducible chest pain or hypotension, and the ECG response to exercise, particularly the heart rate threshold and post-exercise duration of stress-induced ST-segment depression. These variables cannot be assessed when pharmacologic instead of exercise testing is employed.

However, the performance of myocardial perfusion imaging in conjunction with pharmacologic stress testing, either with dipyridamole or with adenosine, has essentially the same sensitivity and specificity for detecting CAD as does exercise myocardial perfusion scintigraphy. Moreover, studies done with both modalities indicate that magnitude of ischemic defects induces by exercise is not underestimated by those induces by pharmacologic stress. A normal scintigraphic study in association with pharmacological stress is associated with a same low risk of cardiac events as is a normal exercise myocardial perfusion study. Dipyridamole or adenosine SPECT is commonly employed as the pharmacological stress agent, given its ease to use. Myocardial perfusion imaging (MPI) can also be performed in conjunction with dobutamine or arbutamine stress, but is generally reserved for patients with asthma or chronic lung disease. Despite the theoretical advantages of exercise in assessment prognosis, excellent risk stratification has been reported with adenosine SPECT imaging, with results similar to those observed with exercise.

Based on the strongly documented prognostic efficacy, MPI has emerged as a key guide for major medical decisions involving patients with suspected or known CAD

Myocardial perfusion imaging provides incremental prognostic value, particularly in patients and an intermediate or high pretest likelihood of CAD or patients with stable CAD and mild symptoms. Patients who exhibit normal myocardial perfusion and function on gated SPECT have an excellent prognosis and should be referred for non cardiac evaluation for determining etiology of the presenting symptoms. Conversely, patients with high-risk scans may benefit from an early invasive strategy with a view toward revascularization depending on coronary anatomical finding. A substantial number of patients undergoing SPECT perfusion imaging will have mild ischemia without a multivessel disease scan pattern. If patients with mild ischemia have good exercise tolerance, they should be considered as candidates for intense medical therapy with follow-up exercise SPECT imaging possibly at 1 year. Unpublished data from the Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluations (COURAGE) trial seem to indicate that many ischemic defects may markedly improve with aggressive lowering of abnormal lipids an the other pharmacological interventions. Hachamovitch and colleagues reported patients with the mildly abnormal scan had a 0.8% annual cardiac death rate compared with 0.9% for those who underwent revascularization. The death rate in medically treated patients who had moderately abnormal scans was 2.3% versus 1.1% for such patients undergoing

revascularization. Finally, patients with a severely abnormal scan treated medically had an annual cardiac death rate of 4.6% versus 1.3% for such patients who were revascularized. In the second study, these investigators showed that medically treated patients who had greater than 20% of the total myocardium rendered ischemic had higher annual cardiac death rate (6.7%) compared with 2.0% for patients with this degree of extensive ischemia who underwent revascularization. For patients with 10% or less of the total myocardium rendered ischemic, there was no difference in outcome between medical therapy and revascularization (2, 12, 13, 17, 18).

Exercise myocardial perfusion imaging is a valuable adjunct for separating high to low risk patients who present symptoms consistent with stable CAD, or in patients who have known disease and in whom further prognostication is warranted. Multiple high-risk nuclear imaging variables can be identified, and the greater the extent of exercise/induced ischemia, the greater the risk of cardiac events. Adjunctive variables, such as transient ischemic cavity dilatation and functional assessment with evaluation of regional wall thickening or wall motion and left ventricular ejection fraction greatly assist in the risk stratification process.

Nuclear cardiology is uniquely placed to address all the major determinants of prognosis in CAD can be assessed by measurements of stress-induced perfusion or function. These measurements include the amount of infarcted myocardium, the amount of jeopardized myocardium (supplied by vessels with hemodynamically significant stenosis), and the degree of jeopardy (tightness of the individual coronary stenosis). Recent evidence in large patient cohorts has revealed that factor estimating the extent of left ventricular dysfunction (left ventricular ejection fraction, extent of infarcted myocardium, transient ischemic dilatation of the left ventricle and increasing lung uptake) are excellent predictors of cardiac mortality. However, measurements of inducible ischemia are the best predictors of the development of acute coronary syndromes. Several reports have shown that nuclear testing yields incremental prognostic value over clinical information with respect to cardiac death, or the combination of cardiac death and nonfatal myocardial infarction as isolated endpoints. Now it is possible to tailor therapeutic decision making for an individual patient based upon combination of clinical factors and nuclear scan results. Patients with severe perfusion abnormalities on their stress image may have a five- to ten-fold higher likelihood of cardiac death versus patient with a normal myocardial perfusion SPECT. If the defects perfusion determined as a culprit lesion, invasive therapy (PCI) is an optimized outcome for that patient (2).

7. Clinical evaluation of MPS

The power of myocardial perfusion imaging (MPI) for predicting future coronary events has been demonstrated in a large number of high-quality studies and in many thousands of patients. It is perhaps the area of nuclear cardiology where the evidence is most strong. The most important variables that predict the likelihood of future events are the extent and severity of inducible ischemia. In general, markers of left ventricular dysfunction tend to predict cardiac mortality and inducible ischemia predicts acute coronary syndromes. MPS has incremental prognostic value even after clinical assessment, exercise electrocardiography and coronary angiography. In other words, patients who appear to be high risk after coronary angiography can be separated into higher and lower risk groups by MPS. In addition, several studies have indicated that a negative SPECT study confers an excellent prognosis with an annual cardiac event rate of <1% for the general population. In the setting of a normal myocardial perfusion study in a low-risk patient, it takes 9 years for

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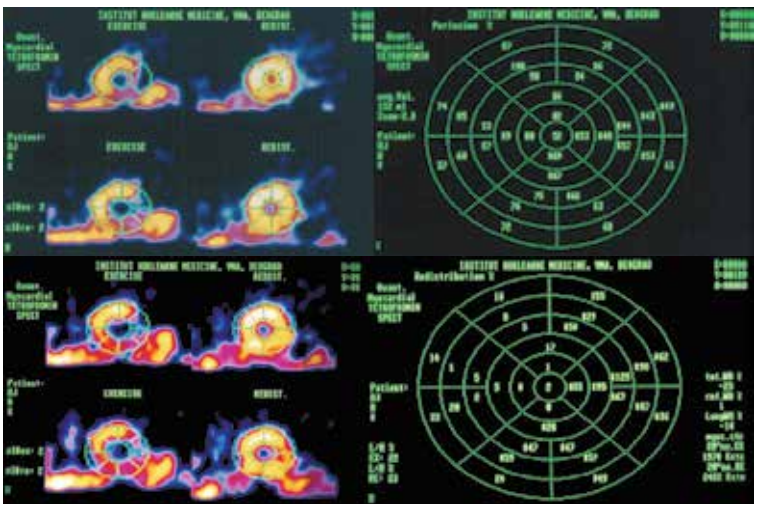

Fig. 1. Culprit lesion on the lateral and inferior segments on the short axis after SPECT MPI with AdenoEx protocol. In the rest study (right side) we showed normal finding of perfusion in the same area. We indicated invasive strategy.

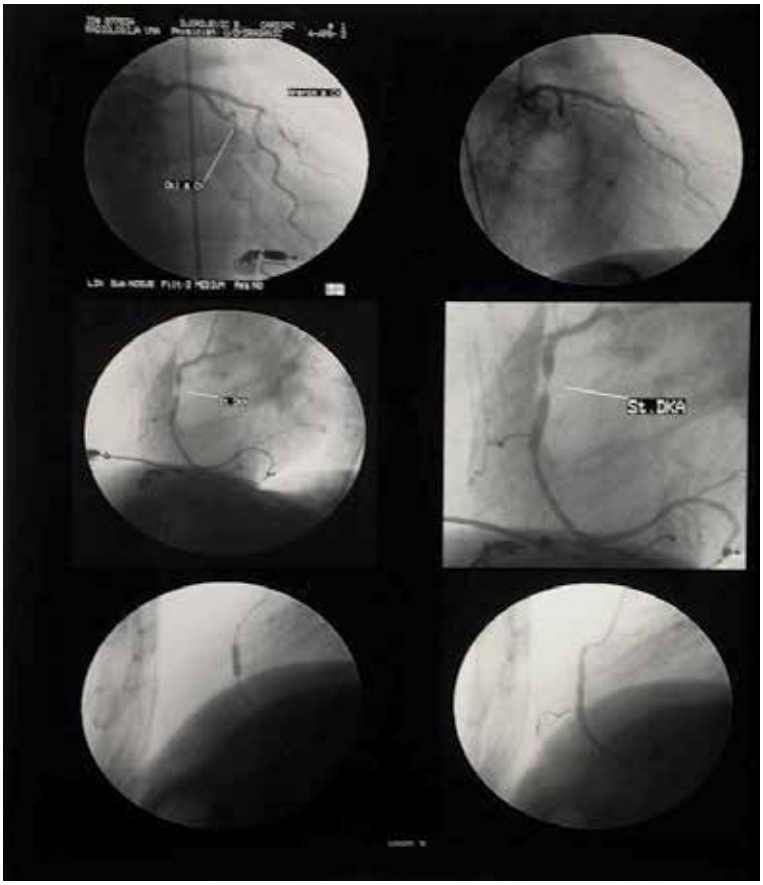

Fig. 2. Occlusion ACx, and subtotal stenosis RCA. In the same acts we perfiormed PCI with stent implantation

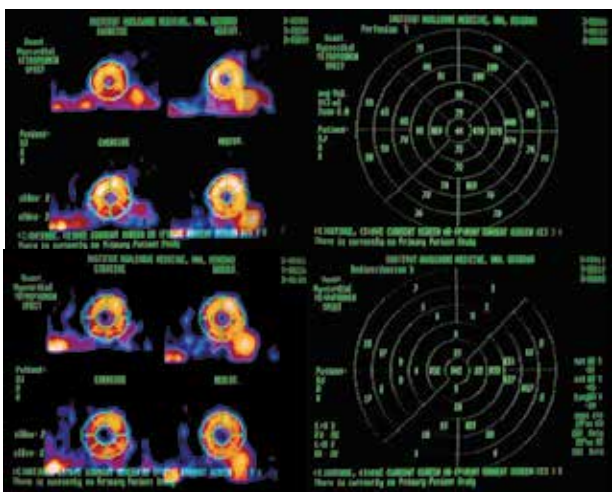

Fig. 3. MPI nearly after PCI (two weeks) showed normal findinig

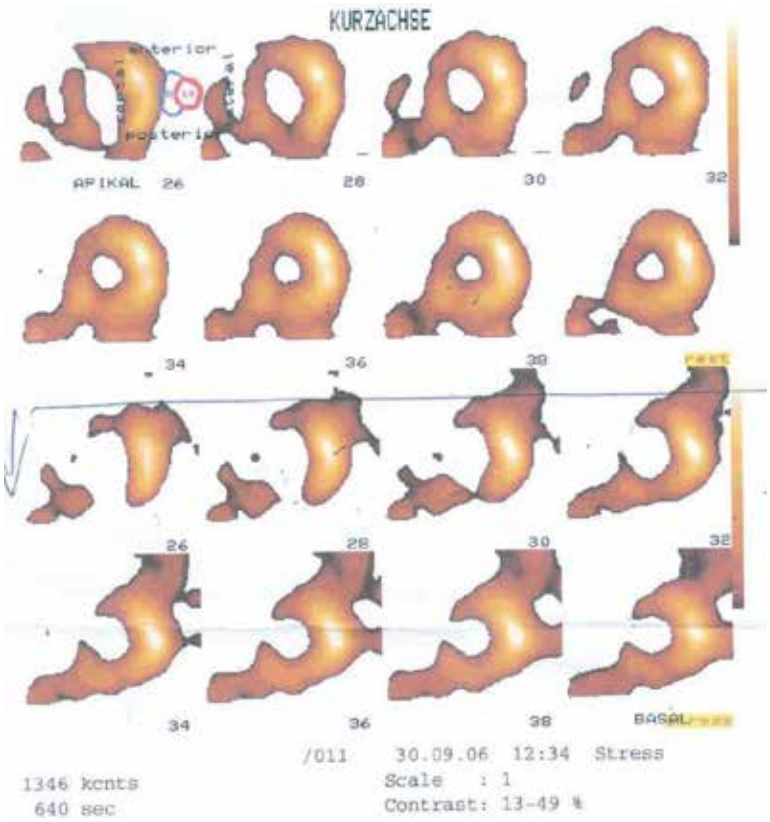

Fig. 4. Rest study showed normal MPI finding; **stress two lines short axis below;** culprit lesion in the anterospetal segments

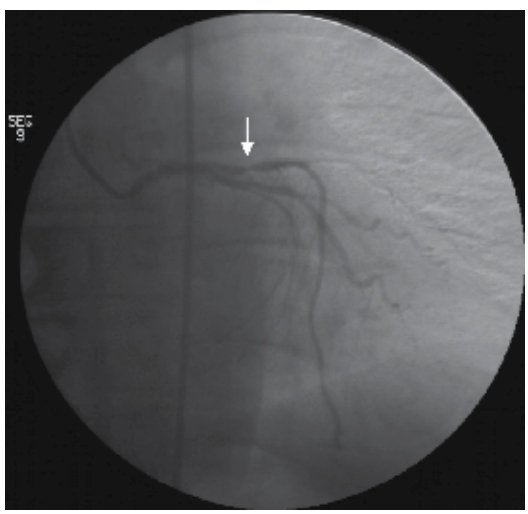

Fig. 5. Coronarography finding; subtotal stenosis LAD

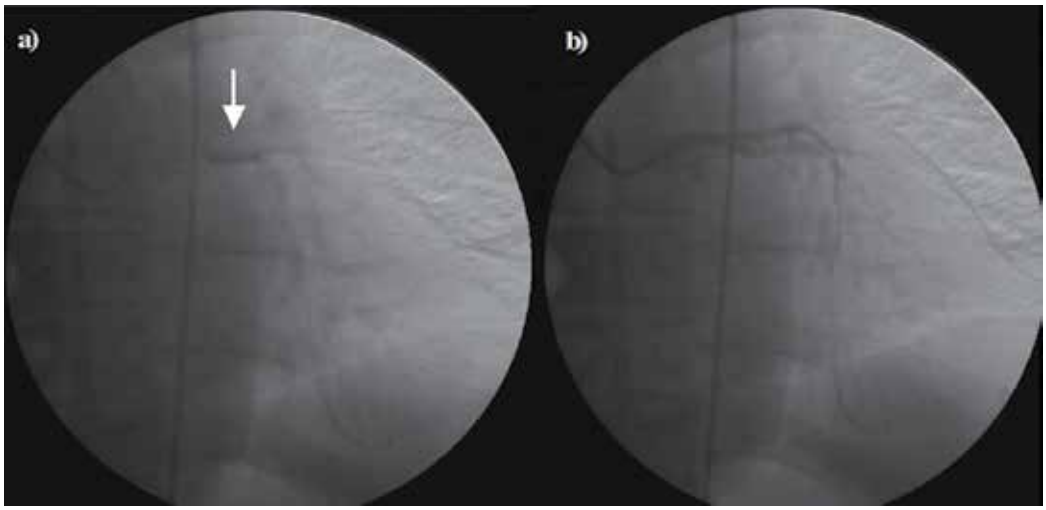

Fig. 6. In the same acts we performed PCI with stent implantation

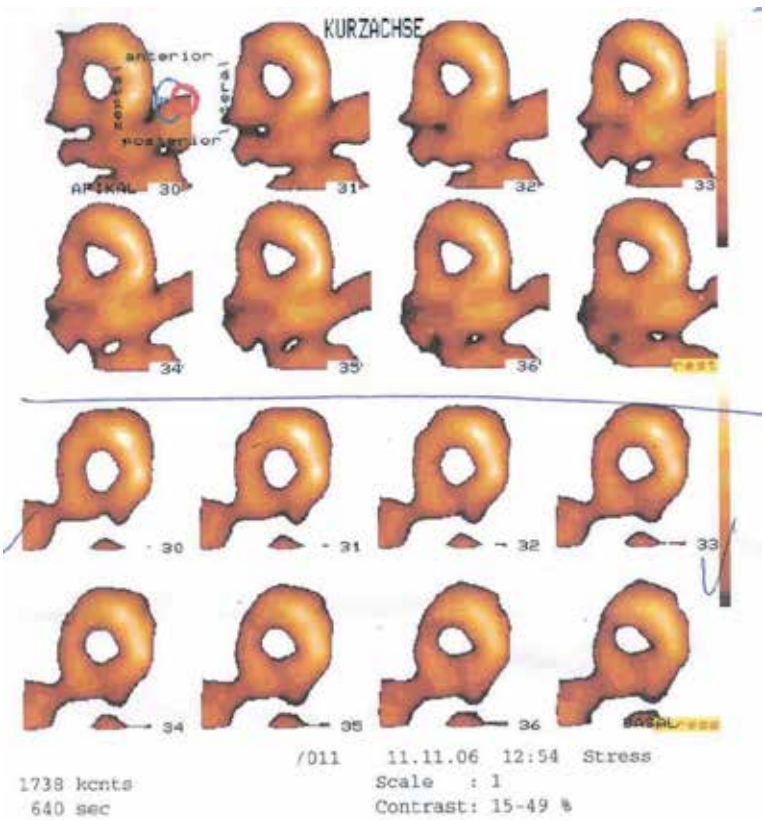

Fig. 7. Two months after we performed MPI for assessment elective PCI theraphy. We fnded normal perfusion in the rest (up) and AdenoEx study (below two lines short axis)

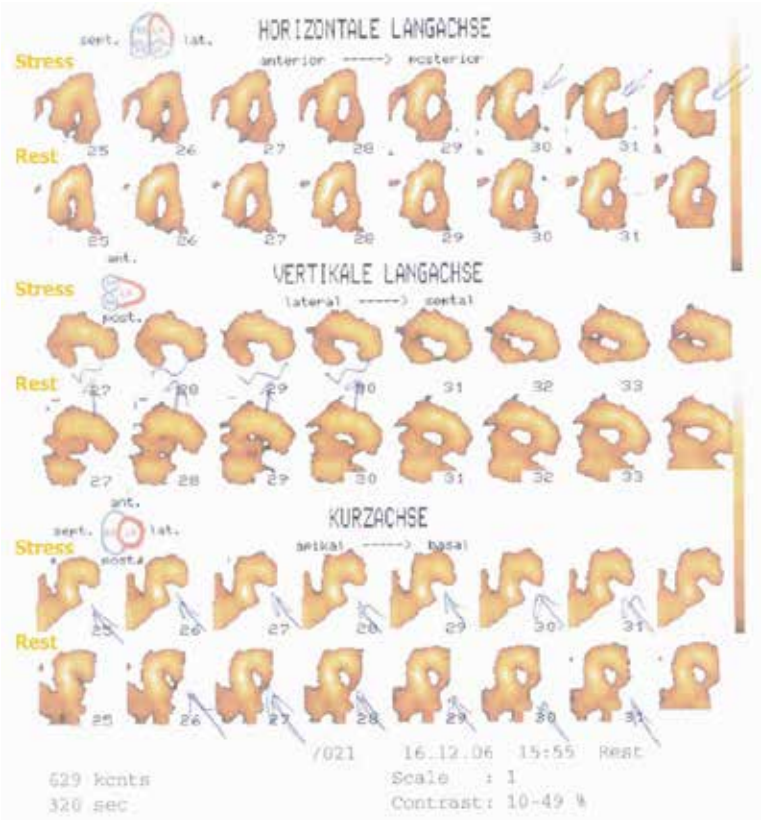

Fig. 8. Culprit lesion in the inferolateral segments in the AdenoEx (up line slices) MPI study

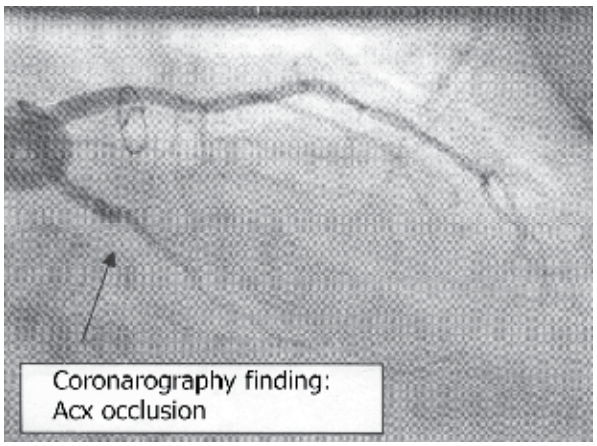

Fig. 9.

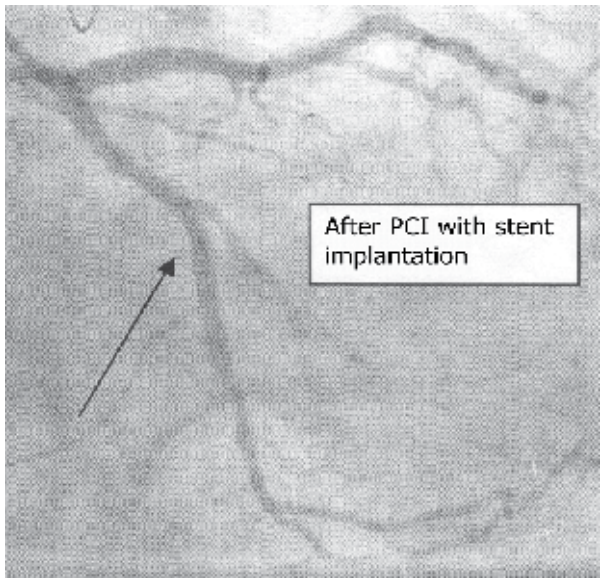

Fig. 10.

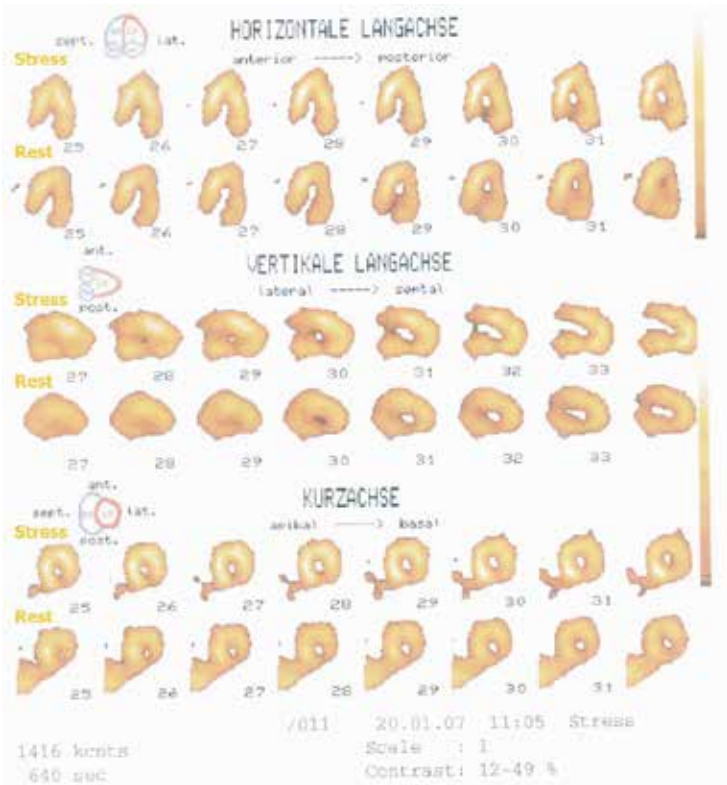

Fig. 11. Nearly after elective PCI intervention we performed MPI with normal finding of perfusion

8. Conclusion

Myocardial perfusion imaging by SPECT, with pharmacologic stress test AdenoEX significantly improves sensitivity, specificity, and accuracy for determination and localization culprit lesion in patients undergoing elective percutaneous coronary intervention.

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New Noninvasive Modalities in Coronary Angiography - Diagnostic Values of New Biomarkers for Cardiovascular Disease

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1. Introduction

The early identification of susceptibility to adverse cardiovascular outcomes and risk stratification amongst asymptomatic individuals, as well as amongst those with overt disease continues to be one of the major priorities of clinically-orientated research in the field of atherothrombosis (Dotsenko et al., 2008). Conventional cardiovascular risk assessment is based on traditional risk factors such as serum cholesterol concentrations, blood pressure levels, smoking, and diabetes mellitus. However, available data from epidemiological studies indicate that these classic risk factors do not fully explain the distribution of risk in the general population. On the other hand, classic risk scores may provide variable results in different people groups (Blankenberg et al., 2010). Besides, coronary artery disease often occurs in the absence of traditional risk factors (Yilmaz et al., 2007). Therefore, it is becoming increasingly clear that the newer laboratory measures may be useful to refine risk estimates in the general population. The pressing need for the development and clinical implementation of new markers of atherothrombotic disease has fuelled rapidly expanding research into cardiac biomarkers (Dotsenko et al., 2008; Le & Wilson, 2010) .

Cardiovascular biomarkers have the potential to augment clinical risk stratification by aiding in screening, diagnosis and assessment of prognosis. However, most current biomarkers have only modest predictive value, and there is a need to identify additional biomarkers from new biological pathways (May & Wang, 2008). Biomarker research is actively developing new testing strategies trying to improve upon current approaches, but it is often unclear how to assess the incremental prognostic information that a new test provides (Wood & Greenland, 2009). Some individual biomarkers such as C-reactive protein (CRP) have demonstrated consistent associations with incident cardiovascular events across multiple studies, but the magnitude of these associations is modest, and only small improvements in discrimination and reclassification are seen. One attractive solution to the limitations of individual biomarkers is to combine nonredundant biomarkers into panels to enhance risk assessment. However, results of studies testing multiple biomarkers for risk prediction in primary prevention populations have not provided a clear picture, with some studies showing qualified promise and others suggesting limited value (de Lemos & Rohatgi, 2010). This chapter provides an overview of the way of biomarker

discovery and selection for cardiovascular disease (CVD) and the practical considerations that are a prerequisite to their clinical use .

2. What is a biomarker and how do we find the best biomarkers for cardiovascular disease?

Biomarkers are one such tool to better identify high-risk individuals, to diagnose disease conditions promptly and accurately, and to effectively prognosticate and treat patients with disease (Dotsenko et al., 2008). The term biomarker (biological marker) was introduced in 1989 as a Medical Subject Heading (MeSH) term and in 2001, a National Institutes of Health (NIH) working group standardized the definition of a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Vasan, 2006). (Table1).

Ambiguity concerning decision limits frustrates clinicians and negatively affects the clinical adoption of a biomarker. At the time of clinical introduction, a new biomarker ideally should have well-characterized decision limits that (a) are pragmatic to apply, (b) have undergone validation in multiple studies, (c) have been evaluated in the relevant population(s) and application(s), and (d) have achieved synergy between available scientific data and regulatory labeling (Morrow & Cook, 2011).

Biological marker (biomarker): A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.
Risk factor: A risk factor is associated with a disease because it is in the causal pathway leading to the disease.
Risk marker: A risk marker is associated with the disease (statistically) but need not be causally linked; it may be a measure of the disease process itself.
Clinical end point: A characteristic or variable that reflects how a patient feels, functions, or survives.
Intermediate (nonultimate) end point: A true clinical end point (a symptom or measure of function, such as symptoms of angina frequency or exercise tolerance) but not the ultimate end point of the disease, such as survival or the rate of other serious and irreversible morbid events.
Validation of a biomarker (assay or method validation): A process for assessing performance characteristics (ie, sensitivity, specificity, and reproducibility) of a biomarker measurement or an assay technique.
Qualification of a biomarker (clinical validation): The evidentiary process linking a biomarker to disease biology or clinical outcome.
Evaluation of a biomarker: A process of linking biomarkers to outcomes, often with a view to establish surrogate status.
Adapted from Reference : Vasan, (2006).

Table 1. Biomarkers: A basic glossary.

How do we find the best biomarkers for cardiovascular disease? Cardiovascular risk estimation by novel biomarkers needs assessment in disease-free population cohorts, followed up for incident cardiovascular events, assaying the serum and plasma archived at baseline (Blankenberg et al., 2010). Composite end points are often used to increase the number of events in the comparison with persons who do not develop events. Such composite end points might include the development of acute coronary syndrome , cardiac failure, cerebrovascular disease, and intermittent claudication. When possible, it is probably best to focus the research interest on discrete events, such as the occurrence of a first coronary heart disease event (Le & Wilson, 2010); or perhaps research should be done considering the severity of the disease, namely the degree of coronary stenosis evaluated by coronary angiography. Besides, diagnosing coronary artery disease (CAD) in patients at risk can be challenging and typically requires coronary angiography as the gold standard (Rosenberg et al, 2010).

When a new biomarker X is evaluated, it is important to remember that the question of interest is not whether X is a better predictor of disease than a previously known biomarker Y. Rather, the pertinent question is whether X improves the predictive accuracy of the best available model (representing the standard of care for that disease) that incorporates several known predictors of disease including Y. Thus, the relative added values of new biomarkers is best evaluated by estimating the increment to the c statistic compared with that from a model that incorporates other previously known predictors (Vasan, 2006). The c statistic, or area under the receiver operating characteristic (ROC) curve, achieved popularity in diagnostic testing, in which the test characteristics of sensitivity and specificity are relevant to discriminating diseased versus nondiseased patients (Cook, 2007).

Although it is generally believed that new biomarkers should add to the c statistic to be useful, there are exceptions to this rule. Novel biomarkers (eg, homocysteine) that are not incremental to known risk factors may be measured in select clinical situations such as in the following: asymptomatic individuals without obviously elevated conventional risk factors but with very strong family history of vascular disease; patients with premature vascular disease but no obvious risk factors; and patients with aggressive recurrent vascular disease in the face of well-controlled levels of conventional risk factors (Vasan, 2006).

Most of the cardiovascular biomarker studies that test the utility of a large number of laboratory tests are hypothesis-generating in nature, and validation of the results is important. Several validations of a new biomarker may be needed to demonstrate that the new test provides information that could help predict risk in the population or in specific subgroups of the population . When the usefulness of a new test for predicting disease is assessed, it is important to bear in mind whether the test adds or replaces information concerning risk or prognosis for a specific clinical or subclinical outcome.The test must also be reproducible, be standardized, have a high diagnostic sensitivity and specificity, and have a high predictive value (Le & Wilson, 2010).

3. What are the new biomarkers for CVD?

In recent years, a number of new candidate risk factors or markers have been proposed as significant predictors of atherosclerosis and its complications (Table 2) (Hackam & Anand, 2003). In a contemporary publication of the MONICA (Monitoring of Trends and Determinants of Cardiovascular Disease) investigators, Blankenberg and colleagues evaluated the potential contribution of 30 novel biomarkers to the 10-year cardiovascular

disease risk in 2 population cohorts (Blankenberg et al., 2010). These biomarkers were part of the MORGAM (MONICA, Risk, Genetics, Archiving, and Monograph) Biomarker Project (Evans et al.,2005) and were representative of 9 distinct metabolic processes linked to atherosclerosis . They include (a) lipid-related biomarkers, (b) renal function markers, (c) metabolic markers representing glucose and obesity pathways, (d) markers of vascular function and neurohumoral activity, (e) inflammation markers, (f) markers of oxidative stress and antioxidants, (g) coagulation markers, (h) angiogenesis markers, and (i) necrosis markers ; classified similar to the risk factors in Table 2 (Blankenberg et al., 2010).

In recent years, the importance of inflammation and increased reactive oxygen species (ROS) for the pathogenesis of atherosclerosis is well recognized (Kaneto et al, 2010; Mayer, 2000; Tabit et al, 2010). Consequently, "non-traditional factors" such as high-sensitive C-reactive protein (hs-CRP), total homocysteine (t-Hcy), as well as oxidative stress, have been proposed as risk factors for the development and progression of atherosclerosis and atherothrombotic cardiovascular disease (Djoussé et al,2001; Eren et al,2002; Lee et al,2003; Pence et al, 2003). Therefore, this chapter will focus on total-homocystein, hs-CRP and the underlying oxidative stress mechanism of CVD or atherosclerosis.

Inflammatory Markers	**Lipid-Related Factors**
C-reactive protein Interleukins (eg, IL-6) Serum amyloid A Vascular and cellular adhesion molecules Soluble CD40 ligand Leukocyte count	Small dense low-density lipoprotein (LDL) Lipoprotein(a) Remnant lipoproteins Apolipoproteins A1 and B High-density lipoprotein subtypes Oxidized LDL
Hemostasis/Thrombosis Markers	**Other Factors**
Fibrinogen von Willebrand factor antigen Plasminogen activator inhibitor 1 (PAI-1) Tissue-plasminogen activator Factors V, VII, and VIII D-dimer Fibrinopeptide A Prothrombin fragment 1+2	Homocysteine Lipoprotein-associated phospholipase A(2) Microalbuminuria Insulin resistance PAI-1 genotype Angiotensin-converting enzyme genotype ApoE genotype Infectious agents: Cytomegalovirus, Chlamydia pneumonia, Helicobacter pylori, Herpes simplex virus Psychosocial factors
Platelet-Related Factors	
Platelet aggregation Platelet activity Platelet size and volume	

Table 2. Novel risk factors for atherosclerotic vascular disease (Hackam & Anand, 2003).

4. Diagnostic values of homocysteine, C-reactive protein and bilirubin for coronary artery disease.

C-reactive protein (CRP) is a circulating acute-phase reactant that is increased many-fold during the inflammatory response to tissue injury or infection (Pepys & Baltz, 1983). This protein has received substantial attention in recent years as a promising biological predictor of atherosclerotic disease (Pearson et al., 2003). On the other hand, it has been postulated that mild to moderate elevations of homocysteine in the general population predispose to atherosclerosis in a manner akin to the classic risk factors (Hackam & Anand, 2003). Additionally, the adaptive and protective responses of arterial vasculature against oxidative stress are important in the prevention of atherosclerosis (Hoekstra et al, 2004).

4.1 CRP or hs-CRP

An evolving body of work suggests that even small increases in CRP within the normal range are predictive of future vascular events in apparently healthy, asymptomatic individuals (Ridker et al, 2002). Danesh et al reported a meta-analysis of 14 prospective long-term studies of CRP and the risk of nonfatal myocardial infarction or death from coronary heart disease (Danesh et al., 2000). The attention in this protein stems in part from a recent shift in thinking about the pathogenesis of CVD, an entity once primarily considered to be a bland lipid storage disease. Inflammation is now widely accepted as central to every aspect of the atherosclerotic process, from its initiation to its progression to plaque rupture, the latter being the essential event underlying the acute coronary syndromes (Hackam & Anand, 2003). Debate exists about the utility of CRP as a marker of cardiovascular risk, given its role as an acute-phase reactant and hence its elevation in the presence of any inflammatory focus or injury. This has been countered somewhat by the development in recent times of an ultrasensitive assay, which has been shown to have a degree of measurement stability similar to that of total cholesterol (Davison & Davis, 2003). In other words, the guidelines identify CRP (as measured by a high-sensitivity [hs] assay--hence the name hs-CRP) as the inflammatory marker of choice for cardiovascular risk stratification. Although a number of other inflammatory markers such as serum amyloid A, white blood cell count, and fibrinogen have been investigated, the "hs-CRP" level has the most stability, assay precision, accuracy, and availability (Shishehbor et al, 2003).

4.2 Homocysteine

Interest in homocysteine as a causal factor was spurred by the observation that more than 50% of children with the genetic disorder homocystinuria die of premature vascular disease and strategies that reduce homocysteine levels in these children have been shown to decrease vascular event rates (Humphrey et al, *2008).* Mechanistic studies have demonstrated that homocysteine might induce vascular damage by promoting platelet activation, oxidative stress, endothelial dysfunction, hypercoagulability, vascular smooth muscle cell proliferation, and endoplasmic reticulum stress (De Bree et al., 2002; Mangoni & Jackson, 2002). In their meta-analysis, Humphrey et al evaluated homocysteine levels as a predictor of new CAD events in persons without known CAD. Their review showed an association between elevated homocysteine levels and CAD that was independent of Framingham risk factors. In the overall analysis, the risk of any CAD event increased approximately 20% for each increase of 5 μmol/L of homocysteine. Consequently, elevated

homocysteine levels independently and moderately increased the risk of developing CAD either in a causal manner or as a risk marker by approximately 20% (Humphrey et al, *2008).*

4.3 Oxidative stress and bilirubin

Imbalances in the redox status in which excess oxidation occurs or reducing power cannot be maintained (e.g. in inflammation, age, smoking, high lipid content and oxidation) creates a state in which molecular and tissue modifications progress rapidly, leading to development of lesions and full-blown atherogenesis. Oxidative stress does not replace the recognized role of lipids and cholesterol in atherosclerosis, but rather underline that role. Indeed, quantifying redox processes may well elucidate some molecular mechanisms by which lipids mediate atherogenesis (Gamkrelidze et al., 2008). Bilirubin has been considered an antioxidant, with capacity to remove reactive species of oxygen. Recent data advocates the idea that an increased bilirubin level promotes protection against atherosclerosis (Ghem et al., 2010).

The heme oxygenase responsible for the degradation of heme grouping of hemoglobin is a stress inducible enzyme with antioxidative properties . The products of its reaction (bilirubine, carbon monoxide and iron) develop a potential protective role against atherosclerosis (Hoekstra et al, 2004). Studies have suggested that different circulating forms of bilirubin have the capacity to remove a variety of reactive species of oxygen, and inhibit the oxidation of LDL particles and the chemotaxis of monocytes, all of which are crucial steps in atherogenesis (Ghem et al., 2010).

In a study we have done , we assessed the diagnostic performance and relationship of bilirubin with hs-CRP and t-Hcy for cardiovascular disease in men and women in an angiographically documented design. The study demonstrated that patients with angiographically confirmed coronary artery disease (CAD) had significantly higher serum hs-CRP and t-Hcy levels than non-stenotic patients (patients with normal angiogram) and the apparently healthy control group.Optimal cut-off levels and the associated diagnostic performances (sensitivity, specificity and diagnostic value) of serum bilirubin, hs-CRP, t-Hcy, based on ROC analysis, are given in Table3. Optimal cut-off levels for bilirubin, hs-CRP and t-Hcy providing the maximum efficiency found in patients (n = 319) with CAD were 0.59 mg/dL, 1.09 mg/dL and 12.1 μmol/L respectively.

Variable	Cut-off level	Sensitivity (%)	Specificity (%)	Diagnostic value (area under the curve)	+LR	-LR
Bilirubin	0.59 mg/dL	70.9	40.4	0.507	1.19	0.72
High-sensitivity C-reactive protein	1.09 mg/dL	50.0	80.7	0.648	2.59	0.62
Total homocysteine	121 ^mol/L	76.8	70.2	0.781	2.67	0.29

+LR = positive likelihood ratio
-LR = negative likelihood ratio

Table 3. Optimal cut-off levels and associated specificity, sensitivity and diagnostic value of concentrations of biomarkers for the diagnosis of angiographically documented coronary artery disease

These data strongly suggest that serum t-Hcy helps to identify individuals at risk of atherosclerosis (AUC value 0.781), especially among those with elevated hs-CRP and decreased bilirubin levels. t-Hcy showed the highest AUC value (0.781) compared to hs-CRP (0.648) and bilirubin (0.507). Area under the curve (AUC) values in receiving operating characteristics (ROC) curve (as a measure of discriminating efficacy) were used for comparison of the diagnostic values of different analyses (including only the CAD and non-CAD groups, using angiography as the gold standard). ROC curve-based sensitivities of bilirubin, hs-CRP and t-Hcy levels were 70.9%, 50.0%, 76.8% respectively. The specificities of bilirubin, hs-CRP and t-Hcy were 40.4%, 80.7% and 70.2% respectively (data of ROC curves are shown in Figures 1–3).

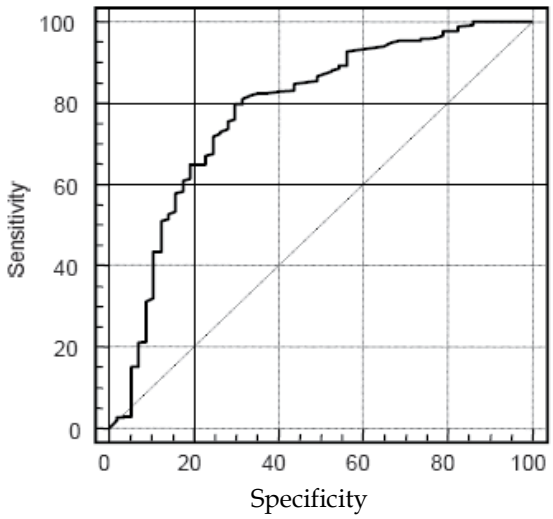

Fig. 1. ROC curve for total homocysteine.

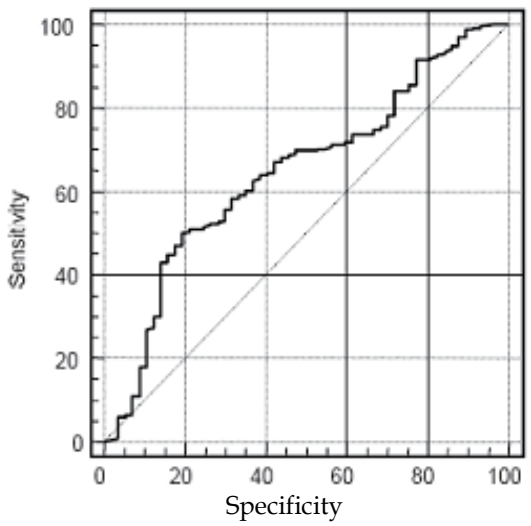

Fig. 2. ROC curve for hs-CRP.

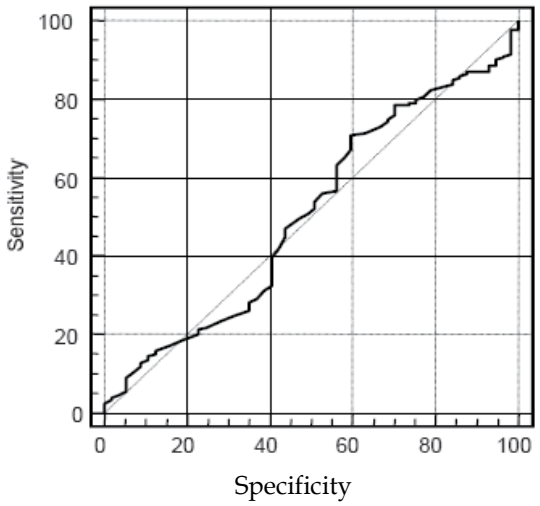

Fig. 3. ROC curve for bilirubin.

In agreement with previous reports, we found that the bilirubin levels in serum were significantly lower in the patients with CAD than in age- and sex-matched controls (Madhavan et al., 1997; Schwertner et al., 1994; Vitek et al., 2002). We found that a serum bilirubin concentration of 10.0 μmol/L (0.58 mg/dL) discriminated between high and low cardiovascular risks .

Additionally, we found that the number of stenotic coronary arteries was significantly associated with elevated serum t-Hcy and hs-CRP concentrations. Several researchers have investigated the risk of myocardial infection in individuals with the *UGT1A1*28* allele (Bosma et al., 2003; Schwertner, 2003). According to the "oxidative modification hypothesis", which suggests atherogenesis is initiated by oxidization of low-density lipoprotein particles, it has been suggested that increased physiological concentrations of serum bilirubin may reduce atherogenic risk by reducing oxidation. An involvement of bilirubin in immune reactions and inflammatory processes has also been documented (Delores et al., 2000; Kronenberg et al., 2002; Lin et al., 2003).

Earlier studies have reported differences in the levels of t-Hcy, ranging between 13.9–20.1 μmol/L in persons with CAD (Yu et al., 2000; Zylberstein et al., 2004). We found a mean t-Hcy level of 19.4 (SD 8.73) μmol/L in the CAD group, 10.7 (SD 5.14) μmol/L in the healthy group and 13.0 (SD 8.61) μmol/L in the non-CAD group. Some differences between reported serum t-Hcy levels may be related to analytical methods and ethnic differences. A study in 19 centres in Europe reported high homocysteine levels and increased risk of CAD in smokers (Graham et al., 1997). We found that the t-Hcy levels tended to increase in the presence of more cardiovascular risk factors, i.e. male gender, older age, diabetes mellitus, hyperlipidaemia and certain chronic diseases. As expected, traditional coronary risk factors were more prevalent among those participants with elevated levels of t-Hcy and hs-CRP in our study, as in other studies (Abdemouttaleb et al., 2000; Sesso et al., 2003; Siri et al., 1998).

5. What is new about hyperhomocysteinemia?

Homocysteine is an accepted independent risk factor for several major pathologies including cardiovascular disease, birth defects, osteoporosis, Alzheimer's disease, and renal

failure. Interestingly, many of the pathologies associated with homocysteine are also linked to oxidative stress (Suszynska et al., 2010). Evidence indicates that hyperhomocysteinemia, which occurs in 5–7% of the general population is a risk factor for CVD, but how? (Jakubowski, 2004). Hyperhomocysteinemia is accused of being responsible for elevating oxidative stress as a result of formation of Hcy- thiolactone or Hcy-thiyl radical, which may lead to impairment of cell signaling and cause pathology (Doshi et al., 2001; Lang et al., 2000). Many researchers, and especially Jakubowski, suggested that metabolic conversion of Hcy to Hcy-thiolactone followed by subsequent spontaneous protein N-homocysteinylation by Hcy-thiolactone might contribute to Hcy toxicity in humans. (Jakubowski, 2002). Hcy-thiolactone is a reactive intermediate that causes protein N-homocysteinylation through the formation of amide bonds with ε-amino groups of protein lysine residues; in the event, homocysteinylated proteins may lose their biological activities (Jakubowski, 2003).

Whereas epidemiological data indicate that elevation of plasma homocysteine is not associated with a significant change in plasma total cholesterol, some studies have reported a negative correlation with HDL concentrations (Ciaccio & Bellia, 2010; Domagała et al., 2006; Williams & Schalinske, 2010). Because low plasma HDL concentration sometimes is associated with increased risk of CVD, whereas other conditions with low plasma HDL concentration are associated with improved prognosis, it seems that it is not only the concentration per se but also the function of the HDL particles that is important for its antiatherogenic effects (Bełtowski, 2005; Mikael et al.,2006). HDL particles are susceptible to structural modifications mediated by various mechanisms, including oxidation, glycation, or enzymatic degradation, affecting their functional properties .Moreover, in vitro studies have shown that homocysteinylation of HDL may reduce the activity of the enzyme Paraoxonase (PON), which is associated with human HDL, thus rendering the HDL particle more susceptible to oxidative damage. Formation of inflammatory HDL has been suggested to correlate with decreases in the activities of various HDL associated enzymes, such as PON, a multifunctional enzyme with antioxidant capacity, and the ability to detoxify the homocysteine metabolite homocysteine thiolactone (Bełtowski, 2005; Liao et al., 2007) .

Paraoxonase is thought to influence serum homocysteine concentrations, at least in part, due to its homocysteine thiolactonase activity and to play a role in atherosclerosis. (Yang et al, 2006). Hcy- thiolactonase activity is influenced by both PON1 and MTHFR genotypes and there is a direct relation between Hcy and Hcy- thiolactone levels. In relation to this matter, Jakubowski et al. hypothesized that high thiolactonase associated PON1 R and L alleles should confer significant cardiovascular protection in subjects with high Hcy levels (Jakubowski et al., 2001).

6. Conclusion

The availability of well-validated decision limits is vital to optimal integration of a new biomarker into clinical practice. Approaches to internal validation and data-mining methods lead to overfitting and overestimation of risk relationships and are generally not sufficient for selecting final clinical cutpoints. Such methods, when applied correctly, can be reasonable for suggesting cutpoints for external validation. Biomarkers that have monotonic linear relationships with risk are best handled as continuous variables when incorporated into comprehensive risk models. As consistently demonstrated in clinical practice and professional society guidelines, however, practitioners will almost always seek thresholds to provide structure for clinical decision-making, such as those existing for cholesterol.

Therefore, such cutpoints warrant development and validation. Although the approach is demanding, we recommend assessment of clinical decision limits by external validation in 2 or more data sets that are appropriate to each of the proposed clinical application(s), with attention paid to the possibility of differences in risk relationships in clinically relevant subpopulations (Morrow & Cook, 2011).

To conclude, there is little evidence of an association between the serum concentration of bilirubin and atherosclerosis. In contrast, the concentration of novel (t-Hcy and hs-CRP) and traditional risk markers may be stronger markers for atherosclerosis in CAD patients. Additional studies are still necessary to confirm and demonstrate the association of these findings with clinical outcomes.

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The Role of Inflammatory Biomarkers in the Assessment of Coronary Artery Disease

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1. Introduction

Coronary artery disease (CAD) is the world's leading cause of illness and death. In 2005, approximately 7.6 million deaths were attributed to coronary artery disease, accounting for almost 13% of the total deaths (WHO, 2011). From a pathophysiological point of view the disease could be considered as a severe clinical manifestation of atherosclerosis (Mallat & Tedgui, 2001). The disruption of an atherosclerotic plaque with superimposed thrombosis had been identified as the main cause of acute coronary syndromes, including acute myocardial infarction (AMI), and sudden death (Gensini & Dilaghi, 2002; Shah, 2003).
The initially silent progression of arterial plaque, prompted by classic risk factors (including hypertension, diabetes mellitus, dyslipidaemia, age, stress, physical inactivity, dietary habits and cigarette smoking), is followed by a phase of acute or chronic progression toward an increasing degree of stenosis that eventually causes thrombosis (Gensini & Dilaghi, 2002; Fuster *et al.*, 2005). Plaque disruption and/or endothelial activation represent the main trigger event for AMI, through exposure of plaque thrombogenic components to platelets and to clotting components of flowing blood. In this phase, haemostasis related risk factors and platelet status play a crucial role. However, current research supports the view of atherosclerosis as an inflammatory process that initiates and promotes lesion development to the point of acute thrombotic complications and clinical events. Thus, the time has come to embrace inflammation as a common pathway for atherogenic risk factors and for providing new opportunities for therapeutic intervention (Libby, 2003).
Acute myocardial infarction is a critical clinical presentation of coronary artery disease in many asymptomatic patients and often the event is fatal. Establishing the presence of coronary lesions either in asymptomatic patients or in symptomatic patients with acute or chronic chest pain can be a challenging task. Consequently, major clinical research efforts

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have been dedicated to the identification of patients at higher risk and to the diagnosis of coronary artery disease.

Angiography remains the undisputed standard in interventional cardiology. Succeeding breakthroughs for cardiac imaging from every branch of radiology technology offer remarkable views and are providing new insights into coronary pathology. Although angiography is a first-line test for coronary artery disease, particularly for screening symptomatic patients, the evaluation of asymptomatic individuals currently relies on the identification of risk factors (e.g., hypertension, dyslipidaemia, diabetes mellitus, stress and smoking habits) (Conroya *et al.*, 2003; Kotecha *et al.*, 2010). However, neither the absence of high-grade stenosis provided by imaging modalities such as angiography assure the lack of future cardiac events, nor the cardiovascular events are readily explained by cardiovascular risk factors (Fisher *et al.*, 2000; Kern, 2000; Hadamitzky *et al.*, 2009; Marwan *et al.*, 2009). The understanding of the cellular biology of the unstable plaque remains poorly known, and the crucial question is still the identification of the factor(s) that play a significant role in the plaque disruption.

Rupture of atherosclerotic plaque has been identified as the proximate event in the majority of cases of acute ischemic syndromes. Plaque rupture exposes thrombogenic components of the plaque, activating the clotting cascade and promoting thrombus formation. Future culprit lesions are difficult to identify, however, and angiographic assessment of stenosis severity is prone to underestimation. Compared with plaques that cause severe luminal stenosis, vulnerable plaques may cause relatively minor stenosis, although they account for more cases of rupture and thrombosis. Such unstable, vulnerable plaques may be associated with outward remodeling of the vessel. Because severely stenotic plaques are more likely to stimulate collateral circulation to the post-stenotic segment, plaque rupture and thrombosis at such sites may be clinically silent. Characteristic histomorphologic features of vulnerable plaques include a high lipid content, increased number of inflammatory cells, and extensive adventitial and intimal neovascularity. These cells are mostly monocyte-macrophages and they are probably recruited into the atherosclerotic plaques by adhesion molecules, especially intracellular adhesion molecule (ICAM)-1 and P-selectin, and chemokines such as monocyte chemoattractant protein (MCP)-1. Another potential avenue for the entry and recruitment of inflammatory cells inside the atherosclerotic lesion may be through the adventitial neovasculature, which is enhanced in atherosclerosis. In addition, other factors that may contribute to the recruitment of inflammatory cells and their activation in atherosclerosis include oxidized lipids, cytokines such as tumor necrosis factor alpha (TNF-α), increased angiotensin II activity, elevated arterial pressure, diabetes, chronic infections remote from the arterial wall, possible infectious organisms in the vessel wall and activation of the immune system (Shah, 2003). In addition, interaction between inflammatory cells, vascular smooth muscle cells, endothelial cells and extracellular matrix may contribute to the development of plaque and its rupture. TNF-α, mainly secreted by macrophages, influences many aspects of atherosclerosis by increasing the permeability of endothelial cells, promoting monocyte adhesion, inducing macrophage differentiation and probably promoting vascular calcification (Trion & van der Laarse, 2004). The calcification of arteries resembles the bone formation (Trion & van der Laarse, 2004). Activated monocytes produce TNF-α and other osteoinductive factors that stimulate the differentiation and mineralization of cardiovascular cells (Trion & van der Laarse, 2004).

New approaches claim that measurements of carotid intima-media thickness or coronary artery calcium obtained by non-invasive techniques can be used to identify vulnerable patients at a time when risk factor modification can slow or stop the atherosclerotic process. Nevertheless, the uncertainty about the functional significance of these markers in the unstable plaque context has not yet been overcome. The crucial questions still are the identification and characterization of the vulnerable plaque in hopes of identifying morphologic and physiological features that predict plaque rupture.

An increase body of literature associates plaque rupture with inflammatory mediators, such as tissue factors, cell adhesion molecules and cytokines, expressed by vascular and immune cells (Shah, 2003; Fuster *et al.*, 2005; Mauriello *et al.*, 2005; Armstrong *et al.*, 2006a).

White blood-cell count (WBC), the most widely available and inexpensive measure of systemic inflammation has been associated with cardiovascular mortality both in primary and secondary prevention settings. In apparently healthy individuals, a high white blood-cell count has been associated with increased cardiovascular mortality and incidence of cardiovascular disease, independently of traditional atherosclerotic risk factors (Folsom *et al.*, 1999; Margolis *et al.*, 2005; Shankar *et al.*, 2007). In a study of patients with acute coronary syndromes, higher baseline white blood-cell counts were associated with greater extent of coronary artery disease, lower thrombolysis in myocardial infarction (TIMI) flow and myocardial perfusion grades during coronary angiography in addition to a higher 6-month mortality independently of other risk factors including ST-segment deviation and troponin levels (Sabatine *et al.*, 2002a). A high neutrophil count and a low lymphocyte count may carry most of this increased risk, as reported for patients assessed for coronary artery disease by coronary angiography (Horne *et al.*, 2005).

Thus, for prognostic purposes, clinicians have focused on white blood-cell, neutrophils or downstream products such as C-reactive protein (CRP). Neutrophils, however, live only for hours and generate no memory of their engagement, which is carried out through inherited receptors that are similar in all hosts, while lymphocytes are long-lived cells that can survive for decades, and the lymphocyte repertoire is tailored for each individual. When mobilized in immune responses, lymphocytes undergo clonal burst, differentiate into distinct types of effector cells, and memorize information about the antigen (Bodi *et al*, 2008). Little is known regarding the role of lymphocytes, which play an important role in the control of the inflammatory system and in the pathophysiology of coronary disease (Bodi *et al*, 2008). Several studies had demonstrated that the adaptative immunity plays an important role in the pathogenesis of coronary artery disease (Blum & Yeganeh, 2003; Methe *et al.*, 2005; Han *et al.*, 2007; Packard *et al.*, 2009; Hansson, 2009). In the culprit lesions of patients with acute coronary syndromes the percentage of activated T lymphocytes is significantly increased, and experimental results (Caligiuri *et al.*, 2000) suggest the existence of antigenic stimuli in these lesions. These findings lead to the paradigm that the transition from a stable to an unstable plaque includes immunological activation and may be T cell-dependent (Steppich *et al.*, 2007).

The interactions between leucocytes, activated platelets and activated endothelial cells, mediated by P-selectin, E-selectin and ICAM-1 whose soluble forms can be released in circulation, associate with the initiation of arterial thrombus (Price & Loscalzo, 1999). The expression of ICAM-1 in activated endothelial cells and of P-selectin in activated platelets, seems to have key roles in binding and rolling of leukocytes along the activated endothelium, and in platelet aggregation and platelet-leukocyte adhesion.

The majority of soluble P-selectin appears to be derived from activated platelets, as its levels are correlated with other established platelet markers but not with endothelial markers (Price & Loscalzo, 1999). Stimulated platelets and immune cells express membrane proteins, such as integrins, CD40 and its ligand (CD40L) (Henn *et al.*, 2001), and eventually secrete products with pro-inflammatory properties, such as TNF-α, and also soluble forms of adhesion molecules. Clinical data relating soluble P-selectin, ICAM-1 and TNF-α to coronary disease are limited and have been derived primarily from randomized clinical trials, cross-sectional or retrospective studies in patients with acute coronary syndromes. While, increased levels of soluble P-selectin have been consistently associated with acute coronary syndromes (Ridker *et al.*, 2001), the measurement of sICAM-1 is not consensual. Prolonged high levels of sICAM-1 were associated with unstable angina and acute myocardial infarction (Mulvihill *et al.*, 2000), although a stronger predictive information for sICAM-1 could not be found (Haim *et al.*, 2002; Hartford *et al.*, 2006). Increases in TNF-α and in some of its soluble receptors were related to primary cardiovascular events and to mortality in heart failure subsequent to myocardial infarction (Ponthieux *et al.*, 2004; Valgimigli *et al.*, 2005).

C-reactive protein is a down-stream marker of inflammation produced in the liver. Its production appears to be regulated, during the acute phase response, by several cytokines, including TNF-α (Calabrò *et al.*, 2009). Though it was originally proposed as a nonspecific marker of inflammation, several reports suggest that CRP may play a direct pathophysiological role in the development and progression of atherosclerosis. Proposed mechanisms include induction of endothelial dysfunction, promotion of foam cell formation, inhibition of endothelial progenitor cell survival and differentiation, and activation of complement in atherosclerotic plaque intima and ischemic myocardium (Armstrong *et al.*, 2006b). CRP is a robust clinical marker because of its stability, reproducible results, and ease of assay (Armstrong *et al.*, 2006b). An increasing variety of literature has been propose CRP as a major cardiovascular risk factor. Elevated baseline concentrations of this acute phase-protein are associated with the risk of atherosclerotic events in general populations (Calabrò *et al.*, 2009) and show a predictor value in terms of cardiovascular risk associated with both primary and secondary prevention of coronary artery disease (Ikonomidis *et al.*, 2008).

Although a great pool of information concerning systemic inflammation markers has been so far collected in different cardiovascular conditions, the evolution of coronary syndromes is not well depicted.

An approach with multiple inflammatory markers might be of interest since different markers may enhance or initiate different and not always overlapping inflammatory pathways, leading to atherosclerosis and cardiac events and may indicate different stages of disease (Ikonomidis *et al.*, 2008). Therefore, the relationship of inflammatory molecules and cells with the coronary disease severity and extension, and with the physiological response of the cardiovascular system may evidence the underlying mechanisms of inflammation responsible for plaque instability.

The main objective of this study was to investigate several inflammatory markers in coronary artery disease. The circulating levels of CRP, sP-selectin, sICAM-1, TNF-α, and inflammatory blood cells were assessed as they express cell adhesion, cell activation and inflammation processes which are crucial in thrombosis and secondary tissue remodelling as the cause of ischemia and necrosis.

This combined evaluation in a well-defined group of patients that had undergone angiography to precisely define coronary artery phenotype, may reveal the relevant roles of those markers in coronary disease and in the processes involved in lesion vulnerability.

2. Materials and methods

2.1 Study groups

To achieve the proposed objective it is necessary to unveil the effect of coronary occlusion, ischemia, and necrosis. Therefore, patients with different stages of coronary artery disease were included in the study.

A total of 177 subjects (53 women and 124 men) were recruited at the Cardiology Service in Santa Marta Hospital (Lisbon, Portugal).

Among them, 65 patients with acute myocardial infarction constituted the acute myocardial infarction (AMI) group. Those patients were diagnosed with ST-elevation myocardial infarction (ST-element changes and creatine kinase >3 times normal; n=56) or non-ST-elevation myocardial infarction (creatine kinase >3 times normal and without ST-element changes; n=9). All AMI patients were enrolled in the first 24 hours of hospital admission, and were submitted to primary percutaneous transluminal coronary angioplasty as reperfusion therapy. The time period from the onset of chest pain to the intervention was less than 9 hours for the majority of AMI patients.

Fifty-five patients with angiographically confirmed coronary artery disease suffering from chest discomfort, were also enrolled in the study and constituted the CAD group.

A coronary control group (CC) was established. This group was constituted by twenty-nine age-matched patients with chest discomfort complain but without coronary artery disease confirmed by coronary angiography.

A reference group (REF) of 26 healthy non-smoking volunteers was also established to help on inflammation baseline interpretation. Inclusion criteria for reference controls was absence of any history of coronary disease, dyslipidaemia or hypertension, any mobility limiting conditions, life threatening diseases, or any other disease or condition that would impair compliance. These 26 volunteers were not submitted to coronary angiography.

Informed consent was obtained for all subjects enrolled, and the study was approved by the local Ethical Committee.

2.2 Criteria used for patient's selection

Exclusion criteria were age above 85, significant co-morbidities as peripheral artery disease or carotid artery disease, known antecedents of malignance or infectious diseases, chronic renal insufficiency and previous myocardial infarction in the last 5 years. Concurrent inflammatory disorders, malignant neoplasm or infection were also excluded.

All patients were clinically and biochemically characterized, by a battery of systemic indicators.

The patients' characterization was accomplished with anthropometric data (body mass index, waist perimeter and blood pressure) and biochemical data consisting of a battery of systemic indicators, such as glucose, haematocrit, albumin, triglycerides, total cholesterol, cholesterol of LDL and HDL. The cardiac function enzymes creatinine kinase and cardiac troponin T (cTnT) were also determined, and ventricle electrolytic regulation assessed by the determination of N-terminal pro B type natriuretic peptide (NT-proBNP).

Furthermore, detailed in-hospital data was registered, including: demographic (such as age and sex), and coronary risk factors data (such as, smoking, previous diagnostic of diabetes mellitus, hypertension and hyperlipidaemia) and personal history and family history of coronary artery disease. Current, in-hospital and after angioplasty medication was also recorded: insulin or other anti-diabetic drugs; antiplatelet and anti-aggregant drugs, such as aspirin, clopidrogel or glycoprotein IIb/IIIa receptor antagonists; β-blockers; ACE inhibitors and statins.

2.2.1 Definition of risk factors, clinical signs and syndromes

Diabetes was diagnosed on the basis of fasting plasma glucose concentration ≥7.0 mmol/l (126 mg/dl) or 2–h plasma glucose ≥11.1 mmol/l (200 mg/dl) or confirmed as clinically known and treated diabetes mellitus. Subjects were diagnosed hypertensive if they were documented to have systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or were already on anti-hypertensive therapy. Dyslipidaemia was identified in subjects who had been given lipid-lowering medication or having total serum cholesterol level ≥190 mg/dl or serum triglycerides ≥180 mg/dl.

Smoking was defined as the inhaled use of cigarettes, cigars or pipes in any quantity. Subjects who smoked within the previous 1 year were also defined as smokers.

Several inflammatory markers such as white blood-cell count, CRP and TNF-α levels have been extensively associated with cardiovascular disease risk (Ikonomidis *et al.*, 2008). In fact, several studies established cut-off values associated with cardiovascular risk and to the occurrence of adverse cardiovascular events for these biomarkers (Sabatine *et al.*, 2002a; Sabatine *et al.*, 2002b; Grau *et al.*, 2004; Ridker & Cook, 2004; Ikonomidis *et al.*, 2005; Ridker *et al.*, 2005).

Based on these documented limits values, we establish risk classes for white blood-cell count, CRP and TNF-α levels. For the white blood-cell counts the cut-off value (low/high risk) considered was $>10.1 \times 10^9$/l (Sabatine *et al.*, 2002a; Grau *et al.*, 2004). The classes of cardiovascular risk associated to CRP were considered as low and high risk based on the cut-off value of ≥3 mg/dl (Sabatine *et al.*, 2002b; Ridker & Cook, 2004; Ridker *et al.*, 2005). Finally, for the TNF-α concentrations the cut-off value considered for low/high risk was ≥3.61 pg/ml (Ikonomidis *et al.*, 2005).

2.2.2 Angiographic data

AMI and CAD patients were clinically characterized for the extension of coronary artery disease through the characterization of lesion morphology data to define the coronary stenosis, the number of diseased vessels, flow characteristics of responsible vessel such as the thrombolysis in myocardial infarction (TIMI) risk score (from 0-3 referring to the levels of coronary blood flow assessed during coronary angiography), lesion length and the presence of calcium and/or thrombi in the lesions. The number and type of stents (bare metal or drug eluting stent) positioned in patients undergoing coronary angiography were also recorded.

A coronary stenosis was considered clinically significant (high-grade) above 70% narrowing in the luminal diameter. Multivessel disease was defined when more than one major coronary artery presented high-grade stenosis. Patients were classified according to the class of stenosis based on TIMI risk score: TIMI 3 – patients having normal flow and complete perfusion; and TIMI < 3 – patients with some degree of occlusion. In the later the TIMI scores considered were TIMI 2 for flow partial perfusion and TIMI 0 for no perfusion.

Additionally, patients were divided into 2 subgroups according to the lesion length using a cut-off of 15 mm. Small lesions were considered ≤15 mm and large lesions >15 mm.

2.3 Study protocol

A longitudinal study was carried out in the AMI patients. Patients were assessed at hospital admission before the administration of IIb/IIIa inhibitors and coronary angioplasty intervention, 2 and 40 days after the initial intervention. At these three time-points biochemical parameters and circulating inflammatory markers were measured. CAD and CC patients were only assessed at hospital admission before percutaneous intervention.

2.4 Blood sampling and laboratory assays

Blood samples were drawn from all patients into pyrogen-free blood collection tubes without additives and immediately centrifuged (2500 rpm for 10 minutes). The serum was collected after centrifugation and aliquots were stored at -80ºC until analysis. Sample storage period did not exceed 6 months. Samples were thawed only once.

Lipid profile, glucose levels, albumin, creatinine kinase, troponin T, NT-proBNP, blood cells count and levels of high-sensitivity CRP were routinely measured on hospital.

Soluble concentrations of ICAM-1 and P-selectin were measured by enzyme-linked immunosorbent assays (ELISA) commercial kits (R&D Systems, Minneapolis, USA). The concentrations of TNF-α were assessed using a commercial available high sensitivity ELISA (R&D Systems, Minneapolis, USA). All the assays were performed on serum according to the manufacturer's recommendations. Each sample was measured in duplicate; the intra-assay variation among the duplicates for all samples was <10%.

2.5 Statistical analysis

Data were summarized as mean and standard deviation (SD) or median and quartiles for continuous variables and as proportions for categorical variables. Non-continuous variables were analysed using a 2x2 table and χ^2 test. Differences between the four study groups were compared using a general linear model analysis of variance (ANOVA) followed by post hoc procedures (Tukey and Scheffé tests) to identify differences. ANOVA adequacy was weighted by checking the variance homogeneity using Levene's test and by verifying the significance of F distribution with the Welch statistics. For the variables white blood-cell, neutrophils, lymphocytes and monocyte counts, sP-selectin, sICAM-1, CRP, total cholesterol, LDL-cholesterol, triglycerides, glucose, troponin T and NT-proBNP the homogeneity of variance was not reached, thus variables were logarithmic transformed before performing ANOVA analysis.

Associations between inflammatory mediators, and with risk factors, co-morbidities and angiographic data were evaluated using non-parametric Spearman correlations.

The study of the repeated measures of inflammatory parameters in AMI patients through time requires specific statistical methods as common analysis of variance or non-parametric correlations are inappropriate. The inflammatory parameters and mediators, CRP, sP-selectin, sICAM-1, TNF-α, and blood cell counts, were measured in day 0 at hospital admission before percutaneous transluminal coronary angioplasty, and repeated at day 2 and day 40 after the initial percutaneous transluminal coronary angioplasty in the same patient. Consequently, the observations are inter-correlated and classical statistical techniques do not account for this type of variability. For that reason, a non-linear regression

algorithm that accounts for the effect of repeated measures was applied, the linear mixed effects model. The procedure of lineal mixed effects models the concentrations of the inflammatory markers measured through time considering that measures for each patient were not independent (Twisk, 2006). Using this algorithm the concentrations of the inflammatory parameters CRP, sP-selectin, sICAM-1, TNF-α) and blood cell counts can be modelled as a response variable on time. This statistical model describes the longitudinal variations of each patient for each variable by calculating slopes and averages of the variables in each time point. Therefore, it allows to estimate the differences in average slopes between baseline (day 0) and the other time points, giving a measure of the variation of each blood marker over time. In order to apply the lineal mixed effects model, variables should comply with normality distribution. A logarithm transformation was used for the variables CRP, sICAM-1, white blood-cell, neutrophil and lymphocyte counts, while a square root transformation was used for monocyte counts, sP-selectin and TNF-α.
Values of $p<0.05$ (two-tailed) were considered statistical significant.
The calculations were performed using SPSS (version 19.0) and R (version 2.11.1) software.

3. Results

3.1 Demographic and clinical characteristics of the study groups

The demographic characteristics and clinical features of the study groups including risk factors, co-morbidities and previous-event medication intake are summarized in Table 1.
The age of subjects enrolled ranged between 27 and 81 years, having the three groups matching ages. There were no significant differences between AMI, CAD and CC subjects in body mass index (BMI), waist perimeter and blood pressure, except for sex ratio. As can be inferred from Table 1, AMI patients were mostly men (80%), while in CAD and CC groups the percentage of the masculine gender decreased (70% and 60%, respectively). The three groups also had similar prevalence of risk factors as diabetes, dyslipidaemia and hypertension, however smoking was more frequent in AMI patients.
Since coronary disease risk factors and co-morbidities were exclusion criteria in REF group selection, these subjects were not considered in this comparison.

	CC (n=29)	**CAD** (n=55)	**AMI** (n=65)
Sex, f/m	13/16	18/37	15/50
Age (y)	60±9	64±9	61±15
Body mass index (kg/m^2)	29±6	27±3	27±4
Waist perimeter (cm)	100±13	98±9	94±17
Systolic blood pressure (mm Hg)	140±20	152±23	125±22
Diastolic blood pressure (mm Hg)	77±10	78±10	73±15
Risk factors and co-morbidity			
Hypertension, n (%)	19 (66)	35 (63)	42 (64)
Smoking, n (%)	3 (10)	6 (11)	33 (50) [a,b]
Dyslipidaemia, n (%)	21 (72)	34 (61)	38 (58)
Diabetes, n (%)	7 (24)	16 (24)	12 (21)
Family history of CAD, n (%)	1 (3)	12 (21)	9 (14)

Table 1. Clinical characteristics of the studied groups.

Values are expressed as mean±SD, except otherwise indicated. [a] $p<0.05$ vs CTR group; [b] $p<0.05$ vs CAD group.
Medication intake in AMI group had also into account the medication before hospital admission and the in-hospital and follow-up treatments. There were no significant differences in the use of pre-event medication in AMI patients and patients with CAD or CC subjects (Table 2).

	CC (n=29)	**CAD** (n=55)	**AMI** (n=65)
Previous-event medication			
Aspirin, n (%)	9 (31)	23 (41)	20 (30)
ACE-inhibitor, n (%)	9 (31)	20 (36)	23 (35)
β-blockers, n (%)	7 (24)	15 (27)	11 (17)
Statins, n (%)	13 (45)	17 (48)	19 (29)

Table 2. Pre-event medication in the studied groups.

After admission, in-hospital medication for the AMI patients included aspirin (59%), β-blockers (52%), angiotensin-converting enzyme (ACE)-inhibitors (55%), statins (12%) and antiplatelet inhibitors (79%). During follow-up 80% of patients took antiplatelet inhibitors (clopidogrel and aspirin) in addition to the previous medication referred above. Furthermore, in the course of the angioplasty, stents were implanted in 86% of the patients, being 29% drug-eluting stents.

	CC (n=29)	**CAD** (n=55)	**AMI** (n=65)
Total cholesterol (mg/dl) *	165 (148 – 200)	158 (133 – 205)	191 (158 – 230)
LDL-cholesterol (mg/dl) *	111 (85.8 – 136)	102 (82.0 – 129)	128 (103 – 149)
HDL-cholesterol (mg/dl) *	40 (312 – 50)	42 (35 – 50)	38 (31 – 47)
Triglycerides (mg/dl) *	104 (65 – 137)	104 (68 – 130)	122 (59 – 154)
Haematocrit (%)	40 (36 – 43)	39 (36 – 42)	40 (37 – 44)
Glucose (mg/dl) *	111 (95 – 128)	115 (94 – 134)	139 (116 – 202)
Albumin (g/dl)	3.70 (3.40 – 3.90)	3.6 0 (3.20 – 4.00)	3.40 (3.10 – 3.70)
Troponin T (ng/ml)	<0.01 **	<0.01 **	0.34 [a,b] (0.08 – 1.74)
NT-proBNP (pg/ml)	71 (40 – 126)	102 (52 – 235)	275 [a,b] (137 – 1030)

Data are expressed as median and quartiles (lower quartile-upper quartile). * not compared (see text); ** values below detection limit; [a] $p<0.05$ vs CC group; [b] $p<0.05$ vs CAD group.

Table 3. Biochemical data of the studied groups.

Concerning biochemical data (as listed in Table 3) AMI patients at admission had high levels of troponin T and NT-proBNP. Lipid and glucose data obtained could not be directly compared as fasting-blood tests were only performed for REF individuals.

3.2 Angiographic features

Of the 151 subjects submitted to angiography (CC, CAD and AMI groups), 29 subjects (19%) had at least one episode of chest pain previous to the coronary angiography, whereas 122 (81%) were asymptomatic.

Lesion morphology data, based on angiography, in AMI and CAD patients was obtained for 94 patients (77%), for the remaining patients those data were not possible to be obtained from the hospital registries.

Multivessel disease was found in a total of 42 patients (45%). Analysis of angiographic complexity of the diseased vessels showed impaired flow (TIMI <3) in 44 patients (54%), and large lesions (>15 mm) also in 44 patients.

From all the patients analysed, it was possible to detect the presence of calcium within the lesions in 14 patients (13%) and the presence of thrombi in 25 patients (24%).

The resume of the angiographic data for the CAD and AMI patients is listed in Table 4.

Comparing the morphologic data of patients from the CAD and AMI groups, it is possible to verify that an impaired flow (TIMI <3) is more frequent in AMI patients than in CAD patients. Furthermore, AMI patients had more often thrombi in the lesions than the CAD patients (Table 4).

	CAD	**AMI**
Multivessel disease, n (%)	15 (42)	27 (47)
TIMI		
TIMI 0, n (%)	2 (8)	32 (58)
TIMI 1, n (%)	2 (8)	3 (5)
TIMI 2, n (%)	1 (4)	4 (7)
TIMI 3, n (%)	21 (81)	16 (29)
Impaired flux TIMI <3, n (%)	5 (19)	39 (71) [a]
Lesion length		
Small lesions, n (%)	11 (44)	18 (56)
Large lesion, n (%)	14 (38)	30 (63)
Lesions with calcium, n (%)	9 (20)	5 (8)
Lesions with thrombi, n (%)	2 (5)	23 (38) [a]

Data are expressed as number and percentages. [a] $p<0.05$ vs CAD group.

Table 4. Angiographic data of the patients studied.

3.3 Inflammatory mediators

The blood cell counts and concentration level of inflammatory mediators for the studied groups are presented in Table 5.

	REF (n=25)	**CC** (n=29)	**CAD** (n=55)	**AMI** (n=65)
Blood cell count				
White blood-cells ($x10^9$/l)	5.36 (5.06 – 6.26)	6.81 (5.74 – 8.56)	6.32 (5.39 – 7.88)	11.3 [a,b,c] (7.69 – 13.9)
Neutrophils ($x10^9$/l)	3.00 (2.49 – 3.59	3.54 (3.20 – 4.19)	4.08 [a] (2.94 – 4.95)	8.34 [a,b,c] (5.61 – 11.2)
Lymphocytes ($x10^9$/l)	2.02 (1.71 – 2.26)	2.03 (1.69 – 3.32)	1.77 (1.38 – 2.30)	1.54 (1.24 – 2.24)
Monocytes ($x10^9$/l)	0.37 (0.28 – 0.51)	0.42 (0.28 – 0.58)	0.50 (0.34 – 0.67)	0.60 [a,b,c] (0.42 – 0.81)
Inflammatory markers				
CRP (mg/dl)	<0.32 **	0.66 (0.31 – 0.74)	0.43 (0.18 – 1.29)	0.69 [a] (0.34 – 1.39)
TNF-α (pg/ml)	0.37 (0.12 – 1.14)	1.17 (0.73 – 2.26)	1.57 [a] (0.75 -2.69)	1.57 [a] (0.57 – 2.47)
sP-selectin (ng/ml)	84 (93 – 119)	68 [a] (41 – 83)	53 [a] (42 – 82)	83 (54 – 116)
sICAM-1 (ng/ml)	214 (190 – 246)	247 (204 – 316)	220 (200 – 237)	248 (218 – 258)

Data are expressed as median and quartiles (lower quartile-upper quartile). ** values below detection limit; [a] $p<0.05$ vs REF group; [b] $p<0.05$ vs CC group; [c] $p<0.05$ vs CAD group.

Table 5. Inflammatory mediators in the four study groups.

The results revealed that the circulating counts of white blood-cells and neutrophils in AMI patients at hospital admission were increased relative to the other groups (REF, CC and CAD). Monocyte counts were also increased in AMI patients relative to a normal baseline situation (REF group). The circulating levels of CRP and TNF-α also showed the same trend (Table 5). Furthermore, in CAD patients the neutrophils counts and the TNF-α concentrations were also increased in comparison to REF subjects (Table 5).

Opposite, the concentrations of sP-selectin were significantly decreased in CAD and CC subjects in comparison to the REF subjects (Table 5).

The relationships of white blood-cells, neutrophils, monocytes, lymphocytes counts, inflammatory mediators (CRP, TNF-α, sP-selectin and sICAM-1) and cardiac markers (NT-proBNP and troponin T) concentrations, were assessed using non-parametric Spearman correlations. Several significant associations were found.

Relevant positive correlations were verified between CRP and ICAM-1($r=0.415$, $p<0.001$), neutrophil ($r=0.378$, $p<0.001$) and monocyte counts ($r=0.437$, $p<0.001$). sICAM-1 also showed positive associations with white blood-cell counts ($r=0.342$, $p=0.004$) and TNF-α concentrations ($r=0.415$, $p<0.001$).

Apart from the relationship between the number of neutrophils and monocytes in blood ($r=0.464$, $p<0.001$), neutrophils and monocytes were also associated to the levels of cardiac function markers troponin T and NT-proBNP ($r=0.526$ and $r=0.315$, $p<0.001$, respectively for neutrophils; and $r=0.438$ and $r=0.356$, $p<0.001$, respectively for monocytes). CRP was also significantly associated to the levels of troponin T and NT-proBNP ($r=0.626$ and $r=0.470$, $p<0.001$, respectively).

Given the importance of risk factors and co-morbidities in the disease evolution, hypertension, dyslipidaemia, smoking habits and diabetes were also tested for Spearman correlations with inflammatory markers. Relevant correlations were only verified for sP-selectin and sICAM-1 with cardiovascular risk factors. sP-selectin showed a negative correlation with hypertension ($r=-0.379$, $p<0.001$) and a positive correlation with smoking ($r=0.381$, $p<0.001$). Furthermore, sICAM-1 was also positively associated to smoking ($r=0.373$, $r<0.001$).

Importantly, levels of P-selectin were also negatively associated with age ($r=-0.399$, $p<0.001$). In fact, subjects with age above 65 had lower levels of sP-selectin (55 ng/ml) compared to younger (≤65 years) subjects (78 ng/ml).

As mentioned previously the enrolled subjects were classified in risk classes white blood-cell count, CRP and TNF-α levels. The frequency of each class in the studied population is resumed in Table 6.

	CC	CAD	AMI	Total Population
WBC risk score				
Low risk (≤10.1x10⁹/l), n (%)	28 (97)	50 (89)	24 (36)	127 (72)
High risk (>10.1x10⁹/l), n (%)	1 (3)	1 (2)	33 (50)	35 (20)
CRP risk score				
Low risk (<3 mg/dl), n (%)	22 (76)	38 (68)	51 (77)	135 (76)
High risk (≥3 mg/dl), n (%)	1 (3)	3 (5)	5 (8)	9 (5)
TNF-α risk score				
Low risk (>3.61 pg/ml), n (%)	19 (66)	20 (36)	35 (53)	89 (72)
High risk (≥3.61 pg/ml), n (%)	3 (10)	2 (4) [a]	4 (6)	9 (5) [a]

Data are expressed as number and percentages.

Table 6. Distribution of the enrolled subjects in the white blood-cell (WBC), CRP and TNF-α risk classes.

3.3.1 Longitudinal variations

The blood cell counts, the concentrations of inflammatory mediators and other biochemical markers, in AMI patients assessed at hospital admission, 2 and 40 days after percutaneous transluminal coronary angioplasty intervention are presented in Table 7.

In the overall an increasing trend of the measured concentrations of sICAM-1 and TNF-α through time was observed, while a decreasing one was observed for white blood-cell and neutrophil counts. Monocyte counts and CRP concentration showed an initial increase. To assess the significance of these changes through time a regression model (linear mixed effects model) was applied. As mentioned before (see section 2.5 Statistical analysis), appropriate transformations of variables had to be applied. Therefore, the results from the

	AMI patients		
	Day 0	**Day 2**	**Day 40**
Biochemical characterization			
Total cholesterol (mg/dl)	191 (158 – 230)	162 (136 – 192)	141 [a] (127 – 180)
LDL-cholesterol (mg/dl)	128 (103 – 149)	105 (78 – 122)	81 [a] (70 – 106)
HDL-cholesterol (mg/dl)	38 (31 – 47)	38 (27 – 47)	35 [a] (29 – 41)
Triglyceride (mg/dl)	122 (59 – 154)	95 (90 – 110)	112 [a] (79 – 175)
Haematocrit (%)	40 (37 – 44)	39 [a] (34 – 42)	42 [c] (39 – 45)
Glucose (mg/dl)	139 (116 – 202)	121 (102 – 151)	102 [a] (93 – 135)
Albumin (g/dl)	3.40 (3.10 – 3.70)	3.19 [a,b,c] (3.00 – 3.50)	3.90 (3.60 – 4.20)
Troponin T (ng/ml)	0.34 [b,c] (0.08 – 1.74)	2.40 [a,b,c] (1.70 – 4.10)	<0.01 **
NT-proBNP (pg/ml)	275 [b,c] (137 – 1030)	1324 [a,b,c] (519 – 2955)	611 [a,b,c] (354 – 1009)
Blood cell counts			
White blood-cells ($x10^9$/l)	11.3 [a,b,c] (7.69 – 13.9)	8.48 [a,c] (6.31 – 10.3)	6.68 (5.37 – 7.33)
Neutrophils ($x10^9$/l)	8.34 [a,b,c] (5.61 – 11.2)	5.32 [a,b,c] (4.05 – 6.37)	3.82 [a] (3.12 – 4.85)
Lymphocytes ($x10^9$/l)	1.54 (1.24 – 2.24)	1.79 (1.25 – 2.51)	1.93 (1.54 – 2.23)
Monocytes ($x10^9$/l)	0.60 [a,b,c] (0.42 – 0.81)	0.74 [a,b,c] (0.62 – 0.91)	0.48 (0.40 – 0.58)
Inflammatory markers			
CRP (mg/dl)	0.69 [a] (0.34 – 1.39)	3.49 [a,b,c] (1.52 – 6.92)	0.34 (0.32 – 0.70)
TNF-α (pg/ml)	1.57 [a] (0.57 – 2.47)	1.57 (0.75 – 2.24)	2.10 (1.06 – 3.15)
sP-selectin (ng/ml)	83 (54 – 116)	67 (48 – 79)	70 (62 – 103)
sICAM-1 (ng/ml)	248 (218 – 258)	281 (238 – 309)	298 (224 – 400)

Data are expressed as median and quartiles (lower quartile-upper quartile). ** values below detection limit; [a] $p<0.05$ vs REF group; [b] $p<0.05$ vs CC group; [c] $p<0.05$ vs CAD group (for comparison see Table 5).

Table 7. Biochemical characterization and inflammatory mediators in the AMI patients at the three time-points: hospital admission (Day 0), two (Day 2) and 40 days (Day 40) after percutaneous transluminal coronary angioplasty intervention.

obtained longitudinal models had into account these transformations (as can be observed in Figures 1 and 2). However, for results considerations purposes those transformations will not be further mentioned in the text.

The association between white blood-cells, neutrophils, monocytes and lymphocytes and time were significant ($p<0.05$) (see Figure 1).

Higher white blood-cell, neutrophil and monocyte counts were observed in the acute phase of AMI (Table 5). The high white blood-cell and neutrophil counts at admission significantly decrease in the following weeks (Figure 1A and 1B), reaching values similar to those observed in CAD patients and in control subjects (Tables 5 and 7).

By the contrary, slightly low lymphocyte counts were observed in the acute phase of myocardial infarction, although that difference was not significant (Table 5). Those low levels were maintained after percutaneous transluminal coronary angioplasty (day 2) and increased with patient's stabilization (Figure 1C and Table 7).

Monocyte counts reach the highest value at day 2 (Figure 1D), decreasing in the following days (Day 40). At that time-point, the monocyte counts were similar to the counts verified in CAD, CC and REF groups (Tables 5 and 7).

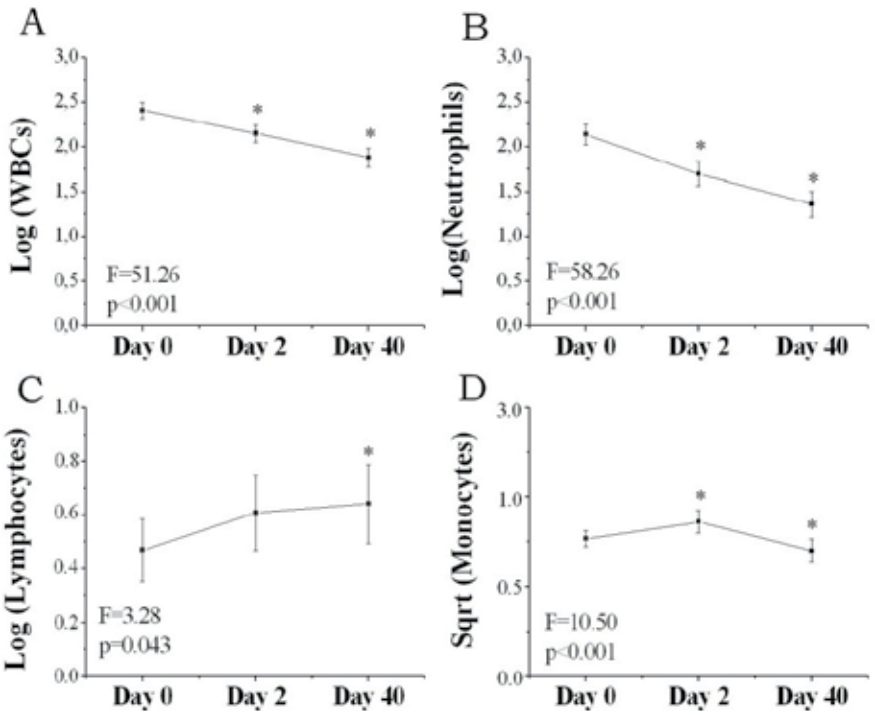

Fig. 1. Longitudinal variations of inflammatory cell counts, white blood-cell (WBC; A), neutrophils (B), lymphocytes (C) and monocytes (D) in AMI patients at hospital admission, two (Day 2) and 40 days (Day 40) after percutaneous transluminal coronary angioplasty intervention. * $p<0.05$ vs AMI patients at Day 0.

The levels of sP-selectin in AMI patients were characterized by a decrease after PCTA followed by an increase to day 40 (Figure 2A). The association between sP-selectin and time was significant ($p=0.003$). Serum levels of this soluble adhesion molecule at inclusion were

remarkably elevated than 48 h later (p=0.001). After that abrupt decrease, sP-selectin levels seem to slightly increase, reverting to the levels observed at hospital admission.
An increasing trend of sICAM-1 to day 40 was observed (Table 7) but the association of sICAM-1 with time was not significant (p=0.085; see Figure 2B).
The levels of CRP were increased in AMI at admission (Table 5), and further increase at day 2 reverting to significantly low levels after 40 days (p<0.05), as verified in Figure 2C. Those concentration levels were not significantly different from those verified in a normal non-inflammatory situation – REF group (Tables 5 and 7).
The serum levels of TNF-α increased from day 0 to day 40 (Figure 2D). TNF-α levels were remarkably elevated through time (p<0.001). TNF-α concentrations were higher at day 2 than at admission (day 0) and continue to increase until day 40 (p<0.001 relative to day 0).

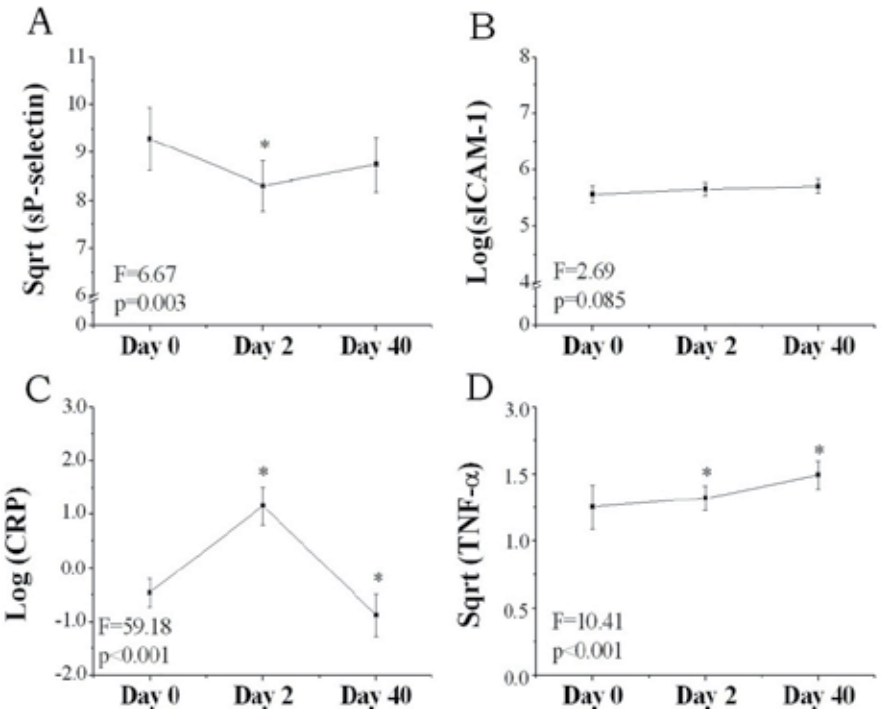

Fig. 2. Longitudinal variations of systemic concentrations of sP-selectin (A), sICAM-1 (B), CRP (C) and TNF-α (D) in AMI patients at hospital admission, two (Day 2) and 40 days (Day 40) after percutaneous transluminal coronary angioplasty intervention. * p<0.05 vs AMI patients at Day 0.

3.3.2 Medication influence

To assess the influence of previous-event medication on inflammatory mediators, subjects that did not take medication before enrolment were compared to the remaining patients that had prescribed medication. Only sP-selectin and sICAM-1 concentrations were significantly affected by the previous-event drug intake (p=0.002 and p=0.014, respectively). Subjects without pre-event medication had higher levels of these molecules (94±32 ng/ml and 280±96 ng/ml, respectively) than those with prescribed aspirin, ACE-inhibitors, β-blockers or statins (61±22 ng/ml and 235±58 ng/ml, respectively).

To further evaluate the possible influence of drug therapy in serial changes of the inflammatory markers, the pre-event, in-hospital and follow-up medication data were categorized to type (antiplatelet inhibitors, ACE-inibitors, β-blockers and statins) and added to the linear mixed effects models as co-variables. None of the drugs administered to the patients before or after admission significantly influenced the neutrophil and monocyte counts, and the CRP, TNF-α or sICAM-1 concentrations (data not shown). However, the sP-selectin variations over time were significantly influenced by pre-event ACE-inibitors ($p<0.001$) and β-blockers ($p=0.019$) intake. Patients that received β-blockers or ACE-inibitors showed minor serial changes and low levels of sP-selectin opposite to those that were not taking those drugs. The same trend was verified for the white blood-cell and lymphocytes counts longitudinal variations with the pre-event and β-blockers ($p=0.024$ and $p=0.25$, respectively) intake. AMI patients that received β-blockers showed minor serial changes and low levels of white blood-cell and lymphocytes counts compared to those that were not taking those drugs.

3.4 Relationships between angiographic features and inflammatory mediators

The existence of associations or variations between the levels of inflammatory mediators and the characterization of lesion morphology was also tested, including the number of diseased vessels (single versus multivessel disease), lesion length (small ≤15 mm versus large >15 mm lesions), TIMI risk score and the presence of calcium and thrombi.

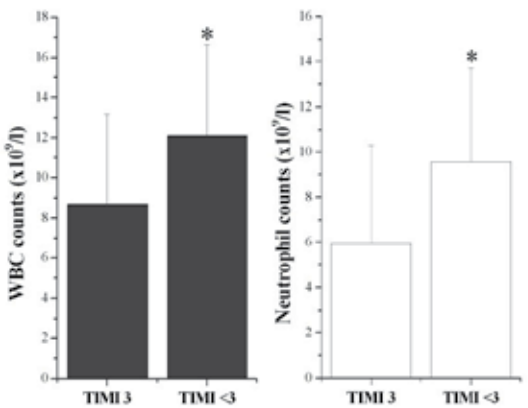

Fig. 3. Variations of white blood-cell (WBC) and neutrophil counts in the two classes of stenosis based on TIMI risk score (TIMI 3 and TIMI <3). * $p<0.05$ vs normal flux TIMI 3.

The neutrophil counts were negatively correlated to the TIMI risk score classes ($r=-0.503$, $p<0.001$) and positively with the presence of thrombi in lesions ($r=0.424$, $p<0.001$). In fact, higher counts of neutrophils (and also white blood-cell) were observed in patients with impaired coronary flux (TIMI <3) than in patients with normal flux (Figure 3). The same tendency of increased levels was verified in patients with thrombi in lesions (Figure 4).

The concentrations of sP-selectin were negatively correlated to the TIMI score ($r=-0.554$, $p=0.041$). No further correlations of inflammatory markers and angiographic features were observed, except for the AMI patients' longitudinal variations.

Considering the presence of calcium in lesions, the lymphocyte counts were lower in calcified lesions than in lesions without calcium (Figure 5).

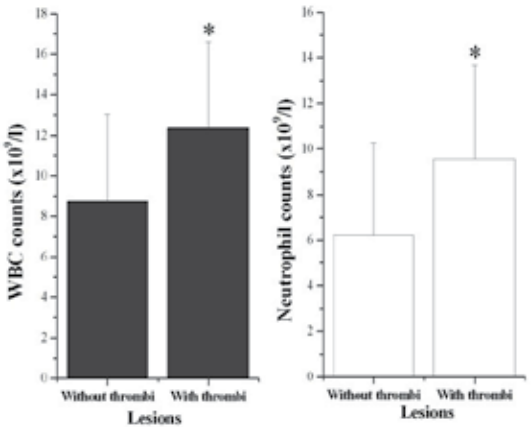

Fig. 4. Variations of white blood-cell (WBC) and neutrophil counts in the presence or absence of thrombi in the lesions. * p<0.05 vs lesions without thrombi.

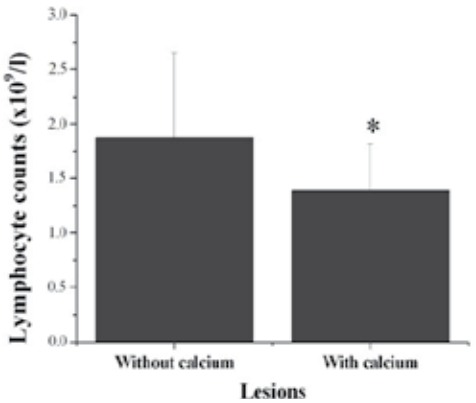

Fig. 5. Variations of lymphocytes counts in the presence or absence of calcium in the lesions. * p<0.05 vs lesions without calcium.

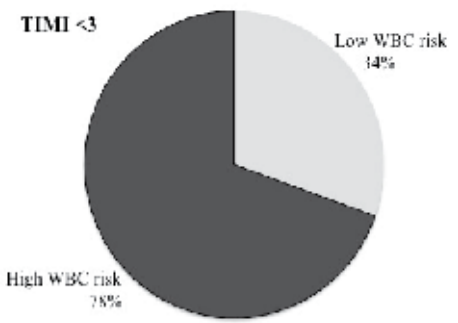

Fig. 6. Distribution of subjects with impaired flux (TIMI <3) through the white blood-cell (WBC) risk classes of cardiovascular events (low/high risk based on the cut-off level of >10.1x10⁹/l).

Examining the longitudinal variations of inflammatory mediators and the angiographic features, no associations were verified for serial changes of white blood-cells, neutrophils, sP-selectin and sICAM-1 ($p>0.05$; data not shown).
In what concerns the disease extension, the concentrations of CRP over time were positively correlated with multivessel disease ($p=0.026$). While, the monocyte counts and TNF-α serial changes were correlated to the lesion length ($p=0.043$ and $p=0.38$, respectively).
The risk classes of cardiovascular events based on white blood-cell and TNF-α cut-off levels were also tested for possible associations to the lesion morphology data.
No correlations were found to the CRP risk class with the lesion morphology data.
By the contrary, the white blood-cell risk class was negatively correlated with the TIMI score classes ($r=-0,439$, $p<0.001$) and positively with the presence of thrombi in lesions ($r=0.460$, $p<0.001$). The distribution of patients within the TIMI score classes differs in the low versus high white blood-cell risk classes. As can be observed in Figure 6, among patients with impaired flux (TIMI <3), there were a higher number of patients with high WBC risk (>10.1x10^9/l) than patients with low WBC risk ($p<0.001$). The same tendency is verified for the presence of thrombi in lesions ($p<0.001$; see Figure 7).

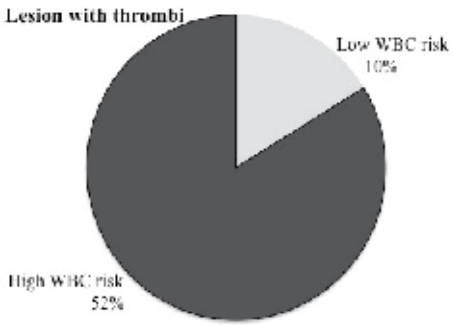

Fig. 7. Distribution of subjects with thrombi in lesions through the white blood-cell (WBC) risk classes of cardiovascular events (low/high risk based on the cut-off level of >10.1x10^9/l).

Furthermore, among patients with calcified lesions there is also a higher frequency of patients with high TNF-α risk (≥3.61 pg/ml) than patients with low TNF-α risk (see Figure 8), although the difference did not reach significance ($p=0.08$).

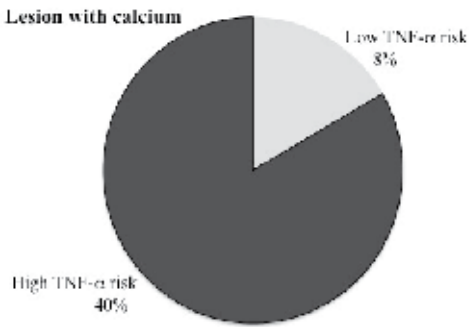

Fig. 8. Distribution of subjects with thrombi in lesions through the TNF-α risk classes of cardiovascular events (low/high risk based on the cut-off level of (≥3.61 pg/ml).

4. Discussion

It is now widely recognized that inflammation plays a critical role in plaque destabilization and vulnerability and that is a key-event in coronary artery disease (Alam *et al.*, 2004). In the last decades, important information about the pathophysiology and mechanism of coronary artery disease and acute myocardial infarction had been extensively studied (Davies, 2000; Frangogiannis *et al.*, 2002; Libby, 2003; VanWijik *et al.*, 2003; Alam *et al.*, 2004; Fichtlscherer *et al.*, 2004; Kumar *et al.*, 2004; Wiviott *et al.*, 2004; Jefferson *et al.*, 2005; Armstrong *et al.*, 2006a; Armstrong *et al.*, 2006b; Libby, 2008; Skyschally *et al.*, 2008). However, the understanding of the interactions between inflammatory markers and between the different cell types involved in those processes remains relatively unexplored, especially in human populations.
Angiography is a first-line test for coronary artery disease, particularly for screening symptomatic patients. The evaluation of asymptomatic individuals relies on the identification of risk factors. However, neither the absence of stenosis provided by angiography assure the lack of future cardiac events, nor the cardiovascular events are readily explained by cardiovascular risk factors (Fisher *et al.*, 2000; Kern, 2000; Hadamitzky *et al.*, 2009; Marwan *et al.*, 2009). Therefore, non-invasive estimation of coronary disease risk is important to screening both symptomatic and asymptomatic patients. Furthermore, the understanding of the cellular biology of the unstable plaque remains poorly known, and the crucial question is still the identification of the factor(s) that play a significant role in the plaque vulnerability.
A multi-parameter approach was used in the present study, which allowed a better understanding of the complex relationships between the studied markers that meant to capture different stages of the inflammatory response involved in the different phases of coronary artery disease evolution and lesion progression.

4.1 Inflammatory markers in CAD

White blood-cell count, the most widely available and inexpensive measure of systemic inflammation has been associated with cardiovascular mortality both in primary and secondary prevention settings. In apparently healthy individuals, a high white blood-cell count has been associated with increased cardiovascular mortality and incidence of coronary artery disease, independently of traditional atherosclerotic risk factors (Ikonomidis et al., 2008).
In acute myocardial infarction two different inflammatory processes can be considered: the coronary arterial inflammation that leads to the pathogenesis of acute myocardial infarction; and the myocardium inflammation after the acute phase that leads to ventricular remodeling and cardiac repair (Cheng *et al.*, 2005). The immune cells can be related to both processes.
The increase in total white blood-cells occurring in AMI patients is considered as an expression of acute-phase reaction (Dragu *et al.*, 2008; Bodi *et al.*, 2008), reflecting the infiltration of leukocytes into the necrotic tissue in response to ischemia and reperfusion. Neutrophils are the first leukocytes to be found in damaged myocardial area (Yu *et al.*, 2009). The prognostic role of leucocytes is supported by observations from thrombolysis trials that identified leukocyte count as a predictor of short- and long-term adverse clinical outcomes, whereas elevated neutrophil count is significantly associated with myocardial infarct extension and the early development of congestive heart failure (Yu *et al.*, 2009).
Monocytes infiltrate the infarct zone where they appear to orchestrate the cardiac repair and remodeling process through a complex cascade involving cytokines and growth factors secretion (Dragu *et al.*, 2008). Their number is reported (Bodi *et al.*, 2008) to increase 2 to 3

days after the acute episode. The elapsed time between neutrophils and monocytes responses was observed in the present study, as monocyte count peak was only reached at day 2 in AMI patients.

No significant variations were observed in lymphocytes count in the four groups studied. However, in AMI patients the slight decrease in lymphocytes number at the onset of the acute event was emphasized in the longitudinal study where their number significantly increased to 40 days. Lymphopenia was previously described in the literature (Bodi *et al.*, 2008). The reason for the fall in T lymphocytes numbers and activity is not yet completely understood (Takeshita *et al.*, 1997), but several authors proposed that could result from a self-protective response in face of an overshoot of pro-inflammatory cytokines and other products with tissue-damage potential (Steppich *et al.*, 2007; Elenkov *et al.*, 2005). A massive lymphocyte apoptosis is proposed as the underlying mechanism (Bodi *et al.*, 2008). This theory gained more attention since hyper-inflammation was disregarded as the body primary response in acute stress situations, such as sepsis (Hotchkiss & Karl, 2003). Severe lymphopenia occurs in the initial stages of sepsis, but as the acute stress conditions evolves the lymphocyte counts return to normal levels (Bodi *et al.*, 2008).

During the acute event important feedback mechanism may be triggered to protect the organism from an "overshoot" of systemic pro-inflammatory cytokines and other products with tissue-damage potential by activated macrophages (Elenkov *et al.*, 2005). As a consequence of those feedback mechanisms no systemic T lymphocytes activation would happen (Steppich *et al.*, 2007), which could partially explain the low counts of lymphocytes verified at AMI onset. After acute myocardial infarction, myocardial necrosis releases or exposes normally sequestered antigenic constituents that may cause activation and proliferation of lymphocytes (Cheng *et al.*, 2005), returning to the normal levels as verified in our study.

After ischemia/reperfusion injury, leukocyte sequestration and the release of cytokines, such as TNF-α, may occur. Injured myocardium (Dawn *et al.*, 2004), several extra-cardiac tissues and immune system activation (Chiu *et al.*, 2005), proved contributing to circulating TNF-α levels. Increasing levels of this pro-inflammatory cytokine after infarct, as measured in AMI patients, has been reported previously (Bauriedel *et al.*, 2003; Barbaux *et al.*, 2001; Blancke *et al.*, 2005). This increasing suggests a continuous systemic inflammatory stimulation that can trigger and/or amplify local inflammatory responses related to ischemia/reperfusion injury (Barbaux *et al.*, 2001). Though a cytoprotective role for TNF-α has also been suggested (Blancke *et al.*, 2005; Zirlik *et al.*, 2007). The reported influence of TNF-α in the upregulation of adhesion molecules is evidenced by its positive association with sICAM-1.

No significant changes were verified in sICAM-1 levels in CAD and CC patients relative to healthy volunteers (REF group). Also, the levels of this adhesion molecule remained unchanged in AMI patients over 40 days after the acute event. Previous medication intake was found to exert no influence on sICAM-1 levels at admission. Also, in-hospital and follow-up medication had no influence on serial changes of this adhesion molecule. However, sICAM-1 levels were positively correlated with white blood-cell counts, suggesting ongoing inflammatory response in coronary artery disease patients that may favor the adhesion of inflammatory cells at injured site of lesions (Mulvihill *et al.*, 2000; O'Malley *et al.*, 2001). Some of the reported data demonstrated increases in circulating sICAM-1 for the first month after the acute event (O'Malley *et al.*, 2001; Haim *et al.*, 2002; Hartford *et al.*, 2006), plausibly reflecting in specific conditions ICAM-1 expression at the

endothelial surface (Mulvihill *et al.*, 2000). Although a stronger predictive information for sICAM-1 could not yet be found (Haim *et al.*, 2002; Hartford *et al.*, 2006).

Similar values of sP-selectin between coronary artery disease patients and controls, as verified in the present study, had already been reported in literature (Barbaux *et al.*, 2001; Blancke *et al.*, 2005; Khare *et al.*, 2005).

In this study, a negative association between soluble P-selectin levels and age was observed, similar to that referred by Barbaux *et al.* (2001). Those authors called the attention for a complex relation between P-selectin and coronary artery disease dependent on age, which could be related to the different effects of P-selectin according to the stage of progression of atherosclerosis. The soluble form of P-selectin could bind to leukocytes via PSGL-1 without triggering their subsequent recruitment on the vascular surface, which limits the excessive activation and extravasation of leukocytes. Therefore, high levels of sP-selectin may be beneficial in some situations by protecting against inflammatory reactions (Barbaux *et al.*, 2001). This may explain the unexpectedly higher levels of sP-selectin found in REF group similar to the levels of AMI patients and higher than the levels observed in CAD patients. In fact, subjects from REF group were younger than those in AMI and CAD groups, 40% of which had more than 65 years old. Similar sP-selectin values between coronary artery disease patients and controls had already been described in literature (Barbaux *et al.*, 2001; Khare *et al.*, 2005).

Furthermore, during the myocardial infarction, sP-selectin levels may be influenced by an intricate network of processes involving inflammatory stimulus of injured myocardium and vascular wall, and administrated medication. The serial changes of sP-selectin shortly after AMI clearly evidenced the fall of activated platelets in consequence of massive anti-platelet and anti-thrombotic therapeutic measures during intervention. The results of the changes of sP-selectin over time evidence a significant influence of medication. Shimomura *et al.* (1998) reported sP-selectin changes in AMI patients at admission and after reperfusion therapy similar to ours. Currently used therapies, such as ACE-inhibitors and β-blockers effectively counteract heightened platelet activation and aggregability (Bauriedel *et al.*, 2003) resulting in decreased circulating levels of P-selectin (Cha *et al.*, 2004; Xiao *et al.*, 2004) as observed in this work. In fact, decreased sP-selectin levels were also found in CAD patients and CC subjects, which had a long-term therapy history.

C-reactive protein (CRP) is considered by many authors as one of the most suitable candidates as nontradicional risk factors, since it meets most of the criteria to be a useful indicator in cardiovascular diseases (Calabrò *et al.*, 2009). Elevated baseline concentrations of CRP are associated with the risk of atherosclerotic events in general populations and show a predictive value in terms of secondary prevention, both in patients with chronic stable angina and acute coronary syndromes (Calabrò *et al.*, 2009). The prognostic significance of CRP has also been shown in apparently healthy adults without cardiovascular disease (Ridker *et al.*, 2003; Dansesh *et al.*, 2004; Ridker & Cook 2004). Our results are therefore consistent with previous variations of this acute-phase reactant described in literature (Fang *et al.*, 2004; Li *et al.*, 2005; Hartford *et al.*, 2006).

Leukocytes and released cytokines may contribute to ischemia/reperfusion injury by interacting with endothelial cells (Xu *et al.*, 2006), linking the thrombotic and inflammatory responses (Libby & Simon, 2001). Thus, temporal and sequential association of events orchestrated by both inflammatory cells, e.g. white blood-cells, monocytes, neutrophils and lymphocytes, and inflammatory markers, such as TNF-α, CRP, sICAM-1, and sP-selectin seem to be crucial in the initiation of inflammatory responses after ischemia/reperfusion injury.

4.2 Inflammatory markers and angiographic features

There is a substantial interest in research and in clinical practice in the development and application of new biomarkers for risk stratification in patients with acute coronary syndromes.

In particular, strategies combining multiple biomarkers that may reflect diverse pathophysiological contributors to the onset and complications of acute events are appealing as an approach to improve risk assessment and effective therapy. Numerous studies have demonstrated independent associations between levels of various inflammatory markers and the presence of angiographically documented coronary artery disease (Sabatine *et al.*, 2002a). Evidences have been established for improved risk stratification using white blood-cells, B-type natriuretic peptide, high-sensitivity CRP, and troponin T, to mention a few, alone or in combination (Cavusoglu *et al.*, 2006; Sanchis *et al.*, 2004; James *et al.*, 2006). However, the relative importance of the various inflammatory markers with coronary disease is still scarce. Comparative evaluation of newer markers is necessary to assess these candidates for integration into present strategies concerning the evaluation of the strongest candidates in order to guide further development as well as potential clinical application.

Acute phase proteins, adhesion molecules and cytokines have appeared among the potential candidate biomarkers of inflammation based upon prognostic performance in studies in patients with coronary artery disease (Armstrong *et al.*, 2006a; Armstrong *et al.*, 2006b; Armstrong *et al.*, 2006c).

In our study various clinical, biochemical and inflammatory markers were correlated with angiographic findings and risk scores. White blood-cells, neutrophils, sP-selectin, sICAM-1, CRP and TNF-α, were found to be associated either with risk scores for stenosis and TIMI or with the presence of calcium and thrombi in lesions. It was also found that high neutrophil and white blood-cell counts were correlated to high-grade stenosis and to the presence of thrombi. In addition, increased risk score based on white blood-cells counts was strongly correlated with high-risk TIMI score.

White blood-cells counts have been associated with the lesion coverage and magnitude of coronary artery disease (Cavusoglu *et al.*, 2006), lower TIMI flow and myocardial perfusion grades during coronary angiography (Sabatine *et al.*, 2002a). Furthermore, white blood-cell count was strongly associated to multivessel coronary artery disease (Cavusoglu, *et al.* 2006). In unstable angina patients the baseline of white blood-cells count proved to be predictive of unfavorable clinical outcomes being highly significant for death within 30 days to six months (Sabatine *et al.*, 2002a). In addition, establishing levels of white blood-cells counts, contributed to improved risk stratification. In patients with low white blood cell counts the predictive of mortality ranged from 1.5% (25^{th} percentile) to 3.6% among patients with an intermediate white blood-cells count (25th to 75th percentiles), to 5.1% among patients with a high white blood-cell count (75th percentile). So far, no association between white blood cells count and new or recurrent myocardial infarction or rehospitalization for acute coronary syndromes could be established.

High neutrophil counts were also associated to high cardiovascular risk (Horne *et al.*, 2005). In the current study, the correlation of neutrophils with acute event manifestations, especially occlusive stenosis and the presence of thrombi in lesions, suggest their involvement in the coagulation management. Neutrophils are large cells that may accumulate in microvasculature after myocardial infarction. They can produce dramatic pathological anomalies as they adhere to capillary endothelium preventing reperfusion

(Bodi *et al.*, 2008). Neutrophils that are recruited to the thrombosis region may be trapped in cloth releasing, during degranulation, myeloperoxidase. Myeloperoxidase is in fact an abundant leukocyte lysosomal enzyme. It was found to be elevated in culprit lesions that have fissured or ruptured in patients with sudden death from cardiac causes. Numerous lines of evidence suggest mechanistic links between myeloperoxidase and both inflammation and cardiovascular disease, therefore linking neutrophils to acute coronary syndromes and highlighting its potential and usefulness for risk stratification among patients with chest pain (Brennan *et al.*, 2004).

Our concurrent results also evidence the ability of white blood-cells and neutrophils as inflammatory entities to predict the presence of angiographic coronary disease and in particular the acute event. The negative correlation of sP-selectin with the class of TIMI (flow from 0 to 3) reinforced the value of combining multiple pathophysiological contributors in the evaluation of angiographic coronary disease. High values of s-Pselectin are linked to limitations in flow. The sP-selectin is an adhesion molecule involved in cell-platelet aggregation and adhesion (Blann *et al.*, 2003; Armstrong *et al.*, 2006c). The verified synchronized rise of sP-selectin, white blood-cells and neutrophils count may be useful to further improve patients' evaluation and prognosis.

Also high CRP levels were associated to stenosis. Inflammation, atherosclerotic plaque rupture or myocardial necrosis, are mechanisms responsible for elevated CRP levels in the circulation. The potential use of CRP in patients' diagnosis and in risk stratification of patients with coronary disease has been largely studied. Although the use of CRP as a prognostic marker still remains controversial (Packard & Libby, 2008). In fact, our results indicated an inverse association of CRP levels with stenosis what may suggest that CRP increases may be governed by other mechanisms. Results may also express the non-relevance of CRP levels immediately after the acute event. Actually, the delay of approximately 48-h after the acute event for CRP increases is well documented in the literature (Fang *et al.*, 2004; Hartford *et al.*, 2006). Biomarkers studied in our work were measured at patient's admission, what for myocardial infarction patients correspond to a maximum of 6-h after acute event. Our findings also pointed out for a positive correlation of high-risk CRP score with sICAM-1, suggesting that endothelial activation may influence CRP expression rather than coronary stenosis.

On the other hand, the presence of calcium in the culprit lesion was associated to higher lymphocytes count, indicating that these cells may express the activity of the atherosclerotic plaque, as mineralized lesions usually indicate more stabilized plaques (Fischer *et al.*, 2000). As referred previously the moderate and non-significant decrease of lymphocytes at the acute phase of myocardial infarction is expressed in the longitudinal study carried out in our work. Lymphocytes are long-lived cells that memorize specialized information about the antigen pool at the individual level (Bodi *et al.*, 2008). Therefore, lymphocytes may keep information about the plaque composition and consequently be associated with the mechanisms of plaque formation during the life span of the individual. These findings are in line with current knowledge on coronary atherosclerotic plaque burden. Plaque burden, and not stenosis severity, was a more important marker of disease. Also, the prognosis of coronary artery disease is more closely related to atherosclerosis plaque stability than the extent of a particular stenosis. The lesion vulnerability is thought to be associated to the plaque composition (Fischer *et al.*, 2000). Lesions contain a lipid core intertwined by fibrous tissue that contributes to the disarrangement of intimal structure. In addition to the presence of macrophages and smooth muscle cells, lymphocytes and monocytes have been identified

in the sub-endothelial region close to the lipid core. The continuous accumulation of extracellular lipids and cell debris promote the atheroma growth with prominent fibrous connective tissue and intimal thickening. Eventually the lipid core or other areas of the arterial wall may calcify and the lesions may present fissures, hematomas and thrombi. Lesions with high fibrotic content are usually more occlusive and ultimately may progress to complete occlusion without participation of acute plaque rupture. Therefore, the susceptibility to rupture is not strictly linked to significant stenosis. Also, the acute coronary syndromes are associated with plaque disruption and associated flow-limiting thrombus that may not be caused by of a non-obstructive plaque.

The extent of coronary atherosclerosis, rather than the severity of stenosis, may be the most important predictor of death due to acute myocardial infarction or sudden cardiac death (Schmermund *et al.*, 1997). The quantification of atherosclerotic burden has become vital to proper risk stratification, especially in the intermediate risk population (Mieres *et al.*, 2005). Established noninvasive methods of evaluating CAD, such as stress testing, generally identify only patients with advanced atherosclerotic disease leading to a flow-limiting coronary stenosis and myocardial ischemia (Greenland & Gaziano 2003; Rumberger *et al.*, 2005). More recently published studies demonstrate a high sensitivity of coronary artery calcium for the presence of coronary artery disease but a lower specificity for obstructive coronary artery calcium depending on the magnitude of the coronary artery calcium (Budoff & Gul, 2008). Coronary calcification is a marker of atherosclerosis that can be quantified with the use of cardiac coronary tomography and it is proportional to the extent and severity of atherosclerotic disease. Coronary artery calcium was found to be a stronger independent predictor of future events than a sum of all of the conventional risk factors combined (Kennedy *et al.*, 1998). Based on multiple observational studies, patients with increased plaque burdens (increased coronary artery calcium) are approximately ten times more likely to suffer a cardiac event over the next 3–5 years (Budoff & Gul, 2008). Opposite, Bauer and coworkers (Bauer *et al.*, 2009) combining angiographic findings of calcified and non-calcified plaque burden and stenosis severity and the myocardial perfusion imaging finding of ischemia proposed that non-calcified plaque burden is a better predictor of the finding of myocardial ischemia at stress myocardial perfusion imaging than are calcium score and degree of stenosis.

A variety of inflammatory factors including the actions of inflammatory cells are thought to play an important role in plaque stability and calcification (Morrow *et al.*, 2008). Vascular calcification is a prominent feature of atherosclerosis but the mechanisms underlying calcification are still unclear. Vascular smooth muscle cells are currently considered to be responsible for the formation of vascular calcifications. Cytokines play an important role in regulation of vascular smooth muscle cells growth and differentiation.

In fact, in our work a strong positive association of TNF-α with lesion length was found. In addition high TNF-α levels (≥3.16 pg/ml) were associated with high calcium percentage in plaques. TNF-α was also found to be augmented at patients' admission (coronary artery disease and myocardial infarction) and progressively increase to 40 days in the infarction evolution. These findings suggest a dual role of TNF-α in coronary artery disease. On one hand TNF-α was associated with vascular calcification, possibly expressing a role in the stabilization of the plaque, and on the other hand TNF-α was related to the inflammatory process after myocardial infarction.

This pleiotropic nature of TNF-α is documented in literature (Trion & Laarse, 2004; Lencel *et al.*, 2010). TNF-α influences many aspects of atherosclerosis by increasing the permeability

of endothelial cells, promoting monocyte adhesion, inducing macrophage differentiation, and promoting foam cell formation. TNF-α is also a regulator of bone formation. In both intima and media, calcification resembles bone formation. This cytokine has indeed been shown to stimulate *in vitro* the expression by vascular smooth muscle cells key enzymes of the mineralization process inducing the calcification of collagen fibrils. TNF-α can also trigger the differentiation of vascular smooth muscle cells and/or mesenchymal stem cells into osteoblast-like cells, by expressing specific transcription factors, eventually leading to formation of a bone-like tissue (Lencel *et al.*, 2010).

5. Conclusion

Reported results support the concept of a differential response of inflammatory markers in coronary artery disease. In acute events the inflammatory response and the interactions between the inflammatory markers observed influenced both the clinical outcome and the vascular remodeling that persist after clinical stabilization as given by the interplay of the studied inflammatory mediators.

The inflammatory multimarker approach used and the differential response observed can contribute to a better assessment of the disease evolution and therapeutic plans.

Only the simultaneous assessment of several markers, as innovatively done in the present study, can give a valuable contribution to the understanding of their importance in coronary artery disease and in the evolution of acute myocardial infarction.

This study was useful both in research and clinical practice approaches. Combining inflammation assessment together with angiographic findings helped unravelling non-invasive markers for the disease.

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Platelet, Fatty Acids, Membrane Viscosity, Depression and Ischemic Heart Disease - Biological-Molecular Path, with Medical-Anthropology Insights

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1. Introduction

This work was born from the need to verify an intuition: could platelets' fatty acids, rather than the fatty acids of other cellular elements, in some way, be potential markers of Ischemic Cardiovascular Disease and Major Depression? For this reason a research work has been set up that, besides the aid of the most advanced statistical methods, has also made use of the Artificial Neural Networks (ANN), in the shape and form of a Self-Organizing Map (SOM). The aim was to compare a group of apparently healthy subjects with two groups of subjects with different clinical diagnoses, one of depression and the other of ischemic heart disease.
From the statistical evidence of the numerous parameters affected, we thought it would be appropriate to develop the SOM. The field of investigation has been narrowed down by reducing it to three of the fatty acids characterizing the two diseases and the pathological subjects (depressed and ischemic) and the normal ones have been placed onto the map obtained by the SOM, clearly distinguishing one from the other.
Naturally conjectures, hypotheses and convictions accrued from long examinations and discussions of the data.
Having at our disposal two neural networks that had proven to be effective in classifying the two pathologies, we were naturally curious to see how the two groups would behave at the moment when their data were fed into the networks, but crossing them (the ones of the ischemic patients in the depression network and those of the depressive patients in the ischemic network).
The result was found to more or less uniformly corroborate the literature data, even if, of course, we are aware of the fact that it will be crucial to recruit from the field significant samples of patients affected by both clinically verified ischemia and depression. Also, we must not forget that in order to get an optimum recruitment, significant samples of normal subjects will also have to be selected, non-cardiopathic depressives and non-depressive heart disease patients. The four groups will obviously have to be balanced for age and sex.
Caution, therefore, but accompanied by the evidence, by the concordances that are constantly observed in the SOM and that will have to be repeatedly examined in the light of new and increasingly more consistent testing, that, even seems to justify the interpretative

possibilities of the connections between depressive illness and ischemic cardiovascular disease.
The fact remains that the markers identified are the expression of the pathology of reference and that anyone who finds himself in the position of the network corresponding to the disease can be recognized to be at high risk. This could be stated much sooner than the moment when the clinical evidence becomes available.
We have been comforted in this evaluation by what has been shown by some patients added to the test for their correspondence between clinical evidence and placing of input vectors of the patients' data in the network.
The investigations performed on other subjects, for example, children, young sportspeople, subjects with morphea, scleroderma etc., have shown unthinkable matches with the literature data relating to the risk of the diseases investigated.
For children, in particular, an absolutely original result has been found. In other words, the fact that the platelet stearic acid is practically double if compared with that of all the other subjects, and this point, takes on great importance, precisely in the safeguarding of the child in respect to ischemic cardiopathy and, probably, other possible pathological phenomena. The child loses this condition when he or she becomes a young adult.
To conclude, we can say that Major Depression and Cardiovascular Ischemic Disease benefit from an unequivocal biochemical typification that can be recognised through the identification of fatty acids markers of the platelets and by measuring its concentration. The platelets bring with them all those elements that mime the neuron and that can alternate the physiological hemocoagulative response, thereby actually becoming the great mediators between the brain and the heart.
Before the new results, it becomes almost maniacal to search for bibliographical references that, for better or for worse, are (but may also not be) comforting for the statements and/or concepts expressed by the researcher.
This is because novelties are always the subject of diffidence.
Before going to press, we have detected, following a very difficult search, some working hypotheses in some way capable of conceptually motivating all of the work performed, which we report hereunder:

- L. S. Schneider, Principles and Practice of Geriatric Psychiatry (Second Edition), 2002:
  It is important to consider that major depression is characterized and defined by descriptive - not biological - criteria, making it unlikely that a neurochemical finding could adequately characterize the disorder. Major depression is heterogeneous in its expression, possessing various phenomenology, family history, and course. In the elderly, a neurochemical characteristic of depression would have to be specific enough to distinguish from dementia, or from secondary depression. In addition, neurochemical differences would be expected between late-onset and early-onset depression, or delusional and non-delusional depression, thus helping to validate these putative subtypes.
- Steven J. Garlow, M.D., Ph.D., Charles B. Nemeroff, M.D., PhD., Emory University, General Clinical Research Center, Period: 10/30/74 - 11/01/02, NIH:
  Platelets from depressed patients are produced in a "unregulated" state, with increased amounts of a number of transcripts that encode platelet specific, the result being the platelets are more reactive and prone to thrombus formation, 2) the platelet serotonergic system, in particular the 5-HT2A receptor, is altered in depression which in turn contributes to the increased platelet reactivity observed in depression, and the relevant alteration may occur in the megakaryocytes and 3) alterations in the concentration of one or more humoral factors (interleukins, cytokines,

stress hormones) orchestrate the alterations in platelet reactivity and serotonergic functioning observed in depression.

- Steven J. Garlow, M.D., Ph.D., Emory University, General Clinical Research Center, Period: 10/30/74 - 11/01/02, NIH:
  We hypothesize that platelet function is altered in depression resulting in platelets that are excessively prone to enter the clotting cascade and hence increase risk of heart disease. We have identified a series of megakaryocytic cell lines that express a number of platelet markers and the 5-HT2A receptor and SERT. We have demonstrated a direct regulatory interaction between the 5-HT2A receptor and the expression of a number of important platelet genes. Gene expression in megakaryocytes is regulated by a series of cytokines and growth factors including IL-3, IL-6, IL-11, TPO, and SCF. These factors are being tested for their ability to regulate the transcription of the 5-HT2A and SERT genes in megakaryocytes.

We think that we have identified, to a reasonable degree of certainty, the biochemical rules that govern the platelets in the diagnostic determination of Major Depression and Ischemic Cardiovascular Disease. Obviously, we are convinced that the disease, in this specific case Major Depression and Ischemic Cardiovascular Disease, is never linked just to biological factors but it is always the synthesis of biology and culture, as every medical anthropology teaches.

2. Medical-anthropology remarks

"Another, not less important, goal could be that of easing the pain in mental disorders. Outside the medical field, the problem of the suffering due to personal and social conflicts seems to find no solutions. Today we tend to make no distinction and to adopt the medical modus operandi to eliminate every kind of inconvenience. The supporters of this trend can make use of the following fascinating observation: if a rise in the levels of serotonin can, for example, not only treat depression but also reduce aggressiveness, making the subject less shy and more confident, why don't we try to make the most out of it? One could say that only a self-righteous spoilsport could deny another fellow human being the benefits of these miraculous medicines…
But obviously the problem is that the choice is not clear for many different reasons. Firstly, we don't know the deeper biological effects of the drugs. Secondly, the possible consequences of a drug large-scale use are still unknown. As a third and perhaps most important point it could be argued that the suggested solution for the problem of personal and social suffering must tackle the causes, the origin, of personal and social conflicts if it is to work effectively in the long-run. Otherwise this solution will work for symptoms but it won't get to the roots of the malaise." (1) (This passage has been independently translated into English from the Italian Edition: A. Damasio, L'errore di Cartesio. Emozioni, ragione e cervello umano, Adelphi, Milano 1995. For the official English version, see the English edition in the bibliography: 1).

This dense passage by Damasio leads to two final reflections:

- the roots of the malaise should never be considered either merely biological or merely psychological, but rather human, deeply human. If you approach the human pain with an auto-referential knowledge or with knowledge displayed as pure, it becomes impossible, ab origine, to understand it (in this sense, the pure biomedicine is due to fail);
- the understanding of each and every human experience, especially painful experiences in all their polychrome, iridescent images, means understanding the meaning, that is to say understanding those biological, existential, ethical, cultural, spiritual dynamics embodied in every single way we live our lives. And yet, the assumption that it is

mainly up to philosophy (because of its very nature), to the arts in all their declensions, to theology and to religion to understand the meaning, doesn't preclude the possibility of science having a say in the matter. Indeed, as the man is *one*, the science is *one,* too, and we are talking about human science, with its wide range of meanings: from a biological and chemical meaning to a spiritual one, following epistemological paths that cross and enrich each other, just because the man is biology and *soulology,* neurophysiology *and* psychology, flesh *and* soul, blood *and* essence. So, philosophical, ethical or theological meanings do exist, just in the same way as biological, chemical or biochemical meanings exist. The biologist or the chemist who's running tests on, for example, the platelet membrane fatty acids is perfectly aware, or should be aware, that all his work can't be separated from the man considered as a bundle of meanings, and that in lab he can grasp, of all these meanings, only the biological one. The same goes for the philosopher or the theologian who is perfectly aware, or should be aware, that the theoretical, the existential or the spiritual analysis takes shape in the biological dimension, whose progressive cognitive exploration, in turn, enriches the spiritual research. But now, after this important remark, let's come back to the biochemical discours.

Platelets, Fatty Acids, Major Depression, Ischemic Cardiovascular Disease

Numerous epidemiological and clinical data suggest that variations in the fatty acid composition of erythrocyte cellular membranes can be correlated with major depression. In particular, an alteration in the Arachidonic Acid (AA)/ Eicosapentaenoic Acid (EPA) ratio has been reported. The fatty acid composition of the cellular membranes determines its fluidity. The physical properties of the receptors and the enzymes, such as adenylate cyclases and the phospholipases, are influenced by the fluidity of the cellular membranes and these effects have a certain relevance in depression, in that the final responses to the neurotransmittor stimulation depends on the membrane's equilibrium. In fact, by virtue of the presence of double bonds in their molecule, omega-3 fatty acids have a folded over structure, they occupy a higher volume and they make the membranes, apparently more fluid (in fact, omega 3 fatty acids are less fluidizing of omega 6 by the saturated chain length from the first double bond). That aspect explains, in literature, why these compounds are thought to be effective as anti-depressives and mood stabilizers (2, 3, 4, 5, 6). The debate is still open and probably the antidepressant function of omega 3 fatty acids may be attributed to other reasons.

This fact is also connected to the neurochemical theories of depressive disease that see the involvement of various neurotransmittor systems; the new research could highlight the fact that the imbalance involves the receptor functions and some secondary intracellular messengers (7). Other authors (8) have found negative correlations between the erythrocyte level of EPA, positive correlations between the AA/EPA ratio in the membranes and the score of the Hamilton Rating Scale, which measures the severity of the depressive symptoms. A high proportion of AA and a low one of EPA in the erythrocyte cellular membranes can bring about a hyperproduction of eicosanoids that derive from the PUFA n6, such as the prostacyclines, leukotrienes and the inflammation mediators with an increase in the oxidative stress (9).

Some further food for thought is the problem of the depression at the outset in advanced age. Depression has a lifetime prevalence of about 15% and most of all it affects the age-range between 18 and 29 years. Henderson et al. (10), using the ICD10 criteria, found a 3.3% prevalence in the population aged over 70 years. Depression is ten times more frequent in

the elderly bearers of organic diseases than in the healthy ones, although specific biological bonds were not evidenced between the diseases. The internal diseases that are most frequently complicated with the outset of depressive symptoms are hypo- and hyperthyroidism, Cushing's syndrome, viral infections, lymphomas, carcinomas of the pancreas. Even some drugs can trigger a depressive episode (reserpine, alpha-methyl-dopa, clonidine, propanolol, digitalis, steroids, levo-dopa, and some anti-tumor drugs). The high prevalence of depression during other neurological diseases such as Alzheimer's also stands out (between 15% and 20% of the patients with AD diagnosis present a major depressive picture, up to 50% present less serious depressive symptoms), and vascular dementia, Parkinson's (40%-50% of patients affected by PD present an accompanying depression that is not in relation to the seriousness of the motor impairment). From a symptomatological standpoint, the depression that occurs in advanced age is prevalently characterized by somatic or anxiety symptoms, rather than by a marked drop in the humoral moral tone, by a marked apathy, obsessive ruminations, sleep disorders. Often the symptoms of a depression at the outset in advanced age mimic the cognitive deterioration typical of dementia (to the extent that we speak of pseudo-depressive dementia), posing large problems of differential diagnosis (11). Although the biological bases of depression in the elderly person are not yet known, it is possible to hypothesize, given the symptomatic confusion with the dementia picture and the high incidence of major depression in the neuro-degenerative diseases, that it is a question of a particular nosographic entity in relation to depression in adult age and that cerebral aging constitutes a factor of vulnerability (if not indeed an etiological factor).

The new frontiers of research take into consideration the addition of omega-3 and other anti-oxidant substances in the diet for the treatment of depression. It has been hypothesized that the omega 3 fatty acids have mood stabilizing properties with a mechanism akin to that of lithium and valproic acid, reducing the turnover of the arachidonic acid and modifying the transduction pathways of the neuronal signal. In their action on bipolar disorders, the omega-3 fatty acids largely resemble lamotrigin, that is, they seem to have stabilizing and anti-depressant properties (12). Biochemical studies have shown that a high-dose oral administration of omega-3 leads to their membrane incorporation. The increase in the concentration of omega-3 in the membranes seems to suppress the transduction pathways of the signal associated to the production of inoxitol-3-phosphate, which is the second messenger associated to numerous neurotransmittor systems, such as the serotoninergic one (rec, 5HT2). Another mechanism proposed is that of the calcium-antagonist, by means of the calcium channel block. Studies on this effect derive from the cardiological literature. Extrapolating the data on the cardiac mechanism to the SNC, an increased activity of the brain phospholipases can be supposed during the maniacal phases, with an increased release of fatty acids as second messengers, which triggers a cascade of events culminating in the release of calcium from the cell deposits (and the consequent activation of protein kinase, which in turn activate various enzymes, amongst which those dedicated to the gene transcription). The blocking of the calcium channels by the omega-3 could reduce a process of hyper-activated signal transduction (*anti-kindling*?). The omega-3 fatty acids also produce a direct inhibition of the protein kinase C (PKC), with an action akin to that of the valproate. Lastly, the omega-3 fatty acids inhibit the production of pro-inflammatory cytokines. The concomitant intake of antioxidant vitamins (Vitamins C and E) would optimize the effect of the omega-3, preventing their oxidation (13). Vitamin E has been used at high pharmacological doses in the treatment of disorders such as Parkinson's, Alzheimer's and retarded dyscinesia. Clinical studies have shown that the use of vitamin E brings benefits in

the treatment of AD, but that it does not have effects in retarding the progression of Parkinson's and retarded dyscinesia (14).
Melatonin also seems to have a 'scavenger' activity *vis-à-vis* the hydroxylic radicals besides its known properties of gonad function and biological rhythm regulation. This fact suggests that the melatonin could interfere with the neurodegenerative processes that affect the formation of free radicals and the release of amino-acid exciters (15).
Depression and the cardiac arrhythmias are linked to the autonomous nervous system as has been seen in the measurement of heart beat variability.
The transport protein of serotonin and the areas promoting the transport of serotonin ligands are shared by the platelets and the cerebral neurons so that central as well as peripheral effects may be expected.
Platelet activation is significantly higher among the depressed patients both in the presence and the absence of heart disease.
All of this shows the existence of a common path to the ischemic event both at the cerebral and the cardiac level by which the depression, more by the cerebral way than mental-behavioural, increases mortality. Although the routine screening for depression in the conditions of primary intervention is controversial, the incidence and the effects of depression among the heart patients and the stroke survivors are persuasive arguments for performing a screening among these groups.
While the post-MI depression has been well documented, depression as a risk factor for CHD remains controversial. Some studies have found a positive correlation between depression and the development of CHD in women alone, but other studies have found that depression was associated to an increase in the CHD risk in men. Confluent variables such as physical functions, hostility, anxiety and somatization involve evidence of a possible relationship between cause and effect. The parameters, the depression symptoms such as fatigue, the lack of interest in activities, the increase or loss of appetite, the psychomotor retardation or agitation, concentration disorders, low self-esteem, a depressed mood, and recurrent suicide attempts, can themselves be the precursors to CHD.
Vast as well as exhaustive scientific research has been performed on the relationship between depression and ischemic cardiovascular disease (16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28), and it has also dealt with the study of the fatty acids evaluated in several bio-humoral fractions of the human organism (plasma phospholipids, cholesterol esters, erythrocytes, etc.) in order to identify the involvement of the different acidic components, in particular the saturated ones, on the determinism of the atherogenetic phenomenon and of those aspects of hemocoagulation that induce a thrombogenetic risk (29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40).

3. Biological plausibility of the platelet role between depression and cardiovascular disease - fragments from the scientific literature

…It is well known that platelets possess metabolic capacities for fatty acid synthesis and that they possess the neurotransmittor receptors. These characteristics make the platelets, in the view of many researchers, an element akin to the neuron. With scientific simplification, a "fragment of circulating brain" and they differ substantially from the erythrocytes … (41)
…The platelets perform an important role not only in the hemostasis but also in the physiopathology of the ischemic coronary disease. Recent results suggest that the platelets are affected by different stress agents including the psychological ones and that the platelets

offer an important advantage in the understanding of the neurophysiology of the various psychiatric disorders.

Research is described relating to the use of platelets as a ground for investigating the relations between stress and cardiovascular disease, apart from psychopharmacological research. There is evidence to propose circulating platelets as a model for bioaminergic neurons. There are many similarities between platelets and neurons relating to the metabolism of serotonin, and it is possible to extend this research model to other neurotransmitters such as dopamine, GABA, glutamate, etc. The reason for such similarities can be traced back to the common embryogenetic origin of the two different cells. Some modifications of the platelet functions have been observed in the psychiatric syndromes and the bond between coronary pathology, stress and platelet function is of interest for future research. Other studies might look to the thrombotic events such as stroke and vascular dementia and their association with stress agents using the platelets as search means or examine the association of certain risk factors of ischemic cardiopathy, with certain personality traits, using platelets as the variable key… (42).

…The comparison with the properties of human platelets and the serotoninergic synaptosomes may be useful as a model in the study of the transport, the metabolism and the release of serotonin by the serotoninergic neurons of the central nervous system (43).

…A marked reduction in the levels of serotonin has been found in patients with major depressive disorder but not in the dystimic disorders. These modifications may represent biochemical modifications suggesting major depressive disorders and cannot be attributed to chronic anti-depressive treatment … (44).

The reduction in the serotonin levels in depressive patients could be traced back to their psychobiological distinction, which involves an abnormal metabolism of the biogenic amines in the brain… (45). The identification of the peripheral markers for the psychiatric illnesses is important if we want to implement an improvement in the diagnosis and the treatment of the diseases. The response of platelet intracellular calcium consequent to the neurotransmitter stimulation has been used as a peripheral marker of psychiatric illness. There is evidence of the appropriateness of extending the use of the platelets as a peripheral marker. The depressed patients show a rise in the basal platelet activation as compared with normal subjects and an elevated susceptibility to platelet activation could be the mechanism that makes depression a significant risk factor for cardiovascular and cerebrovascular disease… (46, 47).

…Fatty acids other than the omega 3 can interact with the metabolism of the eicosanoids and influence the platelet function. For example, there is evidence that diets rich in unsaturated fatty acids such as linoleic and oleic acid can reduce the trend to thrombosis replacing the Arachidonic acid in the platelet phospholipids, diminishing the *in vitro* production of the A2 thromboxanes and the platelet aggregation. Nevertheless, there is little evidence that the platelet function, *in vivo*, is affected by these diets…(48)

There are results that show that the linoleic acid of the diet does not increase the level of arachidonic acid in the plasma and in the platelets besides not contributing in a persistent way to the prostaglandin biosynthesis that is increased by the intake of arachidonic acid with the Western diet… (49). An elevated intake of linoleic acid can be thought to be protective against ischemic stroke, possibly through a potential mechanism reducing arterial pressure, reducing platelet aggregation and increasing erythrocyte deformability… (50).

… Oleic acid has been shown to be a powerful inhibitor of induced PAF platelet aggregation and serotonin secretion. Consequently, in order to understand the molecular action

mechanism of the oleic acid, the effects of this free fatty acid have been sought for in many biochemical events associated with the platelet aggregation induced by the PAF.
… The decrease in the level of [32P] PIP and [32P] PIP2 determined by the oleic acid has been associated with an inhibition in the platelet aggregation induced by the PAF. These results suggest that the inhibition of the PAF response by the oleic acid may be one of the steps in signal transduction …
…Many literature reports suggest that olive oil can inhibit platelet function. This possible effect is of interest for two reasons: it can contribute to the apparently anti-atherogenetic role of olive oil and can invalidate the use of olive oil as an inert placebo in the studies on platelet function … (51).
…After the supplementation with olive oil the platelet aggregation and the release of A2 thromboxane were diminished, the content of oleic acid was considerably increased, and the content of arachidonic acid was significantly diminished. These data suggest that an excess of oleic acid displaces the incorporation of the arachidonic acid in the platelet phospholipids. … it is concluded that the olive oil supplement exerts an inhibitor effect on the various aspects of platelet function, "an effect that can reduce the risk of heart disease, although fish intake can also exert a protective effect…" (52).
…There is relevance in the negative effect low plasma levels of linoleic acid in the long-term prognosis after myocardial infarction … (53).
…The polyunsaturated fatty acids and principally linoleic acid can have a substantially cardioprotective effect that is reflected in mortality. The quality of the dietary lipids seems more important than the quantity of the reduction in cardiovascular mortality in man… (54, 55).

4. The experimental design

Not believing the research performed on the omega 3 fatty acids to be exhaustive in the various experimental conditions of the literature, we have oriented our research towards a complex tissue and in particular that of the platelets, which, for their structural and functional characteristics, seemed to us to better fulfil the working hypotheses of a mediation role between cerebral and cardiac phenomena.
For the experimental aim of providing better knowledge on the fatty acid-depression and ischemic cardiovascular disease, we recruited:

1. 84 subjects (51 females and 33 males, with mean age: 60, 21, SD ± 12.27) with clinical diagnosis of Major Depression. All the patients were interviewed to confirm the diagnosis of Major Depression; the instruments used were: Clinical Global Impression (CGI), Symptoms Check List-90 (SCL-90), Medical and Pharmacological history, BMI, Structured Clinical Interview DSM-IV-SCID-IV (American Psychiatric Association 2000), and Hamilton Rating Scale of Depression (HRSD).
   The severity of the depressive symptoms in the group of patients was determined by the Hamilton Rating Scale of Depression 21 items version (HRSD-21), Hamilton (1960).
2. 50 subjects (17 females and 33 males, mean age: 68, 00, SD: ± 9.50) with diagnosis of Ischemic Cardiovascular Disease confirmed in the hemodynamic diagnostic phase.
3. 60 subjects (38 males and 22 females, with mean age: 33.97, SD: ± 12, 40), apparently healthy, with no known or referred history of depression or cardiovascular disease.

5. Results of the study

The results, regarding the platelet's fatty acid composition, after the statistical analysis, are expressed in Table 1 and 2.

All the ANNs tested gave essentially the same result. However, one type of ANN, known as Self-Organizing Map (SOM), gave superior information by allowing the results to be described in a two-dimensional plane with potentially informative border areas. A series of repeated and independent SOM simulations, with the input parameters being changed each time, led to the finding that the best discriminant map was that obtained by inclusion of the following three fatty acids: Linoleic Acid (C18:2 n-6), Arachidonic Acid (C20:4 n-6) and Palmitic Acid (C16:0) for the depressive condition and Oleic Acid (C18:1), Linoleic Acid (C18:2 n-6) and Arachidonic Acid (C20:4 n-6) for the ischemic condition (56, 57, 58, 59, 60).

The SOM is an unsupervised competitive-learning network algorithm, which was invented by Teuvo Kohonen in 1981–82. According to Kohonen et al. (61, 62, 63) the central property of the SOM is that it forms a nonlinear projection of a high-dimensional data manifold on a regular, low-dimensional (usually 2D) grid. In the display, the clustering of the data space as well as the metric-topological relations of the data items is clearly visible. If the data items are vectors, the components of which are variables with a definite meaning such as the descriptors of statistical data, or measurements that describe a process, the SOM grid can be used as a groundwork on which each of the variables can be displayed separately using greylevel or pseudocolor coding. This kind of combined display has been found very useful for the understanding of the mutual dependencies between the variables, as well as of the structures of the data set. In the context of this definition, a manifold refers to a topological space with welldefined mathematical properties. A particular strength of the SOM map displays lies in enabling relevant information to be 'found' rather than 'searched for'.

The fatty acids values of the 3 groups, administered to the SOM, mixing healthy and pathological individuals and hiding the information on to their own pathological condition,

Fatty acids	Normal (average±SD)	Depressive (average±SD)	p
C 14:0	0.87±0.59	1.03±0.706	N.S.
C 16:0	20.68±2.15	17.92±4.462	< .01
C 16:1	1.48±0.71	2.02±1.571	< .05
C 17:1	0.80±0.540	0.45±0.267	< .01
C 18:0	11.22±3.00	12.7±3.016	< .01
C 18:1 n9	22.19±2.08	21.14±4.134	N.S.
C 18:1 n7	1.82±0.64	1.89±0.870	N.S.
C 18:2 n6	19.40±2.69	16.71±3.359	< .01
C18:3 n3	0.48±0.17	0.73±1.554	N.S.
C 20:3 n3	2.11±0.76	2.29±0.773	N.S.
C 20:4 n6	14.06±2.41	19.03±3.839	< .01
C 22:4	1.62±0.704	1.60±0.820	N.S.
C 22:5	1.16±0.615	0.98±0.564	N.S.
C 22:6 n3	2.09±0.80	1.49±0.802	< .01

Table 1. Platelet fatty acids in MD

Fatty acids	Normal (average±SD)	Ischemic (average±SD)	p
C14:0	0.87±0.59	0.34±0.26	< 0.01
C16:0	20.68±2.15	23.32±3.17	< 0.01
C16:1	1.48±0.71	0.74±0.54	< 0.01
C18:0	11.23±3.00	17.65±2.50	< 0.01
C18:1 n9	22.19±2.08	17.48±2.14	< 0.01
C18:1 n7	1.82±0.64	1.04±0.46	< 0.01
C18:2 n6	19.41±2.69	10.51±3.44	< 0.01
C18:3 n3	0.48±0.17	0.59±0.30	< 0.05
C20:3 n3	2.11±0.76	0.73±0.42	< 0.01
C20:4 n6	14.06±2.41	15.17±3.01	< 0.05
C22:6 n3	2.09±0.80	1.87±0.70	N.S.

Table 2. Platelet fatty acids in IHD

gave, as a result, the separation of the different groups, respectively, Depressive vs. Normal and Ischemic vs. Normal, recognizing as similar those belonging to the same population and, in the meanwhile, different those belonging to one population from the other ones.
An objective, clear and extremely economic diagnostic tool has been built, which beyond the simple healthy / diseased information, offers a range of intensity, starting from a simple blood test.
When this type of situation has been noted, we thought of putting together in a SOM the fatty acid triplets of the subjects with Major Depression (SOM-ADAM), Ischemic Heart Disease (SOM-CAIN) and of the healthy subjects.
We have thus proceeded to the phase of SOM training, which, in the end, provided its self-organised map.
Based on the findings of table 1 and 2, the pathologies have been classified as in figures (1, 2 and 3): in red colour the pathological subjects in green colour the apparently normal subjects.

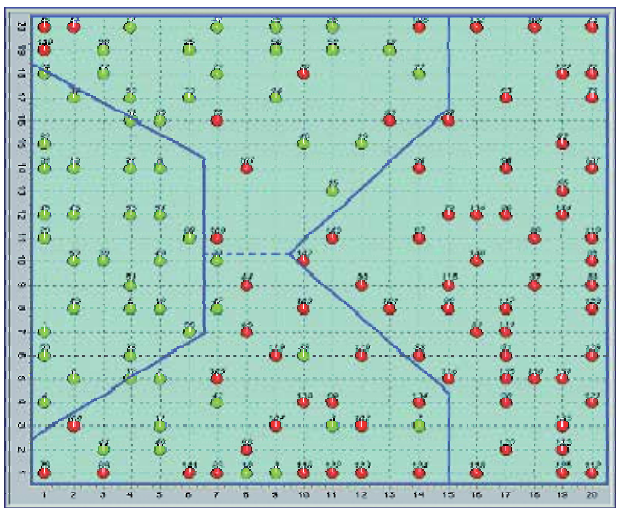


Fig. 1. Classification of the Depressive Subjects

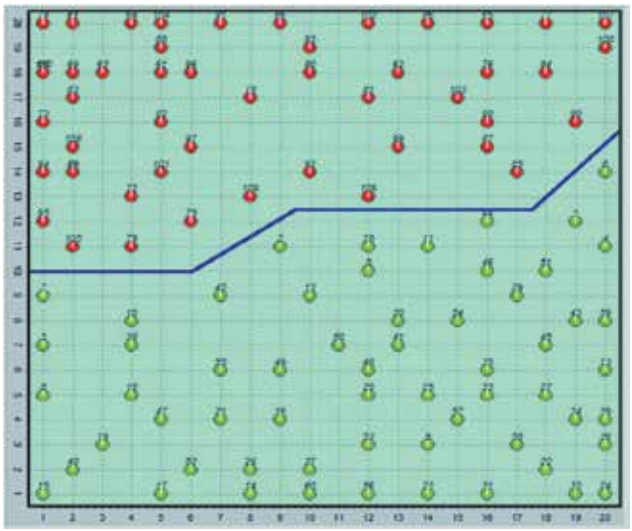


Fig. 2. Classification of the Ischemic Subjects

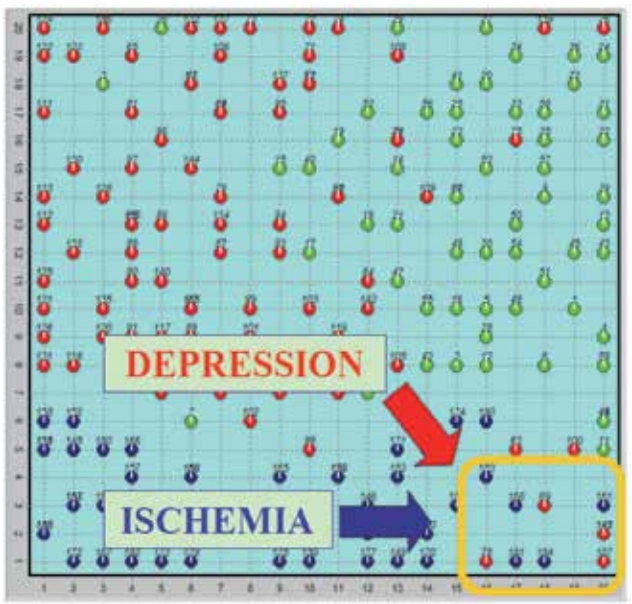


Fig. 3. Simultaneous classification of the three groups and, highlighted, the mixed area depressed and ischemic subjects

The distribution of the 144 subjects affected by the SOM for depression has allowed us to identify four areas: two specific ones (exclusively normal and exclusively pathological) and two mixed ones with different concentrations of pathological subjects and apparently normal subjects of the sample. The two intermediate areas (yellow and orange) have initially been interpreted as different possible levels of staging of the depressive disease and a pathological condition not diagnosed in the normal subjects, as described in the literature,

moreover. The capacity of this mathematical instrument to express the pathology according to a different scale of intensity soon appeared clear. As regards the hypothesis that the positioning of the cases was also the expression of a different chemico-physical condition of the membrane, we addressed our attention towards the possibility of identifying such a condition by only considering the Arachidonic, Linoleic and Palmitic acids. Among the different characteristics observed in these fatty acids there emerged a significant correlation ($r = 0.66$, $p < 0.001$) between the sum of the 2 unsaturated acids (AA+AL) as compared with the saturate (AP). In other words, the sum of the three fatty acids can be considered, to a fair degree of approximation, constant and equal to 53.33 ± 3.43 (average±SD).
Further analysis has led us to identify an effective index (B_2) that relates the saturation characteristics of the set of the three fatty acids (57).
That index has been identified in the following way:

20	2,24	1,17	1,92	1,89	1,92	2,04	2,11	1,85	1,68	1,59	1,58	1,56	1,64	1,60	1,30	0,99	0,50	0,02	-0,49	-1,02
19	1,92	1,94	2,25	2,08	1,98	1,98	2,00	1,71	1,53	1,44	1,37	1,53	1,70	1,54	1,32	0,96	0,48	0,10	0,12	-0,21
18	2,34	2,28	2,21	2,20	2,11	2,00	1,91	1,83	1,55	1,34	1,36	1,50	1,51	1,33	1,29	0,52	0,39	0,36	0,35	0,44
17	2,36	2,37	2,27	2,19	2,22	2,12	2,04	1,85	1,77	1,55	1,39	1,32	1,31	1,30	1,23	0,49	0,39	0,38	0,43	0,51
16	3,03	2,57	2,35	2,38	2,31	2,18	2,09	2,03	1,71	1,47	1,29	1,23	1,31	1,27	1,25	0,97	0,40	0,43	0,46	0,47
15	3,21	3,19	2,59	2,47	2,53	2,39	2,21	2,41	1,67	1,40	1,27	1,14	1,22	1,21	1,22	0,72	0,53	0,47	0,47	0,15
14	3,13	3,24	2,76	2,55	2,79	2,73	2,55	2,57	2,27	1,36	1,15	1,13	1,16	1,16	1,08	0,55	0,54	0,48	0,11	-0,49
13	2,99	3,05	2,53	2,44	2,53	2,47	2,42	2,53	2,17	1,17	1,09	1,11	1,02	1,01	0,67	0,49	0,51	0,18	-0,01	-0,24
12	2,91	2,83	2,35	2,28	2,34	2,18	2,01	2,06	1,97	1,63	1,55	1,48	0,79	0,67	0,60	0,43	0,49	0,12	-0,21	-0,27
11	2,39	2,54	2,44	2,31	2,22	2,12	1,93	1,97	2,22	2,20	2,01	1,91	0,79	0,73	0,63	0,48	0,31	0,07	-0,18	-0,38
10	2,49	2,55	2,45	2,41	2,28	2,13	2,01	1,97	2,27	2,40	2,15	1,68	1,07	0,69	0,55	0,50	0,35	0,05	-0,30	-0,42
9	2,83	2,78	2,54	2,52	2,46	2,26	2,05	1,91	2,02	2,21	1,81	1,57	1,37	0,69	0,56	0,51	0,22	0,04	-0,24	-0,40
8	3,28	3,10	2,78	2,53	2,57	2,43	2,23	2,03	2,01	2,05	1,91	1,41	1,23	0,87	0,58	0,51	0,32	0,19	-0,41	-0,57
7	3,45	3,33	2,88	2,69	2,57	2,50	2,22	1,93	1,97	1,82	1,66	1,38	1,19	0,85	0,63	0,64	0,38	0,32	-0,37	-0,51
6	3,78	3,74	3,07	2,86	2,88	2,59	2,43	2,06	2,07	1,54	1,50	1,40	1,11	0,83	0,76	0,44	0,26	0,08	-0,36	-0,46
5	4,05	3,80	3,40	3,13	2,93	2,76	2,54	2,49	1,99	1,81	1,83	1,57	1,12	0,87	0,75	0,33	-0,05	-0,05	-0,35	-0,54
4	4,60	4,20	3,75	3,18	3,18	2,91	2,85	2,71	2,20	1,99	1,89	1,85	1,23	1,01	0,91	0,10	-0,03	-0,14	-0,70	-0,80
3	4,45	4,20	4,19	3,68	3,45	3,36	2,90	2,69	2,42	2,09	1,93	1,77	1,51	1,34	1,18	-0,01	-0,16	-0,82	-1,07	-1,01
2	7,10	4,47	4,14	3,96	3,63	3,40	3,34	2,91	2,75	2,43	2,08	2,05	1,66	1,19	0,73	-0,14	-0,29	-1,00	-1,53	-1,95
1	8,23	6,58	4,26	3,98	3,36	3,06	3,89	3,07	2,90	2,50	2,16	2,34	1,72	1,03	0,50	-0,03	-0,19	-1,48	-1,79	-2,64
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

Fig. 4. Distribution of the B_2 index on the map
That coefficient was calculated for all the 144 subjects studied. This has allowed us to further classify the subjects on the grounds of this index with statistical significance (comparison between the indices of the normal and the pathological subjects).

$$B_2 = \sum_{i=3}^{3} \left(A_i \frac{mp_i}{mw_i}\right)$$

i	Name	
1	Palmitic Acid	C 16:0
2	Linoleic Acid	C 18:2
3	Arachidonic Acid	C 20:4

Where:
A_i = percentage of *i-th* Fatty Acid
mw_i = molecular weight of *i-th* Fatty Acid
mp_i = melting point of *i-th* Fatty Acid
We then asked the SOM to express the B_2 expected at every point of the map.

6. Cardiovascular ischemic disease

As the fatty acids identified by the network do not represent the majority of all the fatty acids of the acidic spectrum, we tried to identify an index that could express the saturation level that could most significantly be traced back to the CAIN logic (Coronary Artery Ischemic Neural network).
The search for such an index has been performed by assessing the various ratio combinations between saturated fatty and unsaturated acids defining the result with the reported initials (SI = Saturation Index Total and Classic), B2, B_4, B_{tot}. Some of these are already known of in the literature, others are defined by the Authors.
In this way we can define:
SI_{TOT} = (C14:0 + C16:0 + C18:0) / (C16:1 + C18:1n9 + C18:1n7 + C18:2 + C18:3 +C20:3 + C20:4 + C22:6).
$SI_{CLASSIC}$ = C18:0 / C18:1 n9
B_2= f (C16:0, C18:2, C20:4)
B_4= f (C16:0, C18:0, C18:1, C20:4)
B_{TOT}= f (C14:0, C16:0, C18:0, C16:1, C18:1n9, C18:2, C18:3, C20:4, C22:6)
As can be observed in Table 3, all the parameters that express the saturation level are significantly and strongly correlated to each other. For the sake of simplicity of calculation and use, we therefore decided to use the SI Classic (hereinafter SI).
This index, akin to the case of depression with the B2, has been put into the CAIN and, as can be seen (figure 5), it has cadenced the membership of the two groups in the pathological and normal quadrant in a way corresponding to the classification effected by the RNA. For that purpose, the cut-off value proposed for the SI is 0.80.
This SI trend is distributed growingly (from the bottom to the top of the map) and is coherent with CAIN. While the area of the disease is observed with a very gradual distribution, the area of normality appears to be more non-homogeneous. That condition can probably be traced back to three pieces of evidence:

	Correlations (All B) Corr. marked at p < .05 N=300					
	SI TOT	**SI Classic**	**BT**	**B4**	**B2**	**Bcain**
SI TOT	1.0000	.9632	.8296	.9483	.8055	.5992
	p= ---	p=0.00	p=0.00	p=0.00	p=0.00	p=0.00
SI Classic	.9632	1.0000	.7965	.8736	.6806	.4524
	p=0.00	p= ---	p=0.00	p=0.00	p=0.00	p=.000
BT	.8296	.7965	1.0000	.8515	.7304	.5196
	p=0.00	p=0.00	p= ---	p=0.00	p=0.00	0
B4	.9483	.8736	.8515	1.0000	.9206	.7378
	p=0.00	p=0.00	p=0.00	p= ---	p=0.00	0
B2	.8055	.6806	.7304	.9206	1.0000	.8456
	p=0.00	p=0.00	p=0.00	p=0.00	p= ---	0
Bcain	.5992	.4524	.5196	.7378	.8456	1.0000
	0	p=.000	0	p=0.00	p=0.00	p= ---

Table 3. Correlation of the identified saturation index.

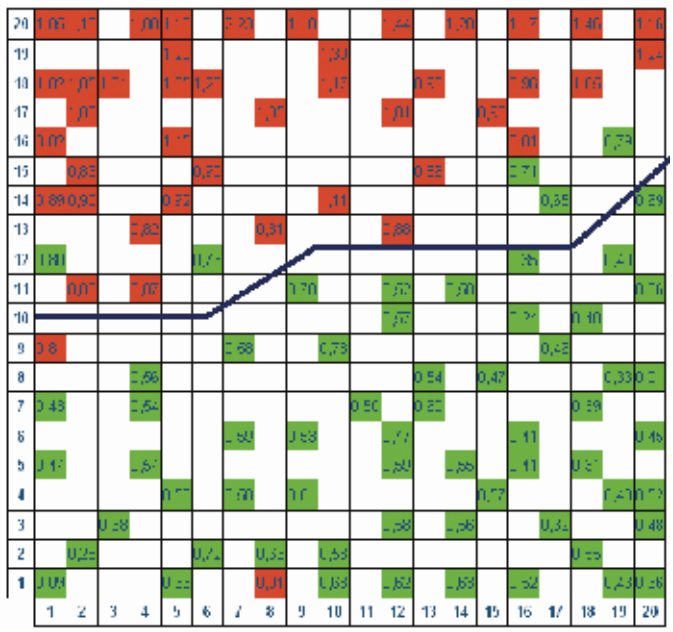


Fig. 5. Distribution of the saturation index (SI Classic) on the CAIN map colouring in red the subjects with SI > 0.80 and in green those with SI <= 0.80

1. The oleic level, in any case distributed in the area of normality, is the guarantee of the same, nonetheless having in the lowest cut-off values;
2. The levels of oleic acid in the area of normality must in any case take account of the distribution of the other fatty acids, CAIN markers. This supports the configuration assumed.

3. In the pathology area, the gradual distribution seems to suggest the position of strength of the oleic acid that leaves aside the value assumed by the other AG.

It is still interesting to see how the characterisation of the oleic acid is also conditioned by a relationship with a fatty acid (stearic) not considered by the conventional statistical methods, neither by the RNA.

7. CAIN and Framingham (The Framingham score)

The Framingham score (61) is one of the best known diagnostic instruments suited to quantifying the presence of the cardiopathy risk. Constructed on a statistical basis, it is made up of 5 items relating to:

a. Subject age;
b. Total cholesterol;
c. Smoke;
d. HDL level;
e. Systolic Blood Pressure;

For each item a score is assigned, according to the value relating to the subject being studied, taking account of the sex (Fig. 6).

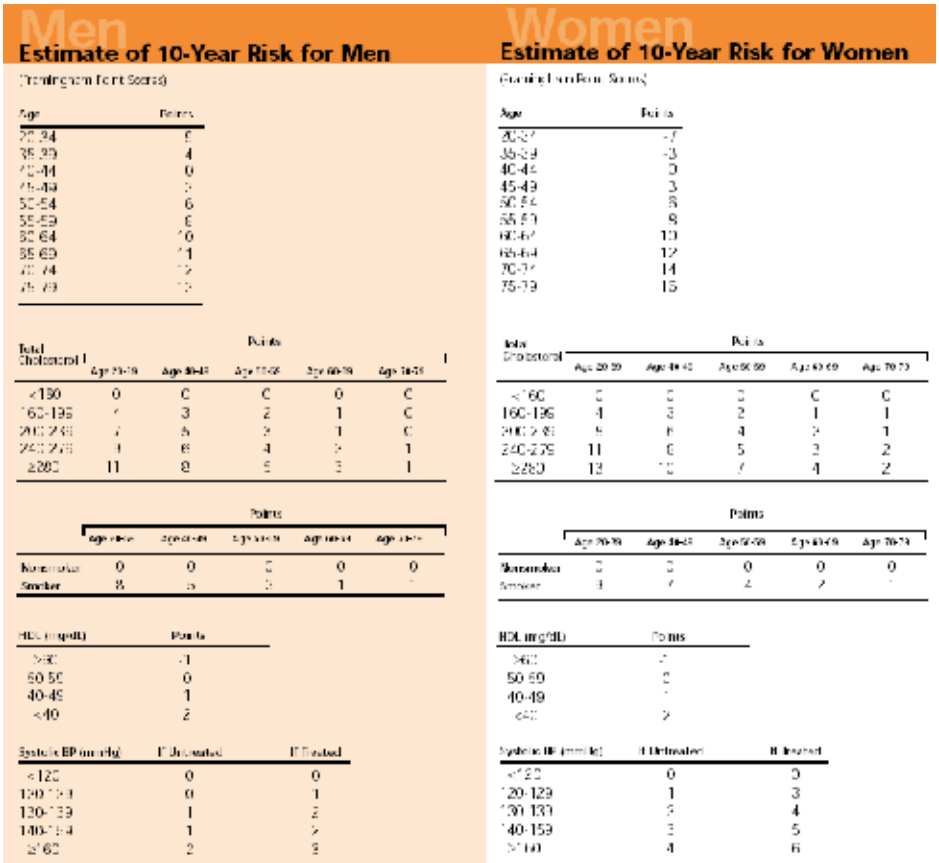
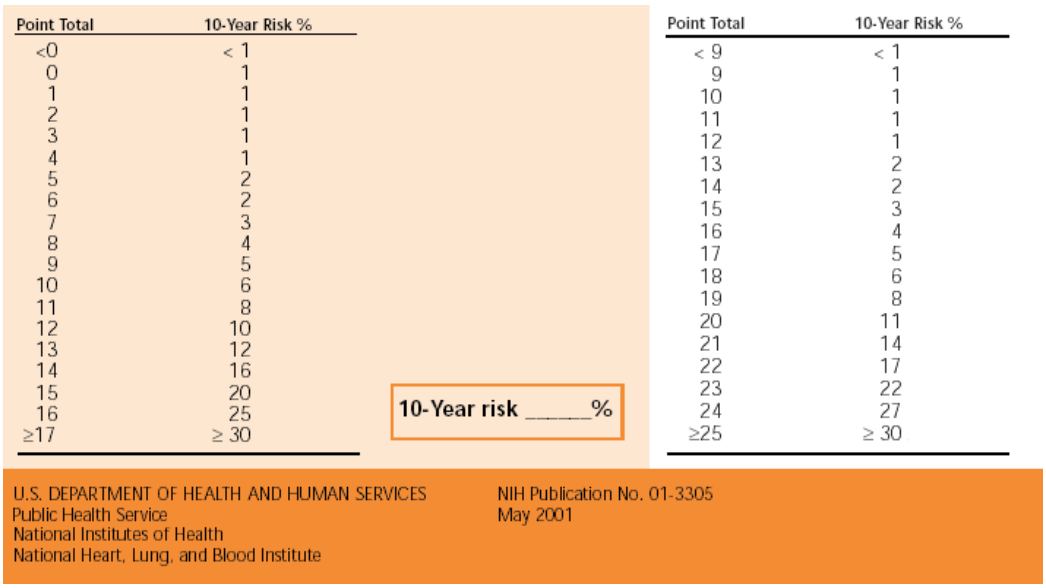


Fig. 6. The Framingham Score

The total score obtained (Figure 7) is converted, by means of a Table (Table 4) into a risk percentage over 10 years.



Point Total	10-Year Risk %
<0	< 1
0	1
1	1
2	1
3	1
4	1
5	2
6	2
7	3
8	4
9	5
10	6
11	8
12	10
13	12
14	16
15	20
16	25
≥17	≥ 30

10-Year risk _____%

Point Total	10-Year Risk %
< 9	< 1
9	1
10	1
11	1
12	1
13	2
14	2
15	3
16	4
17	5
18	6
19	8
20	11
21	14
22	17
23	22
24	27
≥25	≥ 30

Fig. 7. The conversion of the Framingham score to a 10-year risk percentage.

The 10-year risk percentage thus obtained can be further classified, according to what has been suggested by Anderson et al. (64) following the Table reported below.

0 to 5%	low risk
5 to 10%	moderate risk
10 to 20%	average risk
20 to 40%	high risk
> 40%	very high risk

Table 4. Ten-year risk classification

Now, let's suppose we have to examine an ideal, hypothetical subject, who is in excellent condition: a non-smoker, perfect levels of Total Cholesterol, HDL, systolic pressure, etc.
Although his/her health conditions are excellent, according to Framingham, he/she nonetheless has a risk factor: age! In other words, the fact of ageing, irrespective of conditions of health, in itself constitutes a risk factor. The graph of the Framingham score in relation to age of the ideal subject is represented in Fig. 8 (in blue). It should be pointed out that, as the score is different depending on the sex, the graph has been constructed using the mean value. Moreover, for the sake of illustration's clarity, the linear interpolation of the curve has been made to overlap with that of the extrapolated curve (in red in Fig. 8).
Let's now consider the *high risk* and the *very high risk* levels according to Anderson's classification. They are described for a ten-year risk over 20% (see Table 4). That percentage risk, in Framingham's conversion table, read retrospectively, corresponds to a value of 22.5 Framingham points, for the female population. It should be point out that we have chosen the female population because it is characterised by higher scores. It is worth underlining

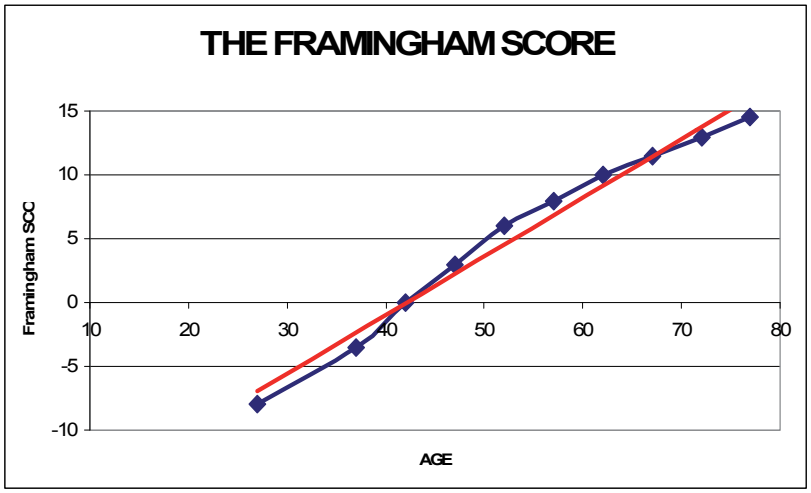


Fig. 8. Graph with the age in the y-axis and the Framingham score in the x-axis. *In blue*: ideal trend of the Framingham score for a ypothetical subject with a single risk factor: age. *In red*: the linear interpolation of the mentioned curve.

that, in any case, by choosing the male population as reference or the mean value of the two sexes, the reasoning that follows does not vary quantitatively and leads to the same conclusion. By reporting the Framingham risk according to age, the *high risk* threshold and thus highlighting the risk area, we obtain the graph reported in Figure 9.

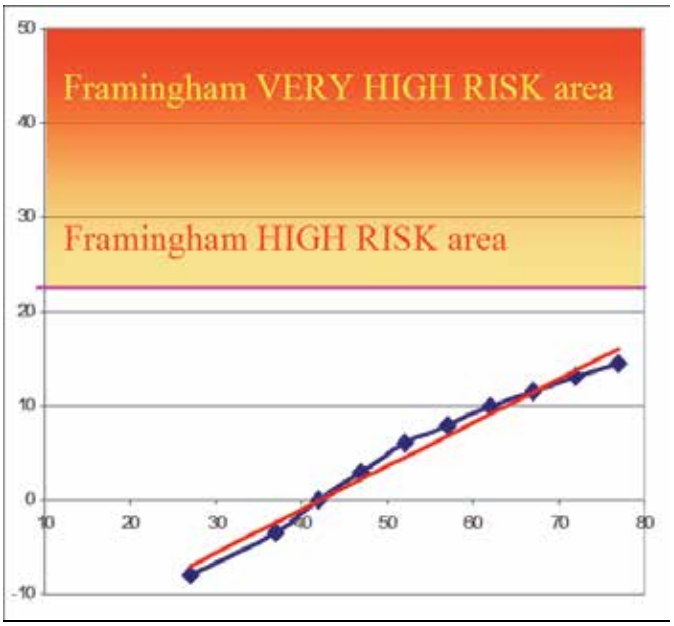


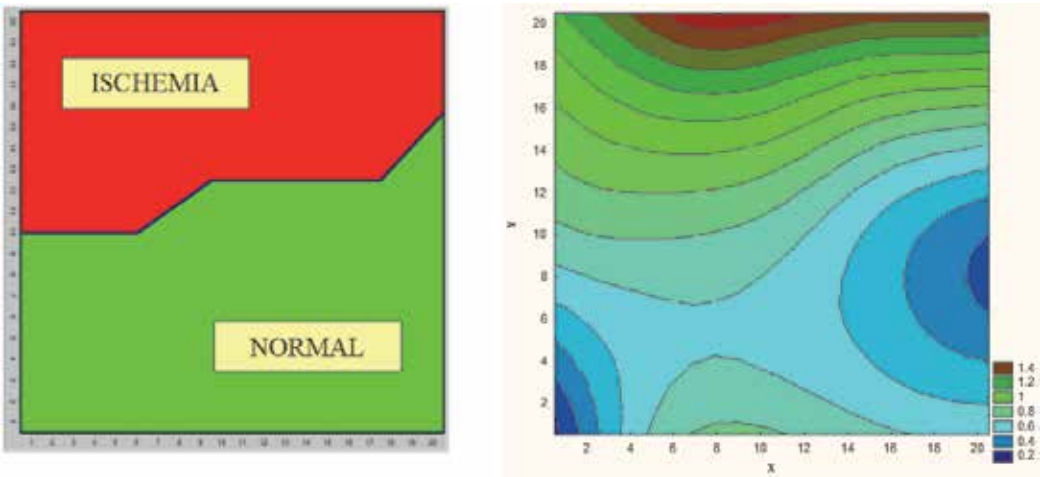
Fig. 9. Graph of the Framingham score by age. In the y-axis: age of the subject; in the x-axis: the Framingham score. Colour gradation highlighting (orange up to red) the risk areas according to Anderson's classification.

8. Saturation Index and CAIN

In order to find a good index to describe synthetically the classification effected by CAIN, several paths have been tried. The most effective one has turned out to be that of the saturation indices, as reported above. In particular, the literature (65, 66) has suggested the Saturation Index (SI) particularly used in the oncology field, which is defined in the following way:

$$SI = \frac{C_{18:0}}{C_{18:1}} = \frac{\text{STEARIC ACID}}{\text{OLEIC ACID}}$$

It is possible to express the SI levels corresponding to each point of the CAIN map and describe then by means of a graph for level curves. In this way it is simpler to intuit the dynamics of SI in CAIN (Fig. 10). It should be remembered that CAIN is constructed using Oleic, Linoleic and Arachidonic Acid while the SI value is calculated by using Oleic Acid and Stearic Acid.



CAIN Map **SI position in CAIN**

Fig. 10. On the left, the clustering of CAIN in the two areas: pathological (at the top) and non-pathological (at the bottom). On the right of the graph for level curves relating to the SI in the CAIN map. Notice how the separation curve of the clusters can be intuitively overlapped in the left-hand map towards the level curve of similar coordinates.

By trying to evaluate the SI trend in CAIN (Fig. 11) we can see that it takes on minimum values in the lower part, corresponding to the "Normal" cluster of CAIN. From the bottom upwards, the value grows gradually until it reaches the maximum values in the upper part of the map, corresponding to the area of pathology, according to the CAIN clustering.

9. The SI of some populations

We have evaluated the mean values of SI in some subject populations. The first two were:

a. "*YOUNG*": Young sports people, supposedly healthy [n= 45; males = 35, females = 10; age = 22.7 ± 3.7 (m±SD)]

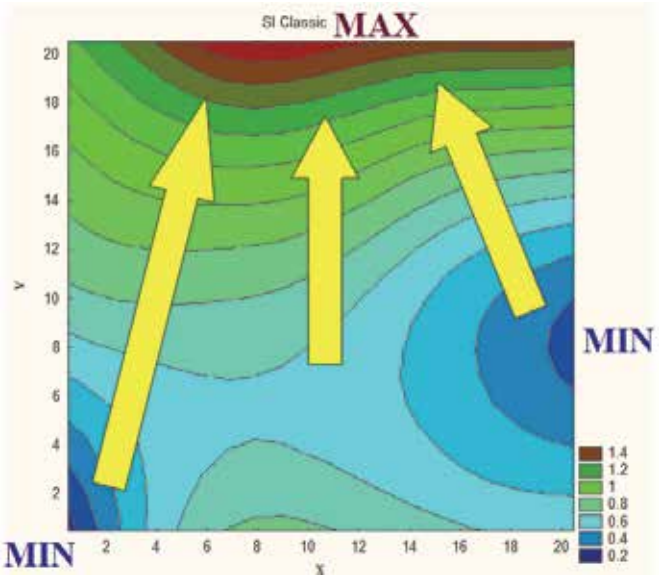


Fig. 11. The SI dynamic in CAIN.

b. "*ADULT*": Adults, supposedly healthy [n= 60; males = 38, females = 22; age = 34.0 ± 12.4 (m±SD)]

By building a graph whose y-axis had the age and whose x-axis the SI value, we obtained, for the two populations cited, the curve reported in Fig.12.

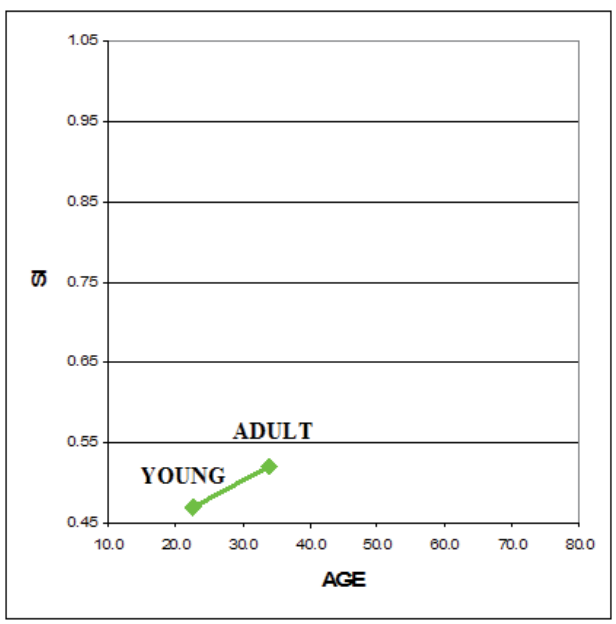


Fig. 12. Graph of the SI values for 2 supposedly healthy populations of different mean ages

Then, a third population was added:

c. "*DEPRESSIVE*": Subjects with clinical diagnosis of Major Depression [n=84; males = 33, females = 51; age = 60.2 ± 12.3 (m±SD)]

In Figures 13 and 14 the resultant graph is shown.

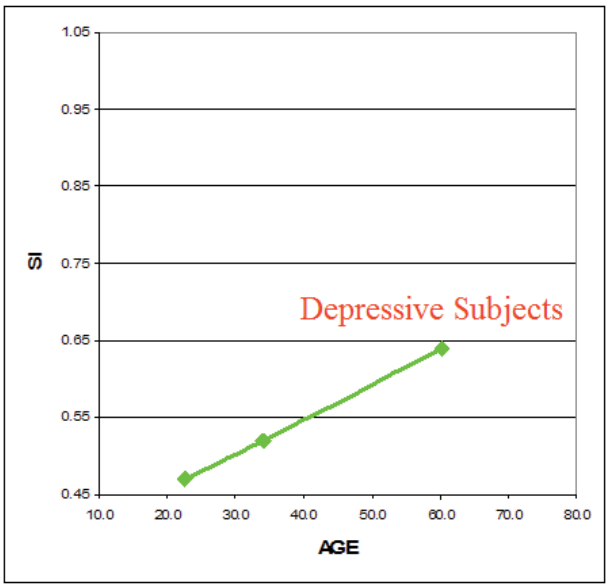


Fig. 13. Curve of the mean values of SI of the 3", "Normal" and "populations: "Young Depressive"

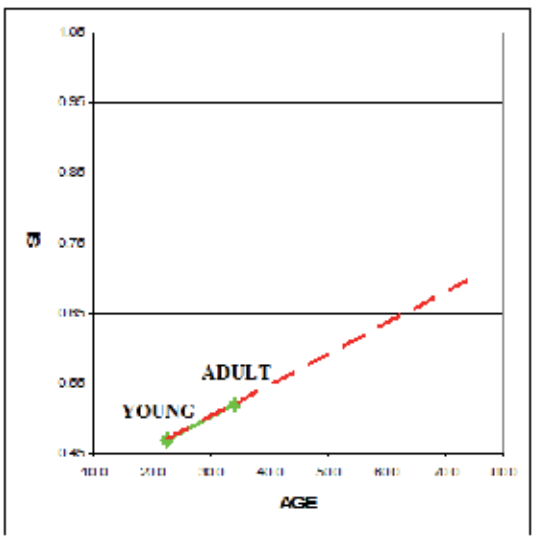


Fig. 14. In red: interpolation (prolonging) of the segment "Young"-"Adult". The interpolation and the curve obtained in Fig. 13 can be overlapped.

The subjects of the third group are positioned in a perfectly overlapping way with the interpolation (prolonging) of the segment that unites the "Young" and the "Adult"

population (Fig. 12). In other words, the means of the three populations are positioned (to a good degree of approximation) on the same line. It should be borne in mind that, while the "Young" and "Adult" populations are healthy subjects, the "*Depressive*" population is composed of subjects with the Major Depression syndrome, characterised, however, by being non-pathological from the cardiovascular standpoint. Hence, a fourth population was added:

d) "Ischemic": Subjects with diagnosis of ischemia [n= 50; males = 33, females = 17; age = 68.0 ± 9.5 (m ± SD)]. Figure 15 reports the resulting graph. It appears evident that the "Ischemic" population does not result to be in line with the other populations examined being characterised by a far superior SI value.

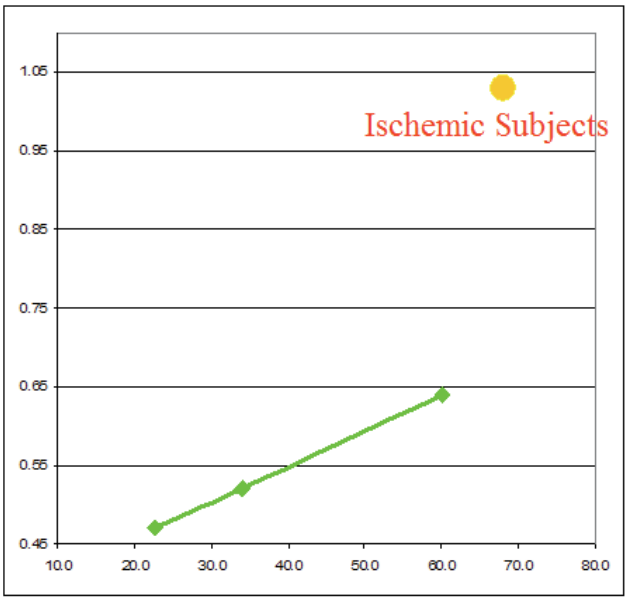


Fig. 15. Curve of the mean values of SI of the four populations: "Young", "Normal", "Depressive" and "Ischemic"

10. SI, CAIN and the Framingham Score

By comparing the graph constructed on the grounds of the Framingham score as opposed to the one relating to the SI, looking at the Figures 16 and 17 we can see some curious congruities.

In fact, we can see that, by matching the curve by age obtained in the SI graph to the age curve obtained in the Framingham, the "Ischemic" population coincides with the "Very high risk" area of the Framingham score. The two graphs seem to let transpire a certain, as yet not well defined, similitude.

But what is the cut-off between healthy and pathological (from the cardiopathological point of view) in the SI graph?

To answer this question it is necessary to ask CAIN for help. It is important to evaluate which SI value corresponds to the subdivision line between the 2 clusters ("Ischemia" and "Normal") that CAIN has formed (Figure 18).

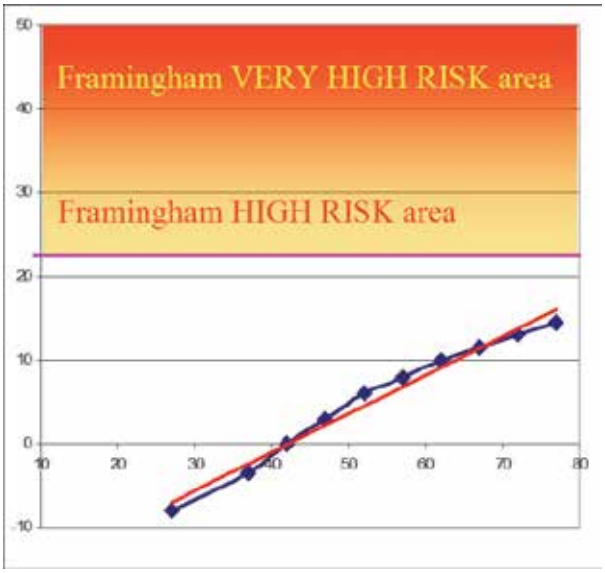


Fig. 16. Framingham score graph

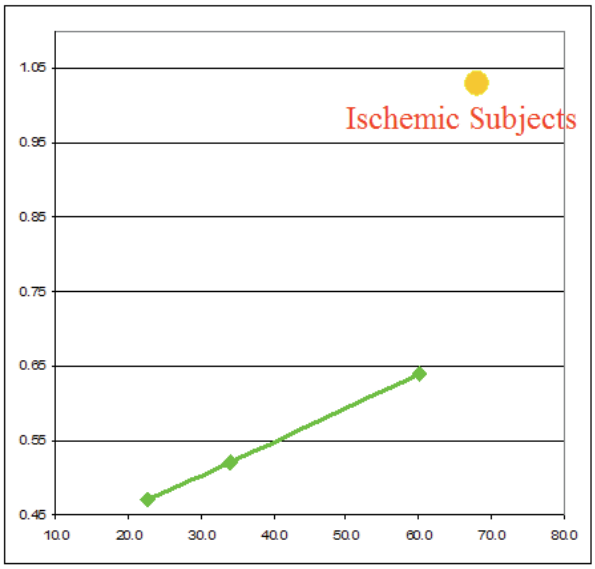


Fig. 17. SI graph

By overlapping the CAIN clustering with the SI level curve graph (Figure 19) we can see that the SI value corresponding to the separation line of the two CAIN areas is indeed SI = 0.8.

Now it is sufficient to report on the SI graph undergoing construction, the SI value corresponding to 0.8. This will be the cut-off of the SI values that subdivides the pathological and non-pathological subjects according to CAIN. Indeed, Figure 19 clearly shows that values lower than 0.8 in CAIN characterise healthy subjects, as opposed to those superior to 0.8 which, on the contrary , identify pathological subjects.

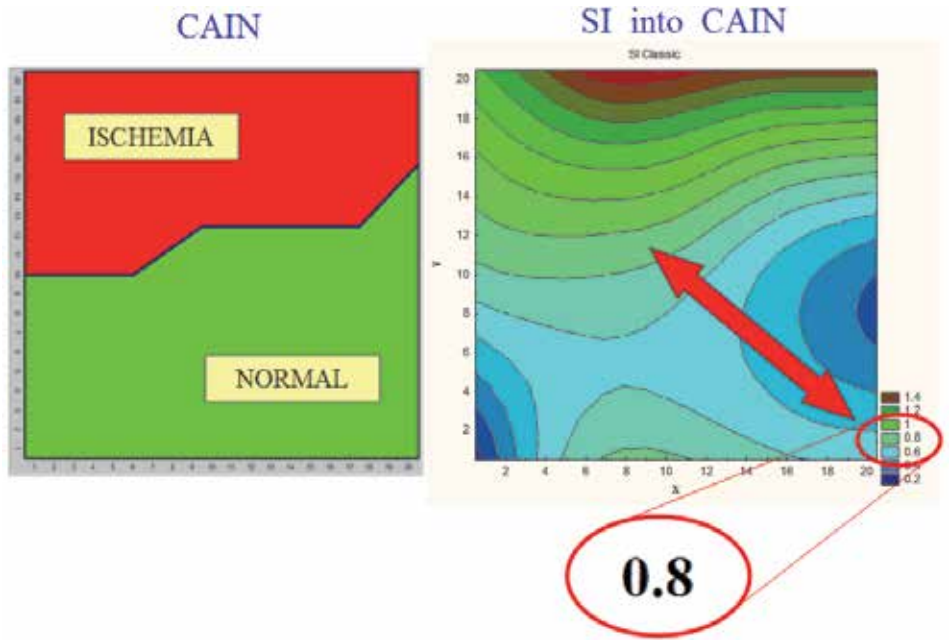


Fig. 18. Evaluation of the SI value corresponding to the clustered subdivision line ("normal" and "Ischemia") of CAIN. By subdivision it is observed that the line corresponds to the level curve of the SI graph equal to SI = 0.8.

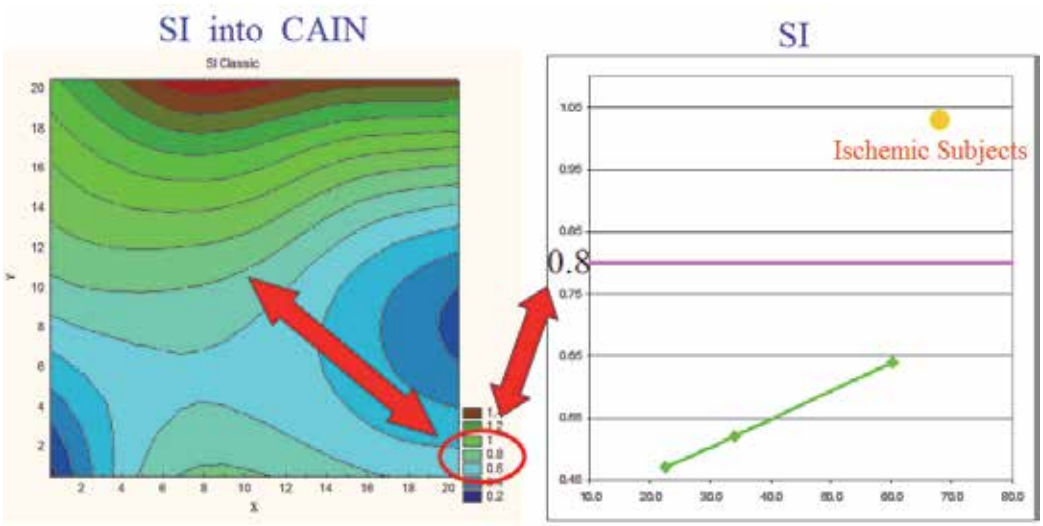


Fig. 19. SI and CAIN graph

Once the areas of pathology and non-pathology are identified in the SI graph, it is juxtaposed with the one obtained from the Framingham (Figure 20). The match is certain.

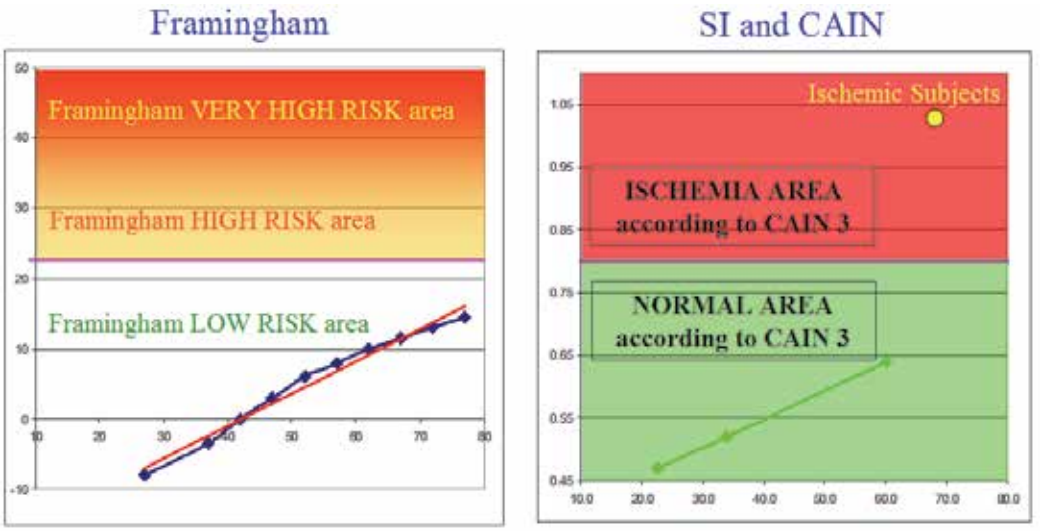


Fig. 20. The Framingham score graph (on the left) and the SI graph (on the right).

In order to better appreciate the overlap between the two graphs we propose (Figure 21) the overlapping obtained by coinciding the two cut-off lines. The two curves submitted overlap in an absolutely coherent way.

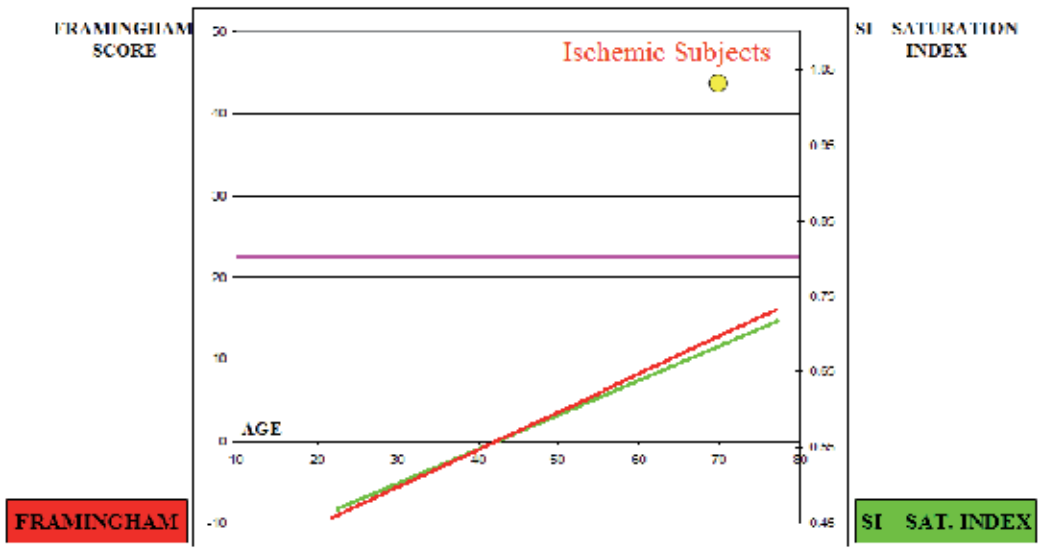


Fig. 21. Overlap of the Framingham Score and the SI graph. On the y-axis the age, on the left x-axis the Framingham score values, with related curve in red; on the right x-axis the SI values with related curve in green.

By overlapping the two graphs, so that the SI cut-off value matches that of the Framingham Score, we obtain a match of the two underlying curves (red and green).
The following figure sums up all the positions of the individuals investigated.

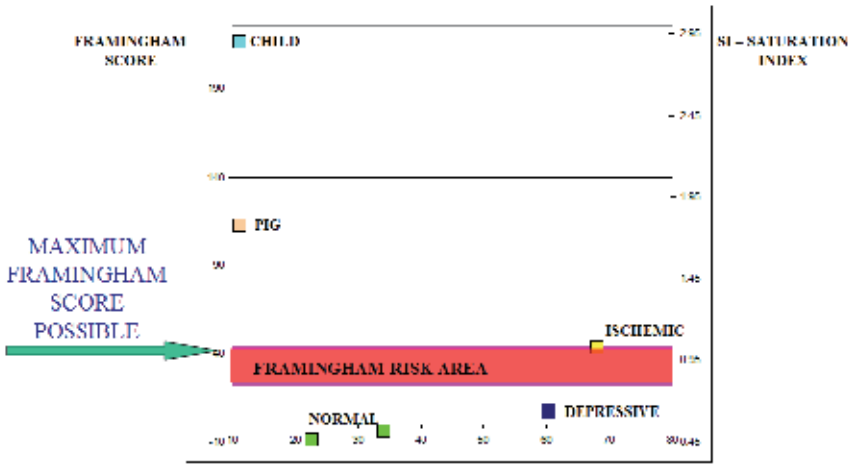


Fig. 22. The Figure represents all the positions of the subjects studied; it can be seen how the swine and the children (SI) occupy an absolutely different position from the adults and even more detached from the ischemic patients.

11. Conclusions

What has been illustrated shows, at least as a first approximation, that the results obtained by CAIN 3 are absolutely compatible with the Framingham Score. Apart from corroborating the results obtained with CAIN even more, this obliges us to make a comparison between the two methods.

The Framingham score, constructed on a statistical basis, offers as a result a pure number that quantifies a risk. Actually, it is one-dimensional. On the other hand, CAIN shows the result on a two-dimensional map, obviously capable of giving more information. In actual fact, for CAIN we have to speak of multidimensionality because once the position of a subject has been identified, there is a great deal of information available. Without citing it all, suffice it to think that it is possible to know if any ischemic pathology is characterised by the low level of oleic or linoleic acid. It is possible to know its SI value straight away and, at least at a first approximation, the Framingham value. In other words, once the coordinates of the subject have been identified, it is possible to decide on which plane to observe it: the SI plane, the fatty acid plane, for example of arachidonic acid, etc. In any case, what is certain is that CAIN offers much more information than the Framingham. Suffice it to think, for example, that for a CAIN value one can go back to its Framingham value (albeit approximate). If, on the contrary, we have the Framingham score we can, in no way, position the subject in CAIN.

These reasons raise a question up: is it plausible to think of CAIN as a new Framingham?

Novel markers for ischemic heart disease are under investigation by the scientific community at international level.

Our work focuses on a specific platelet membrane fatty acid condition of viscosity which is linked to molecular aspects such as serotonin and G proteins, factors involved in vascular biology.

A suggestive hypothesis is considered about the possibility to use platelet membrane viscosity, in relation to serotonin or, indirectly, the fatty acid profile, as indicator of ischemic risk.

In the case of biological membranes we use the terms of "fluidity", "stiffness," permeability "functionality", and "stickiness", related or connected with biological effects of considerable importance.

Fluidity and viscosity are two terms used in physics with specific meaning: the viscosity is a dynamic property of matter and is defined as the skid resistance of two fluid layers between them, in a real system treated as a package of fluid layers superimposed (in slow linear motion), which can vary with temperature for the same molecules, while the fluidity is the opposite.

Rigidity, permeability and function are, however, characteristics of the membrane and are terms used to describe a physical and biological membrane behavior on the physiology of the cell. The viscosity of the membrane, of course, is related to the composition of fatty acids constituting the lipid bilayer (membrane folds). With reference to the membrane folds, they are usually very close and the distance may be very small in the case of saturated fatty acids, distance which tends to increase, replacing these with unsaturated fatty acids, much more as they are unsaturated.

It has been demonstrated that the membrane viscosity is a peculiar characteristic in depressive subjects (67, 68) and that regulates serotonin receptors uptake [69].

Among all its function serotonin is also involved in some aspects of cardiac events such as coronary artery disease, in aggregation and vasoconstriction [70, 71].

Platelet takes up serotonin from plasma by the serotonin transporters and it is, then, secreted by the platelet dense granules during platelet activation, playing a role in platelet aggregation and vasoconstriction of surrounding blood vessels. Recent studies suggest that intracellular serotonin may also play a role in platelet activation through covalent linkage to small G proteins, activating G protein signaling pathways and stimulating platelet aggregation [72].

Total serotonin levels and the number of platelets have been found significantly higher among patients whith coronary artery disease [70].

Independently of the SOM results, among all the fatty acid profiles we have investigated (about 350 subjects), three fatty acids (Palmitic, Linoleic and Arachidonic), unexpectedly, had a constant total amount (53. 33 ± 3.43) representing the larger amount of platelet membrane fatty acids in all cases studied.

If we consider this fatty acid triplet instead of that one which has allowed the classification of the ischemic subjects we can do a further consideration.

It means that we can, also for the ischemic subjects, calculate a new index (B3) according to the B2, as above explained.

Fatty acid	MP/Mwt	Normal subjects (average±SD)	B3 index	Depressive (average±SD)	B3 index	Ischemic (average±SD)	B3 index
C 16:0	0.246	20.68±2.15	5.089	17.92±4.462	4.408	23.32±3.17	5.737
C 18:2 n6	-0.018	19.40±2.69	-0.348	16.71±3.359	-0,301	10.51±3.44	-0.189
C 20:4 n6	-0.164	14.06±2.41	-2.306	19.03±3.839	-3.121	15.17±3.01	-2.488
			2,434		**0.986**		**3.060**

Table 5. Value of the B3 index in the subjects studied

As a result we obtained an index that expresses, on the basis of fatty acids detected, a coefficient of viscosity.
The result is consistent with the knowledge that relates to the membrane viscosity, especially platelets, in the conditions investigated (normal, ischemic and depressive).
This could lead to the hypothesis of the possibility to evaluate the ischemic risk considering each fatty acid concentration, within the same, identical quantity.
As shown in table 1 the B3 index is significantly higher (about 30%) in ischemic than in normal subjects and 3 times higer than in depressive subjects.
For the properties that link membrane viscosity to the platelet serotonin receptor uptake and for the role of serotonin in coronary artery disease, the evaluation of the chemical-physical characteristic should be utilized to forecast the ischemic risk, in agreement with the experimental result obtained in an Ischemic Heart Disease group of subjects through the SOM use.

The Issue of Neural Networks

(Discussion with Kary B. Mullis Nobel Prize), September 21, 2007
Let's try this one more time, Massimo, because somewhere there must be a disconnect in our dialogue on this business of an **un-trained** neural network being able to spot the likelihood of coronary heart disease in a set of patients, where the only information given to the network, is the concentration of three lipids on their platelets, let's just call the lipids 1, 2 and 3.
What I understand is that you are demonstrating that in the absence of a "training set" your program can pick the patients who have a high probability for a coronary problem just from these values.
By a training set I mean that the values for 1, 2 and 3, paired with an independent diagnosis of a coronary artery problem are presented to the computer for a certain number of your initial group. You are claiming that a "training set" is not necessary.
How, I ask, could that possibly work?
Now I do realize that lipid composition on platelets could easily have something to do with coronary artery problems, (and clinical depression). I don't disbelieve that they could. Nobody I ever heard of predicted that, but when you told me it was true, I accepted it. But when you explained to me that your program had discovered this relationship with no reference to some external standard, no training set, I was incredulous and still am.
I simply claim that without some additional data, like a training set of data points wherein the lipid variables were paired with clinical outcomes or some similar KNOWN variable relevant to coronary artery problems, (or a super-exceptional knowledge of physiology), the neuronal net, which is a mathematical object, not an oracle, could know anything except 1, 2, and 3 (the inputs) about the patients.
In my experience, usually, the inputs to neuronal networks, **trained with known data**, are based on something like a thousand RNA concentrations from 100 patients, such that humans would have trouble seeing the connections, thus the need for the computer in the first place. In fact if the relationship that they discover is as simple as some mathematical expression among three numbers, then everyone is surprised but the computer program is no longer necessary. The purpose of the computer was to find those particular three parameters out of the thousand available. The analysis of a particular clinical finding in that case can, thereafter, be done by a calculator. Something like this happened at a company I was consulting for in Savannah, wherein a very uncomplicated solution appeared from a

computer program called a support vector machine regarding the differential diagnosis of acute lymphocytic leukemia and chronic myelogenous leukemia. The input had consisted of several thousands of RNA levels from several hundred patients. After the result had appeared it did not require a computer to make the diagnosis. A human could look at just two relevant RNA levels, and make the call.

Until then, which two, or whether there were only two RNA levels that held the critical data was not known. The support vector machine was instructed (trained) by clinical data, not simply the RNA levels. That's why it worked.

Computers are patient and capable of tedious calculations, but they are not capable, of ever telling you something that given great time and near-infinite patience, you could not have worked out yourself on a calculator. There is no discontinuity between classical mathematics and what is referred to as Turing Machine-like universal calculators. They are just fast, very fast, but not infinitely fast, or perhaps more importantly here, wise. What this means is that we understand how they work. There's no mystery, only speed.

So, if your program can always predict coronary artery conditions from three numbers representing the composition of three different lipids on platelets, without ever having being trained on a set of these variables paired with some clinical indicators, there is no way that a philosopher of science could legitimately say that you had not discovered something that was **unexplainable, but clearly useful**. However, its validity is purely based on induction, that is, it keeps working, unless Lucio can explain how it happened, and the process can be adapted to other applications. Most scientists, on the other hand, and I have to admit in this case that I fall into the latter category, would say you are damned lucky or you are missing something important.

What you are doing isn't in the commonly accepted sense, scientific. It is lacking in the essential quality of being largely explainable as a consequence, however subtle, of known facts, and therefore serving as a guide for other scientists to develop similar methods.

If Lucio has discovered a new principle of neural nets that allows them, independently of a training set, to distinguish healthy coronary arteries from unhealthy coronary arteries on the basis of three numbers, which to a non-biologically informed computer, are only numbers, **that principle** overshadows this particular use of it by a revolutionary leap, and deserves to be published not only in the appropriate medical journal, but in a computer science journal as well.

Given that this is the case: some new principle of neural network programming has been discovered and it has found ratios of three lipids that can be used to predict future cardiovascular problems, then congratulations are in order, BUT even in that case, I don't understand why the program is **still** necessary to make the diagnosis.

In the case I mentioned earlier regarding leukemia, once the relevant genes had been identified, the computer program was no longer necessary.

There is one explanation that I can imagine to account for the facts, but it brings into question your judgement and that of Lucio. Since there were only three variables being considered, it is not impossible that the relationship between them, which correlates with coronary heart disease, is simple enough that it could have been discovered empirically without the use either in the beginning or especially later of a neural net. In fact, how complicated can be the relationship between three numbers? What is the computer doing? Why didn't you compare by eye the three numbers in light of who was a coronary artery risk patient (which as you explained to me from the Framingham data was mostly just the age of the patients.)

Was something preventing you from knowing their potential coronary artery status prior to the computer study, which is not now preventing you from knowing that, and if not, then how do you evaluate your present results? Either you know now the coronary artery risk and can compare it to what your computer says, or you don't. If you do know it now then you can use it to validate your result. If you know it now, when did you learn it? Were you unaware of it before the computer study?
Cordially yours,
Kary

Dear Dr. Mullis,
How are you? And what about Linda and Nancy? Everybody is ok, here in Italy.
Well, let's come to the point. Now I clearly understand (I hope) the misunderstandings between you and Massimo. I'll try to explain.
You wrote:
*"Let's try this one more time, Massimo, because somewhere there must be a disconnect in our dialogue on this business of an **un-trained** neural network being able to spot the likelihood of coronary heart disease in a set of patients, where the only information given to the network, is the concentration of three lipids on their platelets, let's just call the lipids 1, 2 and 3.*
What I understand is that you are demonstrating that in the absence of a "training set" your program can pick the patients who have a high probability for a coronary problem just from these values. By a training set I mean that the values for 1, 2 and 3, paired with an independent diagnosis of a coronary artery problem are presented to the computer for a certain number of your initial group. You are claiming that a "training set" is not necessary.
How, I ask, could that possibly work?
Now I do realize that lipid composition on platelets could easily have something to do with coronary artery problems, (and clinical depression). I don't disbelieve that they could. Nobody I ever heard of predicted that, but when you told me it was true, I accepted it. But when you explained to me that your program had discovered this relationship with no reference to some external standard, no training set, I was incredulous and still am.
I simply claim that without some additional data, like a training set of data points wherein the lipid variables were paired with clinical outcomes or some similar KNOWN variable relevant to coronary artery problems, (or a super-exceptional knowledge of physiology), the neuronal net, which is a mathematical object, not an oracle, could know anything except 1, 2, and 3 (the inputs) about the patients. "
Kary, you are right, we had to start from a "training set". Maybe this is the main point of the misunderstanding. It's simply a problem of words, I believe. Make the call.
Of course we started from a "training set". About Ischemia, it is made up by 60 healthy subjects versus 50 patients with definite diagnosis of Ischemia. So, we had a data base of 110 subjects. For each Subject, we had his Fatty Acids pattern (11 variables) paired with "an external variable": his healthy status related to Ischemia, I mean, if the subject is pathologic or healthy.
So, we absolutely started from a "Training Set".
I think the misunderstanding occurred when we talked about the mathematical method, the **training process**, in particular.
Many mathematical methods, ANNs in particular, are classified into 2 big families:
Supervised ANNs and Unsupervised ANNs, according to the training process they use.

1) ANNs, using a supervised training process, need the complete training set, that is, all variables plus the "external variable". For example, the Multi-Layered Perceptron (usually using the Back propagation algorithm) is a supervised ANN, probably the most common. It studies the Data Base, "learning" the features of each subject, according to his "external variable", which it knows. Mathematically, it builds an n-dimensional error surface linking all variables involved to the "external variable". It's called "supervised" because it corrects itself reducing, in an iterative way, the global error knowing the correct result, as if "a teacher" would correct it, at each time.
2) ANNs using an unsupervised training process, such as the Self Organizing Map (SOM), just need all variables, without the "external" one.
This is because they use a different approach: they are not trained to find the feature of the different "external" value. They just look at the data and try to put together similar subjects without considering the "external variable" (pay attention, SOMs are different from the common "cluster analysis").
A SOM is able to do so, comparing, in a particular way, all the subjects of the data base (if you are interested, I attach an appendix to this paper where I try to explain how a SOM works, but , please, read all this paper before).
I think it could be clearer following this example.
Suppose you want to build up a system able to recognize handwritten characters. You probably start from a Data Base made of characters written by different humans (of course with different handwritings). In this case, the "external variable" is the real alphabetical letter the handwritten character represents.
You could build this system using statistical "supervised" method. But you can also use a SOM. In this case, you have to show to the SOM the different handwritten characters, MIXED AND **WITHOUT TELLING WHICH ONES RAPRESENT "A", "B", "C" AND SO ON.** You just hope the Net will set different characters in different areas, putting the handwritten characters of "A" near to each other, and far from the handwritten characters of "B", "C", and so on. If (as usually happens), once the SOM is trained, all "A" are near, all "B" are near and so on, the SOM is ready: it mathematically realizes that all handwritten "A" are similar and puts them near to each other. The SOM realizes that "A" are different from "B" and put all handwritten "B" far form "A" but, again, near to each other. I mean: the "external" variable in this case is the alphabetic letter, linked to any different handwritten character. The SOM did not know this, when it was training, and when it was producing the final map. Just once the map is done, an external human observes that all handwritten characters, linked to the alphabetic letter "A" are together! And it's the same with all other letters. Just now, the human divides the map into clusters, one for each letter (usually using a well known mathematical method such as the "Voronoi clusterization").
Once the SOM is trained, if we give it a new handwritten character, it will be mapped near to the similar characters, very probably in the correct cluster.
So the whole training set, at the end, is absolutely necessary. It is not, for the SOM training.
By the way, common commercial OCR (Optical Character Recognizer) software, usually use really this method. Actually, a lot of common commercial software use this kind of ANN even for many other purposes.
Let's return to Ischemia.
We had a Training set of 110 subjects. We give to a SOM the 110 subject Data Base, without saying who was pathologic and who was not (exactly as done with handwritten characters). We just told the SOM the 3 fatty acids values.

Once the SOM gave us the result (I mean the map), we just observed that pathological subjects were all mapped on the top while healthy subjects were all mapped on the bottom. But we observe it, not the SOM, which does not know their pathological status. I mean: just once the net drew its answer, I mean the map, we colour healthy with green and pathologic with red, and realize the result. Since, all pathologic subjects are into a cluster, while other are opposite, we can say that:

all pathologic subjects are similar to each other, and different from normal, at least from the 3 fatty acids involved point of view. (statement 1)

This last statement is absolutely scientific. In fact:

- The SOM is a very common neural network, deeply known and widely used even in common commercial software. It appeared to the scientific community more than 20 years ago (T. Kohonen, 1981). Actually, in the biomedical field, SOMs are widely used since 10 years at least, and of course, they are absolutely accepted.
  So, the protocol used, is not a mystery, nothing unexplainable, but well known and accepted from years.
  Maybe it's not so popular as a logistic regression but, at least here in Italy, more popular than Support Vector Machines.
- If anyone, everywhere and whenever he wants, builds up a SOM or buys it (there are hundreds of commercial software developing SOMs) and trains it with our training set (of course, without the information about the pathological status), he will find that pathologic subjects go to a side and healthy, opposite. Probably, he will not find exactly our map, but the same result for sure. In fact, a SOM, like almost all ANNs, depends on random starting weight and on some other parameters: as you know, being familiar with ANNs, each training process is always different from another! In any case, if someone gives the same SOM parameters we used (to tell you the truth, they are those suggested by the literature, so they are the first that a computer scientist would try…) he will have exactly the same map.

As a result of the statement 1, we can add that the 3 fatty acids of the pathologic population are, in some ways, different from those of the healthy population.

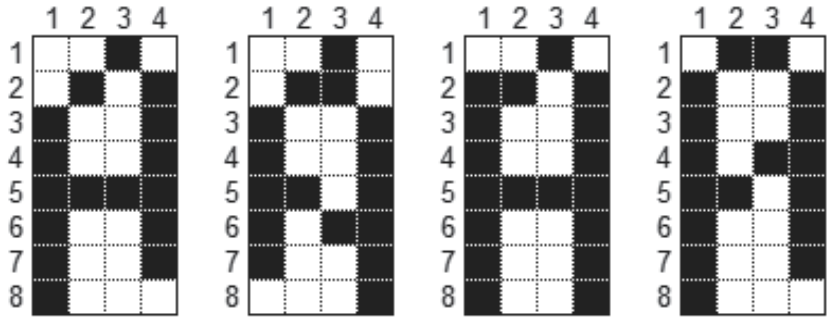
Another result is, like in the handwriting character example, that we can map a new subject, whose status is unknown and, according to his position on the map, according to the cluster he reaches, we can think that he can be considered healthy or pathologic. I E: if his position into the map is in the middle of all other pathologic subjects, he can be suspected to be pathologic, or at least it's very probable.

Now, another point:

*"In my experience, usually, the inputs to neuronal networks, **trained with known data**, are based on something like a thousand RNA concentrations from 100 patients, such that humans would have trouble seeing the connections, thus the need for the computer in the first place. In fact if the relationship that they discover is as simple as some mathematical expression among three numbers, then everyone is surprised but the computer program is no longer necessary. The purpose of the computer was to find those particular three parameters out of the thousand available. The analysis of a particular clinical finding in that case can, thereafter, be done by a calculator."*

Well, this is not our case. In our situation we had 110 subjects, and only 11 variables (coupled with the pathological status, 12th variable). So our main problem wasn't to find those particular parameters out of only 11 variables. Talking about Ischemia (things are quite different about Depression), we identified the 3 fatty acids, quite easily, by means of common conventional statistics (Discriminant Analysis, ANOVA and so on).

Once we found those 3 parameters, we wanted to study their dynamics, we wanted more information, a deeper knowledge of the problem and, a diagnostic tool. So we tried to use different mathematical tools such as "Cluster analysis", Classification Trees, and so on, but in our opinion, the best one has been the SOM.
In fact, it **quickly** led us to a lot of important (according to us) results. We do not use "*a thousand RNA concentrations*" but just a simple SOM. Maybe in our case, the need for the computer is not in the first place, maybe we could reach same results "by eye" but, as you say, the main feature of a calculator is speed and our main interest is the result, secondly the method. In any case, by eyes we can't evaluate the 3 parameters in the same time, with the same speed and precision of the SOM.
Many people are scared by the name "artificial neural network". But ANNs are not strange objects at all! They are not difficult; they are accepted as useful tools from years and commonly used even in everyday life application. Maybe, the only trouble is the different approach they use, a bit different from conventional statistics. But the problem, if it exists, is just in our way of thinking. And, I'm sure, Kary, you have not this kind of problems at all!!!
One more question:
"There is one explanation that I can imagine to account for the facts, but it brings into question your judgement and that of Lucio. Since there were only three variables being considered, it is not impossible that the relationship between them, which correlates with coronary heart disease, is simple enough that it could have been discovered empirically without the use either in the beginning or especially later of a neural net. In fact, ***how complicated can be the relationship between three numbers? What is the computer doing? Why didn't you compare by eye the three numbers in light of who was a coronary artery risk patient*** *(which as you explained to me from the Framingham data was mostly just the age of the patients.)"*
Well, a computer is not necessary to guess a handwritten character!
How complicated can be recognizing a handwritten letter?
But I ask you: can you explain the mathematical rules in order to classify a handwritten character, in an easy way? Or better: a digitized character could be expressed, for example, with a 8x4 matrix of pixels = 32 pixels. We certainly could give a formula with 32 variables in order to classify a character, but all computer scientists find it more comfortable to use a SOM instead of a formula. And I agree with them.



Well, in our problem, there are only 3 numbers, it's true. But, when we found the SOM map, we just understood that using the 3 fatty acid, we could classify a patient but we absolutely did not know the correct rules in order to do that, yet. We realized soon that it was not a so simple problem.

Some examples of handwritten "A" in an 8x4 matrix of pixel

I try to explain. When Massimo and I studied the "rules", we observed that a subject was pathologic if parameter 1 was low while parameter 2 was medium and parameter 3 was high. If parameter 3 decreases, the subject is no more pathologic unless parameter 2 increases too, but just up to a certain value, and so on. I mean that there are a lot of possible configurations, combinations. We agree that we could express them with pencil and paper but we simply find it more comfortable and clearer using a 2 dimensional map. In our opinion, it's just more simple, easy and clear.

For example, let's think about the Body Mass Index (BMI) and its application. It's one of the simplest formulas all over the world. You just have to calculate Height/Weight^2 and than check the result in a reference table. Well, every doctor simply has stupid soft wares that do it. I'm sure this could be done by eye but it's faster and maybe easier to use a computer that shows a graph. Of course, we translated all our results into rules, and we are using them in order to continue our research. But when we have to check the healthy status of a new subject, we find it more comfortable to use the SOM, mainly when a subject falls in our border-line area.

So Kary, as you have seen, there's no mystery, nothing is unexplainable. Everything is, in the commonly accepted sense, scientific. I have not discovered a new principle of neural nets (warning: until now!), unfortunately, because the net we used has been existing since 1981.

Maybe the reason of this misunderstanding between you and Massimo, between you and us, is me. I feel really guilty and apologize, because I was not clear in my explanations with you. You always had to talk of mathematical questions with Massimo, who is neither a mathematician nor a computer scientist! I remember that, even if we didn't have so many occasions to talk together in private discussions, you came to me many times, for questions, remarks and so on. I apologize but my main problem is that it's very difficult to talk with you: I'm neither a "researcher", and I should talk with a Nobel laureate. I know, you were present when I did my first lecture, and year by year you saw me grow.

I am really sorry, Dr. Mullis, but you are considered one of the best minds all over the world, and I feel so small, in front of you… sorry.

Thank you for your time,

Sincerely, Lucio.

Dec 17, 2007

Dear Massimo,

Lucio has clarified the situation for me.

I now understand your work with Lucio and agree that it is a contribution to the field of diagnostics in cardiology which is worth pursuing. There was a communication problem.

I did not understand that after the self-organizing map program had operated in an unsupervised manner on all the data from healthy and at-risk patients, sorting each individual set of the values for oleic, linoleic and arachadonic acid into one of 400 bins, according to the similarity of the relations between their components, then you, knowing which sets were (from independent considerations) at-risk, "colored" the 400 squares according to "healthy" or "at-risk." That input from you took the place of informing the overall procedure as to which of the patterns it had found originated in healthy and not-so-healthy patients, and logical induction allows you to assume that whenever future values fall into one of those categories it will be defined.

Certainly now by examining the program statements that assign the three fatty acid concentrations to particular cells in your program, you could replace the program with a

series of "if then" statements, but I understand that computers are ubiquitous and cheap. Why bother. In addition, by continuing to add more data, which you know is from healthy or unhealthy patients you might refine your colouring pattern if that is called for.
So the mystery is solved.
I'm sorry to have been so much trouble. I think if scientists still refused to talk to each other except in Latin, such misunderstandings might not happen. But then we would all have to learn Latin (even Italians) and agree on how to interpret it.
Give Lucio my best and assure him that I understand now what you are doing as a result of his explanation.
I am looking forward too seeing you next year. My regards to all.
Cordially,
Kary

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18

Acceleration of New Biomarkers Development and Discovery in Synergistic Diagnostics of Coronary Artery Disease

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1. Introduction

The current definition of biomarkers includes "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (Biomarkers Definitions Working Group, 2001)". Accordingly, biomarkers are usually used for detection and establishing the magnitude of a physiological derangement as well as to monitor a treatment.

The role for imaging techniques and biomarkers in the diagnosis and treatment of myocardial infarction (MI) after percutaneous coronary intervention is well-established. Many candidate biomarkers emerging from genomics and proteomics research have the potential to serve as predictive indexes for guiding the development of interventional cardiology (Gerhardt et al. 1991; Katus et al., 1991; Lindpaintner, 1997; Kong et al., 1997). Among them the undisputed role still play cardiac proteins like troponins or creatine kinase-myocardial band (CK-MB) (Alcock et al., 2010; Lim et al., 2011). Less established, however, is the employment of biomarkers to determine long-term, progressive, or dynamic risk over time in patients with advanced coronary artery disease (CAD). Biomarkers offer a means to **track** differential exposure as well as **impact** of exposure. As such, they reflect individual vulnerability, ongoing person-environment interaction, and unmeasured environmental factors that mediate the effect of exposures (Fig. 1). Essential to a vision of **synergistic diagnostics** is a focus on the mechanisms of diseases. Understanding what is happening on a molecular and cellular level, how disease actually begins, how cells begin to express certain proteins, influence other cells and trigger processes (atherosclerosis, thrombosis, calcification) will allow to develop *in vitro* diagnostics and imaging techniques to distinguish these processes. By characterizing a comprehensive set of measurable processes that capture diverse pathogenic aspects of CAD, a real-time systems view of disease activity can be generated to improve decision making.

This chapter summarize a current view on the development of new biomarkers as a prognostic platform among patients at risk of CAD and upcoming complications.

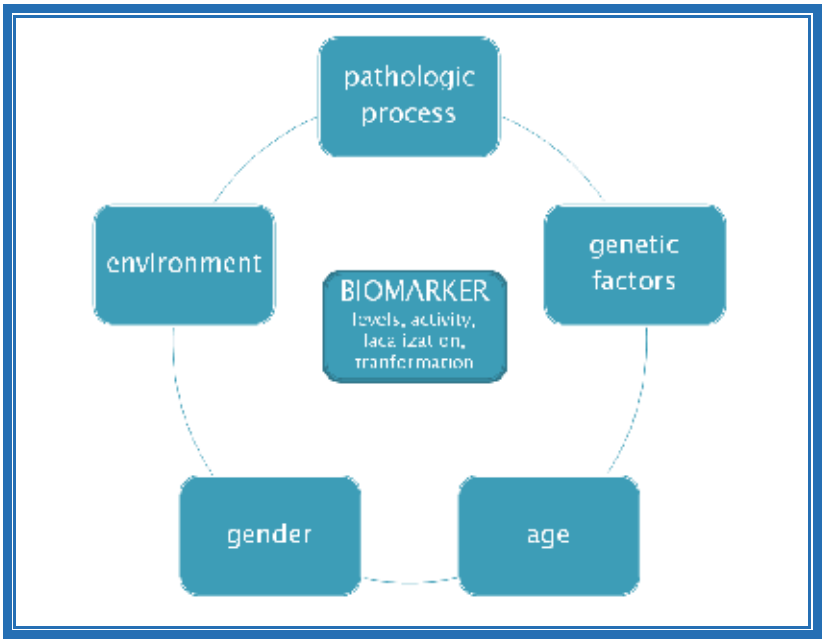

Fig. 1. Model showing relationship of a biomarker with internal and external factors which have an impact on measurable and unmeasurable features of biomarker.

2. Bone remodelling biomarkers

About 10 years ago, the hypothesis that bone remodelling biomarkers might be involved in the progression of **coronary artery calcification** seemed to be tricky and beyond any reasonable expectation. However, in 1995 Boström *et al.* first time proposed the possible mechanisms for bone formation in artery walls involving retention of pluripotent cells or osteoblastic immigration coupled with embryonic-like osteogenic program (Boström et al. 1995). The main reason for understanding the regulatory mechanisms of vascular calcification was firstly related to therapeutic approaches to prevent and possibly reverse vascular mineralization (Demer , 1997; Parhami et al. 1997). The data from clinical studies regularly report an association between bone remodeling biomarkers and the presence, severity and progression of a broad range of cardiovascular diseases. Whether they are biomarkers or rather play a causal role in mediating or protecting against vascular injury is not clear. The mechanisms underlying the postulated role of bone remodelling biomarkers in atherosclerosis probably involve **inflammation** and **calcification** processes.
This section will focus on the prognostic significance of plasma bone remodelling biomarkers levels in stable and unstable CAD.

2.1 Biology of bone remodelling of biomarkers

Vascular biomineralization in an atherosclerotic plaque results from an imbalance in osteoblast- and osteoclast-like cells and the induction of vascular or immune cells differentiation into osteogenic cells (Demer & Tintut, 2008). Osteobalsts, osteoclasts and inflammatory cells are firmly involved in bone remodelling (Fig. 2).

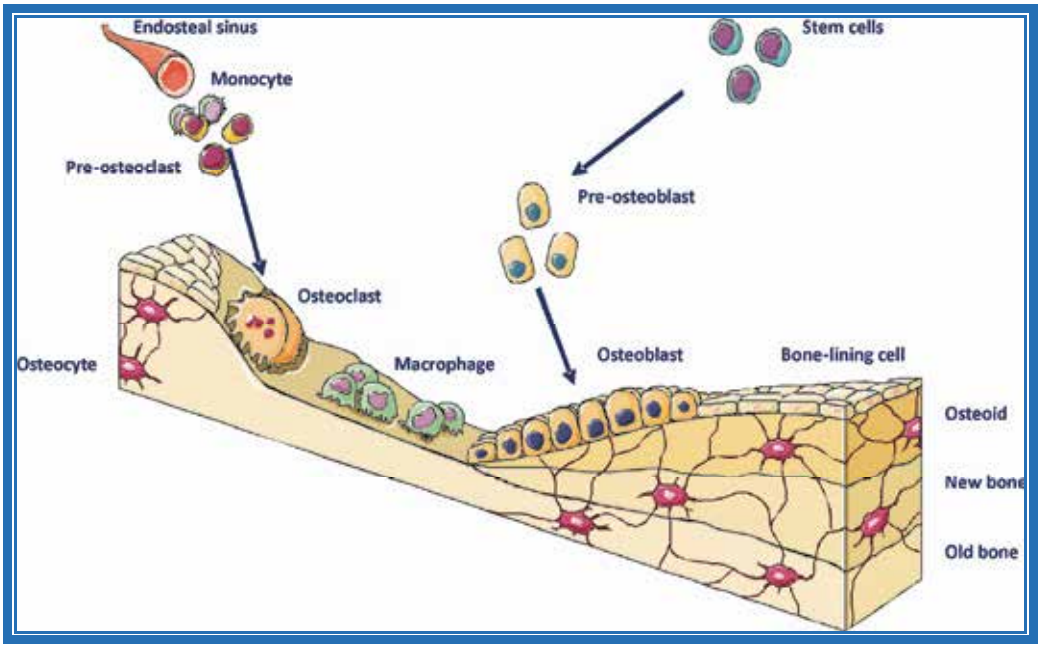

Fig. 2. Bone remodelling osteoblasts and osteoclasts differentiation. Figure was produced using Servier Medical Art.

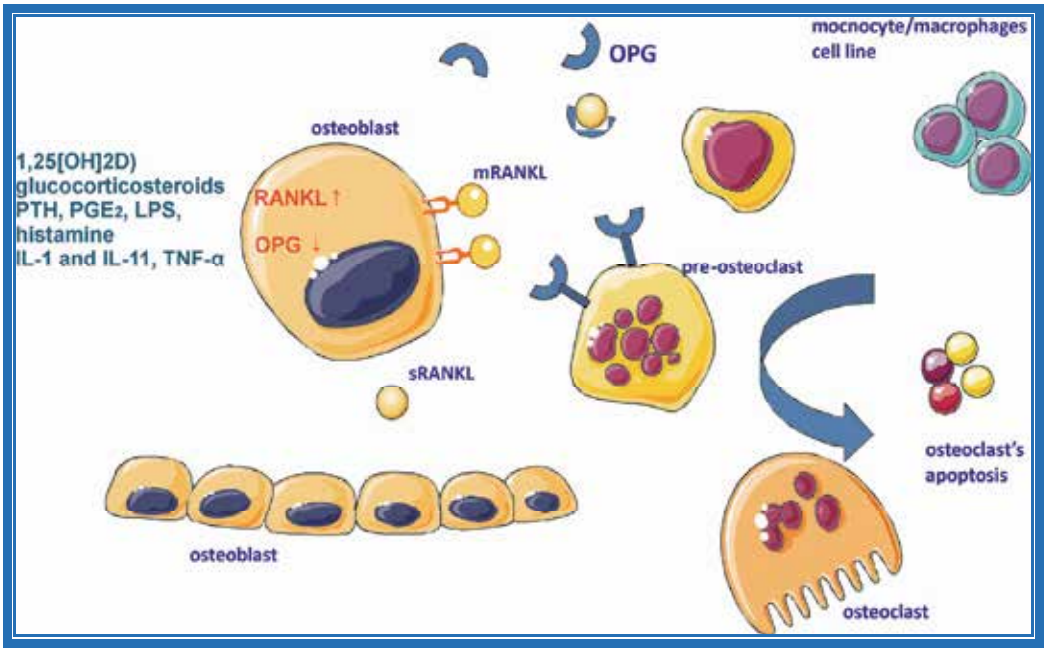

Fig. 3. The role of osteoprotegerin (OPG) in pre-osteoclast differentiation. OPG trap and neutralize a soluble receptor activator of nuclear factor kappa-B ligand (RANKL) which activates osteoclasts by its receptor (RANK). Figure was produced using Servier Medical Art.

Mesenchymal stem cells are precursors for **pre-osteoblasts**. **Osteoblasts** activity leads to bone formation and mineralization, their differentiation and activity is mostly regulated by RANKL (receptor activator of nuclear factor kappa-B ligand) inducers, such as: vitamin D (1,25[OH]2D), glucocorticosteroids, parathormone (PTH), prostaglandins (PGE_2), lipopolysaccharides (LPS), histamine and pro-inflammatory cytokines: interleukins (IL-1 and IL-11), tumor necrosisi factor alfa (TNF-α) and others (Eriksen, 2010). RANKL is a surface-bound molecule (also known as CD254). It is found on **osteoblasts** and serves to stimulate **osteoclasts** by RANK (receptor activator of nuclear factor kappa-B) activation and RANK/RANKL axis has a core regulatory role in osteoblasts and osteoclasts signalling (Fig. 3) (Caidahl et al., 2010).

2.2 Osteoprotegerin and osteopontin as risk factors of coronary artery disease

Several studies suggest the involvement of bone remodeling biomarkers in coronary artery disease and related atherosclerotic disorders (Van Campenhout & Golledge, 2009; Venuraju et al. 2010). Prime regulators of bone remodelling, such as osteoprotegerin (OPG) and osteopontin (OPN), are significantly and independently associated with inflammatory processes and arterial hypertension and may exert substantial influence on the severity of cardiovascular disease. (Stępień et al., 2011)

OPG is a soluble glycoprotein widely expressed in most human tissues including the bone (osteoblasts) and the vasculature (endothelial and vascular smooth muscle cells, VSMC) (Collin-Osdoby, 2004; Schoppet et al., 2002) that is implicated in the regulation of bone and vascular calcification. OPG is a member of the tumor necrosis factor (TNF)-related family and a part of the OPG/RANKL/RANK triad. OPG acts as a soluble secreted decay receptor for a receptor activator of nuclear factor kappa-B ligand (RANKL) and neutralize this essential cytokine required for the osteoclasts differentiation (Hsu et al., 1999) (Fig. 3).

RANKL expressed on osteoblastic, stromal and T cells binds to RANK (osteoclast differentiation factor) on the surface of osteoclasts, monocytic and dendritic cells and mediates a cell-to-cell signal responsible for osteoclastogenesis (Yasuda et al. 1998). Additional roles in immunological responses include the RANK-RANKL binding between dendritic and T cells which enhances the immunostimulatory capacity of dendritic cells and T cell proliferation (Green & Flavel 1999).

It was observed that *opg*-knockout mice (OPG -/-) develop early onset osteoporosis and arterial calcification (Bucay et al., 1998) and the restoration of the gene prevented osteoporosis progression and arterial calcification (Min et al., 2000). Increased OPG level has been observed in men with advanced CAD and plasma OPG level has proved to be an independent predictor of myocardial ischemia in asymptomatic diabetic patients (Avignon, 2007; Schoppet et al., 2003). Moreover, increased OPG has been related to the number and vulnerability of plaques as well as in carotid artery (Kadoglou et al., 2008; Vik et al. 2010) or coronary vessels (Palazzuoli at al., 2008), which suggests its involvement in the coronary disease progression (Mikami et al., 2008; Pedersen et al., 2010). Elevated OPG in plasma is univariable predictors of coronary artery calcification (CAC) progression (Anand et al., 2007). The sensitivity of OPG for detecting of CAC score higher than 200 Agatston units was 80% in patients with predialysis diabetic nephropathy (Schoppet et al., 2003). However, in the large Norwegian study by Pederesen *et al.* (Pedersen et al., 2010), adjustment for conventional risk factors attenuated the risk estimates for OPG levels. Only the subgroup of patients with stable angina pectoris (SA) with levels above the 90th percentile was at risk all-cause mortality: 1.94 (1.18, 3.18), p=0.01; CAD mortality: 2.29 (1.16, 4.49), p=0.02; and MI: 1.76 (1.02, 3.06), p=0.04.

In patients with acute coronary syndromes (ACS) the baseline OPG concentrations were strongly associated with increased long-term mortality (hazard ratio [HR] for log transformed OPG level 1.7 [range 1.5 to 1.9] $p<0.0001$) and heart failure hospitalizations (HR 2.0 [range 1.6 to 2.5]; $p < 0.0001$) but weaker with recurrent MI (HR 1.3 [range 1.0 to 1.5]; $p = 0.02$) and not with stroke (HR 1.2 [range 0.9 to 1.6]; $p = 0.35$). The association remained significant after adjustment for conventional risk markers (Omland et al., 2008). In apparently healthy individuals (the European Prospective Investigation into Cancer in Norfolk – EPIC Norfolk cohort) high serum concentrations of OPG and soluble RANKL were associated with an increased risk of future CAD (Semb et al., 2009). OPG showed a significant association with the risk of future coronary events in both sexes. This association remained statistically significant after adjustment for traditional cardiovascular risk factors (i.e. age, diabetes, systolic blood pressure, smoking, total cholesterol and HDL cholesterol).

OPN is secreted as a calcium-binding glycophosphoprotein that has been implicated in bone remodeling and inflammation as well. Similarly to OPG, osteopontin is widely distributed in different human cells including osteoblasts, lymphocytes, macrophages, endothelial cells and vascular smooth muscle cells (Brown et al., 1992).

OPN is a cytokine and has the ability to stimulate migration of macrophages and osteoclasts (Giachelli et al, 1998; Suzuki et al., 2002) and proliferation of osteoclasts and vascular smooth muscle cells (Giachelli et al, 1998; Liaw et al., 1994). A growing body of experimental evidence suggests that OPN overexpression plays an essential role in modulating compensatory cardiac fibrosis and hypertrophy (Xie et al., 2004; Singh et al. 2010). OPN acts through different integrins and thus has a great potential to regulate populations of different cells on the molecular and cellular levels (Bazzichi et al., 2009; Burke et al., 2009). OPN plays a pivotal role in inflammation and atherosclerotic plaque formation in an animal model (Scatena et al., 2007). Recent data has indicated a high predictive value of OPN for secondary manifestations of atherosclerotic disease (e.g. cardiovascular death, myocardial infarction, stroke, and endovascular interventions) in a 3-year follow-up of patients undergoing carotid surgery (de Kleijn et al., 2010).

Baseline levels of OPN are independent predictors of future adverse cardiac events in patients with chronic coronary syndrome (CCS), and may be useful for risk stratification (Minoretti et al., 2006). Recent data have indicated a high predictive value of OPN for secondary manifestations of atherosclerotic disease (e.g. cardiovascular death, MI, stroke and endovascular interventions) in a 3-year follow-up of patients undergoing carotid surgery. In a prospective study by Gogo *et al*. (Gogo et al., 2006), the association between angiographically quantified coronary artery calcification and OPG was not found. Detection of coronary calcification by coronary angiography may underestimate the calcification burden, thus synergistic diagnostics of coronary calcification should utilize more sensitive techniques of MSCT (Willemsen et al., 2009). However, in patients with CAD undergoing percutaneous coronary intervention (PCI) the highest OPN levels were associated with both plaque progression and restenosis in a stent ($p=0.003$). In addition, OPN, IL-6, and CRP were higher in patients with ACS than in those with CCS (analysis of variance: $p<0.001$, $p<0.05$ and $p<0.05$, respectively) (Mazzone et al, 2011).

A question arises as to whether peripheral vascular function (calcification markers) matches the coronary arteries (calcification) and thus, whether it may serve as a surrogate marker to identify individuals with increased hazard of CAD and mortality (de Kleijn et al., 2010; Lieb et al., 2010; Scatena et al., 2007). Therefore bone-matrix proteins combined with cardiovascular imaging could be potential markers for vulnerable coronary artery plaques.

3. Microparticles

Microparticles (MP) are sub-micron sized cell membrane/cytoplasmic fragments that are released from the cell surface. There are two well-known cellular processes that can lead to the formation of MPs: chemical and physical cell activation (by agonists or shear stress, respectively), and apoptosis (Jimenez et al., 2003). However, the mechanisms that take place during MP formation are still not revealed. It seems that, the flopping of phosphatidylserine (PS) to the outer layer of the plasma membrane is pivotal. Finally, this process leads to the formation and shedding of MPs from activated or apoptotic cells. In resting condition the membrane asymmetry is maintained by an aminophospholipid translocase with **flippase** activity. Bilayer asymmetry is disrupted in the consequence of the inhibition of flippase activity by calcium influx. Increased calcium ions concentrations activate calcium-dependent calpains, which disturb cytoskeleton, promote the shedding of MPs (Morel et al., 2011) and stimulate **scramblase** and **floppase** activities, which lead to the collapse of the membrane asymmetry (Freyssinet & Toti, 2010).

MPs are qualitatively and quantitatively diverse and vary in diameter between 0.1 and 1.5 μm and may harbor a number of cell surface proteins (Fig. 4). MPs are released from various cell types such as circulating blood cells (platelets, lymphocytes T and B, monocytes and erythrocytes) and cells of the vessel wall (endothelial and smooth muscle cells) (Amabile et al., 2010).

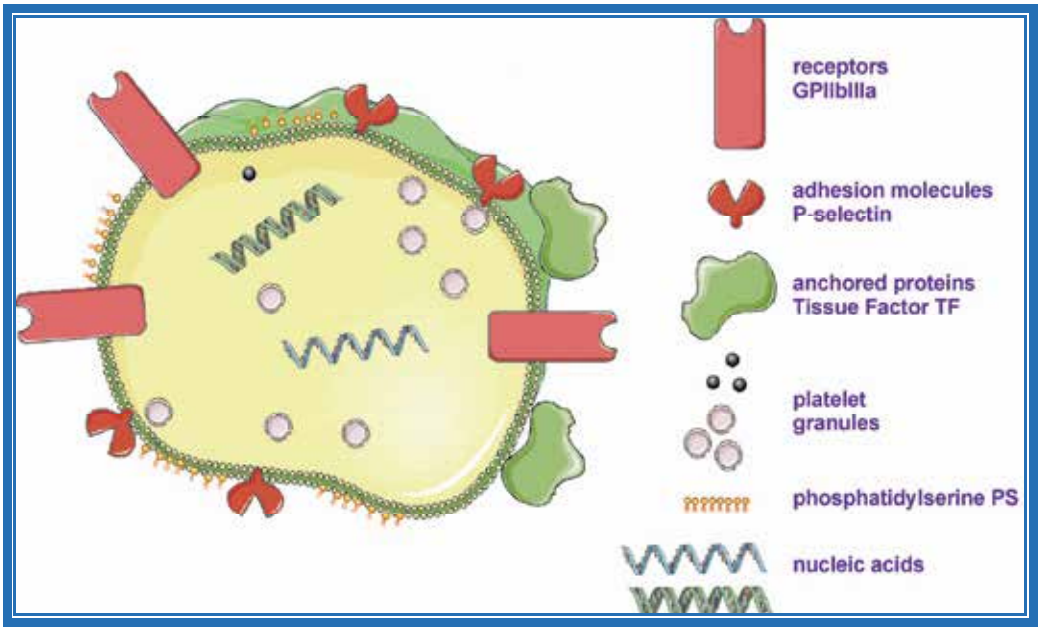

Fig. 4. A platelet microparticle is carrying not only specific membrane adhesion proteins (P-selectin, integrins – e. i. GPIIbIIIa,), but also may harbour and transfer tissue factor (TF) which has its procoagulant potential and other functional effectors (E-selectin, von Willebrand factor, arachidonic acid, thromboxane A2), that can regulate aggregation, adhesion molecule expression, cell proliferation, apoptosis and endothelial migration. MPs may capsule messenger molecules (miRNA, DNA ?), cytokines, growth factors and calpains. Figure was produced using Servier Medical Art.

MPs from numerous cellular sources have been described in human plasma. They have received increasing attention as potential biomarkers of cell damage and activation or biovectors in blood coagulation, inflammation and cancer (Benameur et al., 2009; Hoyer et al., 2010). In several pathological states like dilated cardiomyopathy, chronic renal failure or cerebrovascular disease, MPs were used as biomarkers to identify a disease or to detect complications linked to a given disease (Bulut et al. 2011; Faure et al., 2006; Jung et al., 2009). Numerous clinical studies have evaluated their usefulness in the stratification of patients at risk for vascular disorders and to monitor response to treatment. Circulating MPs may serve as a marker for cardiovascular events in CAD patients or as a predictor of acute allograft rejection after heart transplantation (Morel et al., 2008; Sinning et al., 2010).

3.1 Microparticles discrimination and enumeration

The high level of microparticles' diversity may create a problem with compatible masurement of MPs using different analytical methods. The number of microparticles depend on the detection technique and a wide range of pre-analytical variables, i.e. blood collecting and handling, plasma preparation and storage conditions. Therefore, optimization and standardization of detection methods are important to define microparticles correctly and to avoid falsely high or low quantification. Even minor protocol changes significantly affected MP levels (Ayers et al., 2011).

3.1.1 Flow cytometry in MP analysis

Several research have evaluated the impact of these different parameters to propose a pre-analytical protocol for MP analysis. Three ISTH Scientific and Standardization Subcommittees (SSC Vascular Biology, DIC, and Haemostasis &Malignancy) have initiated a project aimed at standardizing the enumeration of cellular MPs by means of flow cytometry method (FCM). The first collaborative workshop was set to establish the resolution and a threshold levels of the flow cytometers currently used in laboratories. Additionally, the interinstrument reproducibility of platelet MP enumeration in human plasma was analyzed (Lacroix et al., 2010). The study included 40 laboratories and 59 flow instruments were validated according to the protocol based on Megamix beads calibration to discriminate microparticles between 0.5 µm and 0.9 µm using the forward scatter (FS) channeling (FSC) parameter (FS/FSC). After that, selected laboratories received PFP samples prepared as frozen aliquots by the core laboratory, to avoid any preanalytic-linked variability. The authors found high discrepancy among Becton Dickinson instruments, as well within low, medium and high values of MP: coefficients of variation were 78%, 60% and 91%, respectively. Whereas interlaboratory reproducibilities were 30% ,15% and 17% for low, medium and high values among Beckmann Coulter instruments. These data indicate that standardization of platelet MPs enumeration by FCM dependents on intrinsic characteristics of instruments. Moreover, standardization by calibrated beads such is useful tool for MP enumeration, however, calibrated beads do not reflect real condition of MPs in human plasma.

3.1.2 Indirect methods for MP enumeration

Alternative methods for MP enumeration based on TF-activity/antigen or platelet glycoprotein GPIb-integrin have been already described (Huise et al, 2009; Kuriyama et al., 2010). The activity of tissue factor is evaluated using a chromogenic substrate for factor Xa,

thus the ability of MPs to promote factor X activation in the presence of factor VII using a chromogenic activity assay is utilized (Huise et al, 2009). Alternatively, TF antigen or activity can be measured in plasma or whole blood (Key NS & Mackman N, 2010). However, determination of microparticle size is not possible by such approaches.

3.1.3 Pre-analytical variability in MP determination

The analysis of different protocols used in MP preparation showed that washing, centrifugation, filtration of buffer and long-term freezing influenced significantly the MP quantification (Ayers et al., 2011; Dey-Hazra et al., 2010). Freezing samples at -80°C decreased MP levels (Ayers et al., 2011; Shah et al., 2009). The second collaborative workshop was dedicated to propose a common pre-analytical protocol useful for standardization of pre-analytical variables in determination of MPs (Scientific and Standardization Committee 2010).

3.1.4 Specific antigens in MP discrimination

There are two main features of native MPs: the small size and the anionic phospholipid - PS on the outer leaflet of their membrane. In addition, MPs carry surface membrane antigens reflecting their cell of origin, including those induced by cellular activation, cell injury or apoptosis. These properties permit detection of specific subpopulations, such as endothelial, leucocyte or platelet-derived MPs (Diamant et al., 2004).

PS is specifically bound to annexin V and is recommended as a distinguish marker for MP enumeration (Bulut et al., 2009; Shah et al. 2009). However, a number of evidence suggests that some vesicles derived from endothelial cells are PS-negative by annexin-V labelling (Jimenez et al., 2003; Sekuła et al., 2011). In platelet-poor plasma obtained from healthy donors, 80% of platelet-derived MPs failed to bind annexin V (Connor et al., 2010). In this case, a phalloidin-staining of actin filaments could be helpful in discrimination of MPs and other cell fragments (Mobarrez et al., 2010). Washing samples as well as double centrifugation result in decreased annexin-V (Ayers et al. 2011).

3.1.4.1 Platelet MPs

Platelets constitute the main source of circulating procoagulant MPs under many physiological and pathophysiological situations (Geiser, et al., 1998; Huise et al, 2009; Kuriyama et al., 2010). Procoagulant platelet derived MPs are enriched in P-selectin (CD62P), cell surface protein (CD63), integrins: GPIIbIIIa (α2bβ3), GPIIb (α2b, CD41), GPIIIa (β3, CD61) and GPIb (CD42b), tissue factor (CD142, TF) or calpains (Figure 4).

Patients with unstable angina (UA) and AMI had a significantly increased number of procoagulant MPs: GPIIbIIIa-positive, CD62P-positive and CD41-positive (Huisse et al., 2009; Morel et al., 2004; Stankiewicz et al., 2007; van der Zee et al. 2006). The total number of platelet-derived MPs were numerically higher in patients with no recanalisation compared to patients with recanalisation (Huisse et al., 2009). However, we observed paradoxically lower number of CD62P-positive platelets in whole blood obtained from patients with ACS, than from SA patients, but the level of soluble P-selectin in plasma was significantly higher than in those with ACS (Figure 5). We may suspect that soluble P-selectin levels are derivatives of platelet origin MPs (Chung et.al., 2009).

3.1.4.2 Tissue factor-bearing MPs

It was shown by cell sorting with the specific marker CD42b that under resting conditions, blood-borne TF was mainly harbored by platelet-derived MPs (Müller et al, 2003). In acute

coronary syndromes, TF triggers the formation of intracoronary thrombi following endothelial injury, activation of macrophages and apoptotosis of smooth muscle cells (SMCs) and macrophages (Morel et al, 2006). Apoptotic (annexin V-positive) MPs support a number of TF-positive MPs from different origin. Apoptotic macrophages and SMCs are the main source of membrane-bound TF and they contribute to TF accumulation. Formation of TF triggering MPs rich in PS provides a suitable anionic phospholipid surface for assembly of the tenase and prothrombinase complexes and thrombin activation (Del Conde et al., 2005).

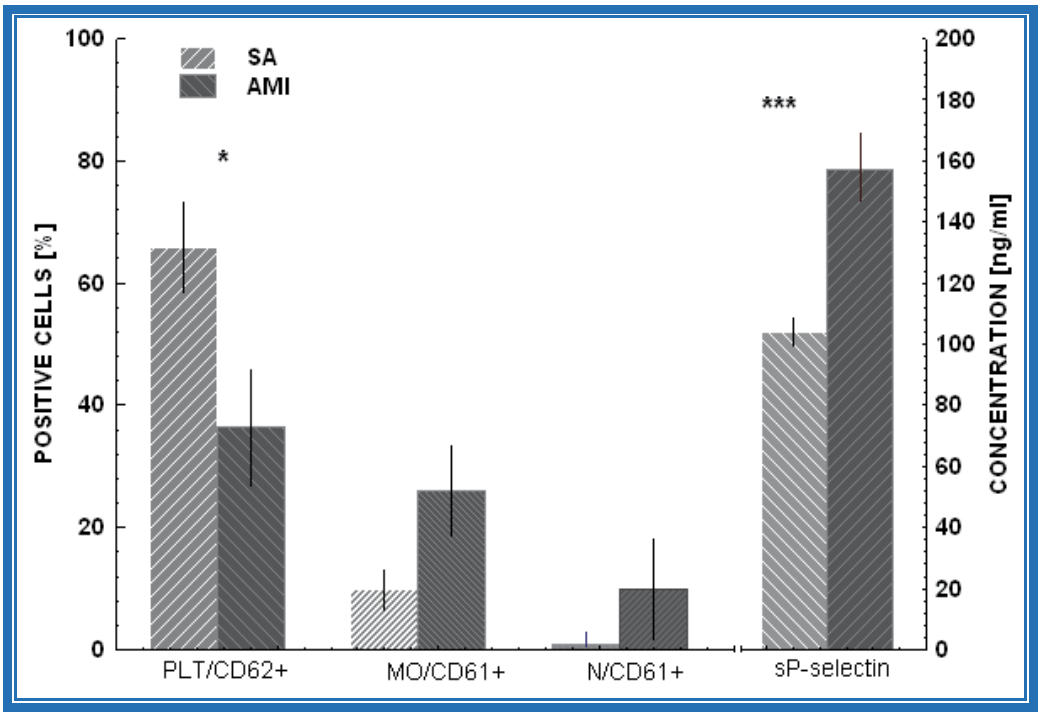

Fig. 5. Platelet activation measured as a percentage of surface P-selectin-positive (CD62+) platelet (PLT), and by monocyte/platelet aggregates (MO/CD61+) and neutrophil/platelet aggregates (N/CD61+) in peripheral blood from patients with stable angina (SA) and acute myocardial infarction (AMI), and by levels of soluble P-selectin in patients with stable angina (SA), and acute myocardial infarction (AMI). Data are expressed as medians. *p<0.05,***p<0.00001 for the comparison.

Additionally, an increased number of TF-positive (CD142-positive) MPs in patients with ACS was observed (Figure 6) (Huisse et al., 2009; Steppich et al., 2005). Moreover, elevated levels of different origin TF-bearing MPs were significantly higher within the occluded coronary artery than in peripheral blood samples (Morel et al. 2009). It suggests their contrubution in coronary atherothrombosis and *in situ* formation of procoagulat MPs.

3.1.4.3 Endothelial microparticles

Endothelial microparticles (EMPs) are an emerging marker of endothelial cell (EC) activation and dysfunction and their circulating numbers are elevated in a number of pathologic states including cardiovascular disease. Many studies suggest that endothelial

cell-derived MPs have a paracrine role and contribute to the development of endothelial dysfunction in most cardiovascular diseases: CAD, ACS, MI, hypertension and congestive heart failure. Moreover, diabetes, end-stage renal failure and pulmonary or venous embolism are strong factors bringing about EMP shedding [Bal et al., 2010; Chirinos et. al., 2005; Faure et al., 2006; Morel et al., 2004a]. In this case patients have marked activation of endothelial, platelet, and leukocyte MPs.

Endothelial-derived microparticles (EMPs) may carry different endothelial originating coagulation factors, for example TF, which contribute to the clot formation and lysis (Chou et al., 2004; Stępień et al., 2007b). Patients with AMI displayed higher levels of all MPs than patients with SA and CD31-positive EMPs appeared the main source of procoagulant MPs (Morel et al., 2004b). In patients with ACS significant correlations between both the total

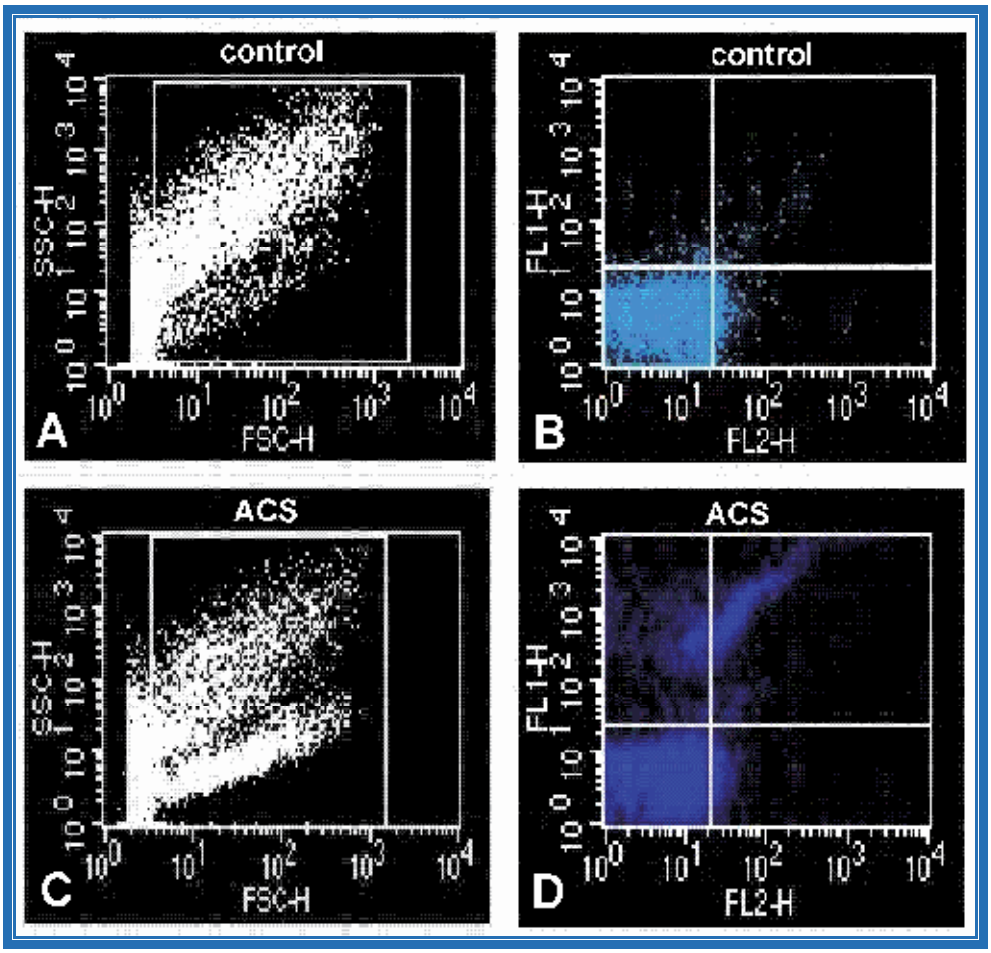

Fig. 6. Representative dot plot of circulating microparticles (MPs) in a patient with acute coronary syndrome (ACS) and in a control voluntary. A, C - flow cytometry gating logic, MPs were initially gated by forward (FCS-H) and side scatter (SSC-H) in logarithmic scale; B, D - fluorescence plots show MPs binding of annexin V-FITC (FL1-H) and anti-CD142-PE (FL2-H) monoclonal antibody.

number as well as the level of CD34, CD51 and CD142 were observed (Stankiewicz et al., 2007). Moreover, increased number of EMPs (E-cadherin/CD144-positive MPs) was an independent predictor of future cardiovascular events (HR 2.42 [range 1.03 to 5.68), p=0.04), but not for all-cause mortality (HR 2.10 [range 0.83 to 5.32] p=0.12) in patients with heart failure (Nozaki et al., 2010) and the assessment of EMPs improved prediction of future cardiovascular events in patients with CAD (Nozaki et al., 2009).

4. Clotting

Clotting is a rapid and highly dynamic process, which involves both platelets and coagulation factors. To monitor the clotting process a lot of instrumentations and methods are engaged: i) clotting times: the activated partial thromboplastin time (aPTT) and the prothrombin time (PT); ii) thromboelastography; iii) assessment of thrombin generation markers and thrombin inhibitors; iv) the real-time monitoring of thrombin generation. This section will focus on the prognostic significance of thrombin generation markers in stable and unstable CAD.

4.1 Markers of thrombin generation in CAD patients

Antithrombin (AT) appears to be the most important stoichiometric inhibitor which forms equimolar complexes with thrombin molecules – TAT (thrombin-antithrombin) complexes. A concentration of TAT complexes measured in peripheral venous blood and in blood collected at the site of microvascular injury reflect thrombin generation. TAT complexes are expressed during clot formation and there are (alike fibrinopeptide A and F 1+2 fragments) markers of thrombin activation (Pelzer et al., 1988; Pelzer et al., 1991). These markers are elevated in pro-thrombotic conditions In patients with cardiovascular disease, the detection of a prothrombotic state may have two major implications: i) to extend the duration and ii) to monitor the dose of anticoagulation after cardiac intervention. The thrombin plasma activity is very firmly associated with CAD.

The potential coagulation activity in plasma can be evaluated by the rate of thrombin formation and the total amount of formed thrombin is measured by means of chromogenic or fluorescence methods (Devreese et al., 2007; Hemker et al., 2002). This thrombin potential in plasma can be assessed by different methods and the Calibrated Automated Thrombogram (CAT) applies a fluorogenic substrate. A chromogenic substrate is used in Behring Coagulation System (BCS). In both methods thrombin generation is activated by diluted recombinant tissue factor (TF), but in the BCS method a non-defined fibrin aggregation inhibitor is present. Both methods are applied in diagnostics. In CAT a calibration factor is measured in a plasma sample identical to that in which thrombin generation is being determined and the course of the calibration factor is assessed during the entire measurement (Figure 7). Thrombin generation assays seem to be useful in endogenous TF assessment (Ollivier et al. 2010; Stępień et al., 2007a).

4.2 Blood sampling for coagulation markers assessment

The most important think in coagulation diagnostics is to apply a reliably sampling method. To ensure accurate measurement samples must be collected in the circumstances under which false elevations of molecular markers of hemostatic and fibrinolytic activation will not occur. Thus, atraumatic antecubital venipuncture into vacutainer containing buffered sodium citrate is essential and the contamination with calcium or magnesium should be

avoided (van den Besselaar et al., 2007; Stegmar et al., 2007). To avoid activation of coagulation by tissue thromboplastin, each collection of citrated plasma should be preceded by a serum tube. Duration of needle puncture, rather than duration of tourniquet use, produced the greatest elevation in plasma levels of TAT and F1+2 (Omote et al., 2008).

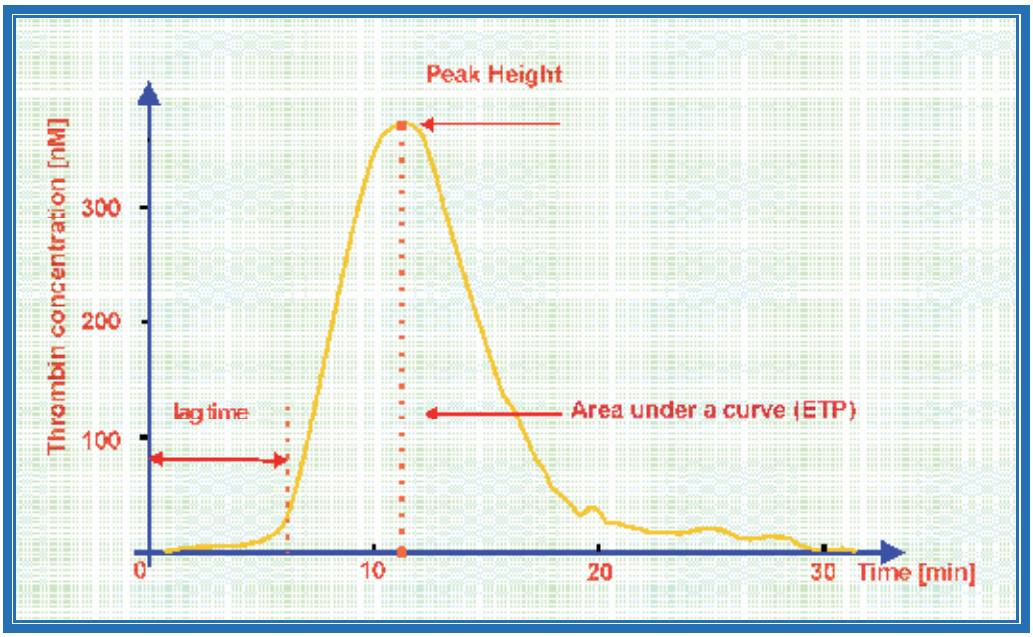

Fig. 7. The rate of thrombin formation is presented as the thrombin concentration against time curve. Three parameters are presented: lag time (T_{lag}), peak height (C_{max}) and endogenous thrombin potential (ETP).

4.3 Prognostic value of thrombin generation in cardiac events

Increased circulating levels of thrombin and its markers characterize ACS (Ardissino et al., 2003; Takano et al., 1991). Plasma F1+2, normally about 1 nM, is roughly 1.5-2-fold higher than observed in SA patients, reaching maximum values in AMI (Ardissino et al., 2001). U-shaped relationship between plasma prothrombin fragment 1+2 levels and the risk of developing cardiac death or renewed myocardial infarction was observed. Intermediate levels (1.5-1.9 nM) were associated with the lowest risk, whereas both higher (>1.9 nM) and lower (< 1.5 nM) values were associated with an increased risk (RR 1.56 [range 1.25 to 2.28] and RR 1.35 [range 1.11 to 1.86], respectively) (Ardissino et al., 2003). Hypercoagulable state measured as thrombin-antithrombin complexes (TAT) levels and as calibrated automated thrombogram reflects vascular impairment in CAD patients (Stępień et al., 2007a). It was observed that high TAT levels may predict mortality in chronic heart disease group after adjustment for classic risk factors (Marcucci et al., 2006). In empirical reconstruction, simulated maximum thrombin levels ($p<0.01$) and rates ($p<0.01$) were 50% higher with ACS while the initiation phases of thrombin generation were shorter than in patients with stable CAD (Brummel-Ziedins et al., 2008). Elevated levels of thrombin derivatives are associated with clinical risk factors for stroke (Lane et al., 1983; Takano et al., 1991). Elevated thrombin concentration reflects hypercoagulable state in patients with hypertension (Hoeper et al,

1998; Kłoczko et al., 1996), hyperglycaemia (Undas et al., 2008) and hypercholesterolemia (Wada et al., 1992; Sanguigni et al., 2005; Undas et al., 2005).

5. Conclusion

Endothelial and platelets activation leading to cardiovascular complications can be evaluated quantitatively by measurement of plasma levels of circulating MPs. Moreover, a multiple biomarkers strategy that includes bone remodeling biomarkers (OPG, OPN) and clotting properties can provide better risk stratification of cardiovascular events. Development and discovery of new biomarkers may improve clinical assessment of patients who might benefit more from treatment. Synergistic strategies in diagnostics seem to be more advantageous than routine method in prognosis and patients' management.

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Biomarkers and Coronary Atherosclerotic Burden and Activity as Assessed by Coronary Angiography and Intra-Coronary Imaging Modalities

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1. Introduction

Coronary artery disease (CAD) is one of the leading causes of death worldwide and it is expected that the rate of CAD will accelerate in the next decade due to overall aging of population and increases in the prevalence of cardiovascular risk factors (type 2 diabetes, obesity, metabolic syndrome) in younger generations (Amborsioni et al., 2003). The mortality associated with atherosclerotic disease is mainly related to the acute coronary syndromes (ACS), including acute myocardial infarction (AMI), unstable angina (UA) pectoris and sudden cardiac death. Inflammation plays a central role throughout the entire disease progression, and it lies at the root of atherosclerosis initiation, progression and its complications (Bonow et al., 2002).

However, recent data support the notion that plaques within the coronary circulation become "more severe" or at "high-risk" (vulnerable plaque) in response to a wide array of local and systemic influences, both inflammatory and non-inflammatory (Alsheikh-Ali et al., 2010; Finn AV et al., 2010). Indeed, plaques may have similar structural features and morphologic assessment, but may differ in their biology, their activity, and thus their likelihood of advancing toward clinical complications. Advances in the understanding of the pathogenesis of coronary atheroslcerosis have stimulated development of novel biomarkers, and expanded their role in the different spectra of their underlying pathophysiology (Hochholzer et al., 2010). In this regard, an emerging approach is represented by the assessment of plaque burden, morphology, and remodeling with in vivo atherosclerosis imaging and its correlation to novel biomarkers (Prati & Zimarino, 2010).

In the past, invasive coronary angiography (CAG) has been the only diagnostic procedure for identifying coronary atherosclerosis. However, newer intracoronary imaging modalities have been developed allowing a more accurate and precise evaluation of coronary atherosclerotic lesions, with regard to specific morphologic criteria, especially concerning vulnerability. Intravascular ultrasound (IVUS) is a catheter-based technology that allows for assessment of vessel wall thickness and structure while coronary angioscopy also allows to visualize the vessel lumen (Kaneda et al., 2010). More recently, optical coherence

tomography (OCT) has been introduced as an invasive technique that provides images of vessel wall morphology and plaque characteristics (Prati et al., 2009; Bezerra et al., 2009).
By using these novel techniques, an array of biomarkers assessing plaque growth and destabilization, myocardial stress and ischemia, along with inflammatory processes, has been developed, including cellular adhesion molecules, cytokines, and proatherogenic enzymes. Importantly, different biomarkers may look at different phases of coronary atherosclerotic disease and CAG, along with IVUS, angioscopy and OCT, may be of great clinical utility in assessing the role of these new biomarkers in coronary atherosclerosis pathophysiology (Libby & Theroux, 2005; Hansson, 2005).
The present chapter will summarize our current understanding of inflammatory and non-inflammatory biomarkers, validated with intracoronary imaging modalities, their presumed pathophysiological role in coronary atherosclerosis and the clinical evidence that supports their prognostic importance.

2. Soluble biomarkers

A dynamic inflammation model has supplanted the previously held view of atherosclerosis as a passive deposition of debris in the arterial wall (Libby, 1995a, 1995b; 2001; 2002; Libby & Theroux, 2005). Numerous mediators contribute to atherogenesis, including chemokines, cytokines, growth factors, proteases, adhesion molecules, hemostasis regulators, and receptors, and their interactions may regulate plaque progression and instability (Blake & Ridker, 2002;).
A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes or pathogenic processes or as a physiologic response to a therapeutic intervention. In clinical medicine, biomarkers are routinely used in disease diagnosis, prognosis, ongoing clinical decision-making, and follow-up to assess effects of therapy. A framework for the validation of biomarkers was proposed by Boissel et al. (Boisell et al., 1992) and subsequently adapted by Espeland et al. (Espeland et al., 2005) in a discussion of the usefulness of carotid ultrasound to measure the clinical efficacy of lipid-lowering medications. Espeland et al. (Espeland et al., 2005), in modifying the terminology of Boissel et al. (Boisell et al., 1992), described clinical and statistical characteristics that a biomarker should have to be considered a surrogate marker of efficacy in atherosclerotic disease. Importantly, the clinical criteria outlined for validating surrogate markers are efficiency, linkage, and congruence. Taken together, these data suggest that four main factors are related to the development of effective biomarkers (Fry, 2010): 1. Analytical validity: the accuracy and precision with which a particular biomarker is identified by the Proposed test; 2. Clinical validity: the accuracy with which a test identifies or predicts a patient's clinical status; 3. Clinical utility: assessment of the risks and benefits, such as cost or patient outcome, resulting from the test; 4. Ethical, legal or social implications.
Several inflammatory and non-inflammatory soluble biomarkers have been widely investigated during the last two decades in the setting of CAD, including both stable and unstable pattern of clinical presentation. On the one hand, many of these biomarkers have been associated with the presence of CAD; on the other hand, they have been associated with the presence of "vulnerable" plaque, thus with an increased risk of cardiac death and non-fatal AMI, eventually stressing both their diagnostic and prognostic role. In this regard, intracoronary imaging modalities may help to understand the pathological role exerted by soluble biomarkers in the pathogenesis of CAD.

3. Inflammation

Recent research has shown that inflammation plays a key role in CAD and other manifestations of atherosclerosis. Immune cells dominate early atherosclerotic lesions, their effector molecules accelerate progression of the lesions, and activation of inflammation can elicit ACS.

3.1 C-reactive protein

C-reactive protein (CRP) was identified more than five decades ago as an acute-phase reactant that was capable of activating the complement system (Tillet & Francis, 1930; Abernathy & Avery, 1941). It was subsequently noted to be one of a number of acute-phase biomarkers, along with the erythrocyte sedimentation rate and complement, which was elevated in AMI (Boltax & Fischel, 1956). After the development of more sensitive, reliable, and readily available assays for CRP, a number of epidemiologic studies were conducted to assess the value of CRP in predicting cardiovascular risk (Ridker et al., 1997, 2001, 2002, 2005a, 2005b; Danesh et al., 2004; Koenig et al., 2004; Cushman et al., 2005). In a post-mortem study of 302 autopsies of men and women with atherosclerosis, median CRP levels were higher with acute plaque rupture than in stable plaques or controls (Burke et al., 2002). The levels correlated with the staining intensity for CRP in macrophages and the lipid core of plaques, and it increased with the number of thin cap atheromas found in coronary arteries. Moreover, plasma CRP levels at the upper end of the reference range in apparently healthy men and women, in the absence of other sources of inflammation, has been previously correlated with increased risk of future cardiovascular events, including MI, peripheral vascular disease with intermittent claudication and stroke (Ridker, 2001). These data support the view that systemic CRP accurately reflects the number of vulnerable atherosclerotic plaques. Liuzzo et al. (Liuzzo et al., 1994) reported that patients with UA and elevated levels of CRP (>3 mg/dl) had higher rates of death, AMI and need for revascularization compared to patients without elevated levels. Of note, this increased risk may be evident as early as 14 days after presentation (Morrow et al., 1998). Importantly, the U.S. Centers for Disease Control and Prevention and the American Heart Association have both advocated the use of CRP as an adjunct to global risk prediction among those at intermediate risk for CAD (Pearson et al., 2003). Thus, among non-specific markers of inflammation, CRP is the most investigated and has widely been associated with an increased risk of future cardiovascular events both in primary and secondary prevention studies (Willerson et al., 2004; Ridker et al., 1997, 2001, 2005a, 2005b; Haverkate et al., 1997). However, the association of CRP levels with angiographic coronary atherosclerotic burden is controversial, whereas its association with coronary instability has consistently been reported in several studies.

More than 15 years ago, Mori et al. (Mori et al., 1995) reported that baseline CRP levels were associated with the severity of CAD, as determined by the Gensini score and this association remained significant even after adjustment for body-mass index, smoking history, hypertension, and total cholesterol. Subsequently, Tataru et al. (Tataru et al., 2000) found a correlation between CRP levels and the number of diseased vessels in patients with a previous history of MI. Zebrack et al. (Zebrack et al., 2002) enrolled 2.554 patients with angina but without AMI who underwent CAG (1.848 patients had CAD and 706 patients did not). CAD was quantified in 5 ways and combined for a CAD score and CRP was measured in all patients who were followed for up to 5 years for death or MI. Interestingly,

CRP correlated with the extent of CAD, but correlation coefficients were low (0.02 to 0.08). Of angiographic measures, the CAD score best predicted future events. CRP retained predictive value within each quintile of CAD score. CRP and CAD independently and additively contributed to the risk prediction: low CRP and lowest CAD score were associated with lowest risk, and high CRP and highest CAD score were associated with the highest risk, with a 10-fold difference between extremes (2.5% vs. 24%). In conclusion, they demonstrated that CRP correlated with extent of CAD, but the degree of correlation was low, however, both severity/extent of CAD and CRP were independent and additive predictors of risk. Azar et al. measured CRP levels in 98 patients with stable angina or an abnormal stress test who were referred for diagnostic CAG. They showed that the CRP level did not correlate with the extent and severity of coronary narrowing measured by angiography (Azar et al., 2000). In the acute setting of CAD, Niccoli et al. (Niccoli et al., 2008) studied 97 consecutive patients with UA undergoing CAG and CRP was measured by an ultrasensitive nephelometric method. Atherosclerotic disease severity and extent were assessed by angiography using the Bogaty score. No significant correlation was found between baseline CRP serum levels and angiographic measures of atherosclerotic disease severity and extent. In this study, the authors demonstrated that in patients with UA, CRP serum levels and coronary atherosclerosis are not correlated, but they were both independently associated with a worse outcome at 6-month follow-up. In UA patients, the association between atherosclerotic background and CRP may be altered by the increase in CRP associated with the acute phase response. On the other hand, in vitro studies suggest that the proatherogenic role of CRP is associated with very high levels of serum CRP (up to 50 mg/L), much higher than that observed in patients with CAD or ACS (Devaraj et al., 2003).

Erren et al. (Erren et al., 1999) previously evaluated 147 patients undergoing semiquantitative CAG and they measured CRP, serum amyloid A (SAA), and the proinflammatory cytokine IL-6; the active and total fractions of the anti-inflammatory cytokine transforming growth factor-b (TGF-b); the macrophage activation marker neopterin; and the infection marker procalcitonin. Compared with 62 patients without either CAD or peripheral artery disease, 57 patients with CAD but no peripheral artery disease showed greater median CRP and IL-6 levels and a lower level of active-TGF-b. Moreover, CRP, IL-6, and neopterin levels showed a positive and the active TGF-b level a negative correlation with the extent of coronary atherosclerosis. Compared with these 57 patients with CAD alone, 15 patients with peripheral artery disease and CAD had higher median levels of SAA, IL-6, neopterin, and total TGF-b. However, these strong univariate associations of markers of inflammation and atherosclerosis were lost in multivariate analysis once age, sex, and HDL cholesterol or fibrinogen were taken into account. Taken together, these data suggest that increased plasma levels of CRP, SAA, IL-6, TGF-b, neopterin, and procalcitonin constitute an inflammatory signature of advanced atherosclerosis and are correlated with the extent of disease but do not provide discriminatory diagnostic power over and above established risk factors. Indeed, as previously mentioned, Lee et al. (Lee et al., 2006) reported on the associations between 3 plasma markers of low-gade inflammation, such as CRP, IL-6 and SAA protein and total homocysteine with CAD and death in a Canadian angiography cohort of 1.117 patients followed for 8.5 years. They found no significant association between elevated levels of these biomarkers and CAD (defined as the presence of any lesion with stenosis greater than 10%), but they did find significant independent associations of

elevated IL-6 (a key pro-inflammatory cytokine) and homocysteine levels with both CAD-related and all-cause death. These findings do not support the hypotheses that these biomarkers play a causal role in atherogenesis. The importance of this study is that no previous studies have examined such associations in a large, prospective angiographic cohort in which CAD was defined using sensitive criteria. However, it is worth noting that when Lee et al. used the definition of CAD as the presence of any lesion of 50% stenosis (which was developed for assessing suitability for cardiac surgery or angioplasty), they observed significantly higher CRP and IL-6 levels in the CAD group (Lowe et al., 2005).

On the other hand, several data suggest that CRP may be a marker of plaque activity (Liuzzo et al., 1994). In a previous report, Arroyo-Espliguero et al (Arroyo-Espliguero et al., 2004) sought to assess whether CRP was an independent predictor of future cardiovascular events after adjustment for CAD severity and whether CRP levels correlate with number of angiographically complex coronary artery stenosis. They studied 825 consecutive anginal patients (700 with chronic stable angina and 125 with ACS without ST-segment elevation). The composite endpoint of non-fatal AMI, hospital admission with class IIIb UA and cardiac death was assessed at 1 year follow-up. CRP levels were higher in chronic stable angina patients with the combined end-point ($p=0.03$) after adjustment for number of diseased coronary arteries. CRP was also significantly higher in patients with ACS compared to stable patients ($p=0.004$) and correlated with number of complex angiographic stenoses ($p=0.01$). Interestingly, in this report, CRP levels predicted future cardiovascular events independently of CAD severity and correlated with number of angiographically complex coronary artery stenosis in patients with ACS, thus suggesting that CRP levels may be a marker of atheromatous plaque vulnerability and CAD activity. This concept was endorsed by studies showing that CRP levels correlate with cardiovascular risk in ACS patients, thus giving further support to the increasingly accepted hypothesis that CRP is not merely a marker of systemic inflammation but may also be a pathogenic mechanism in ACS (Yeh et al., 2001; Pasceri et al., 2000; Zwaka et al., 2001). Additionally, the relation of serum CRP concentrations with adverse cardiovascular events during follow-up in patients with stable angina also suggests that clinical "stability" does not always indicate atheromatous plaque stability (van der Wal et al., 1999). Geluk et al. (Geluk et al., 2008) recently evaluated the population based on the Prevention of Renal and Vascular Endstage Disease (PREVEND) study. Of note, 8.139 subjects without previous documented CAD were followed for the incidence of CAD and coronary events from 1997 to 2003. In the prospective PREVEND study of subjects without previous documented CAD, CRP levels at baseline were associated with angiographic characteristics and clinical consequences of plaque instability during follow-up. Because CRP was weakly correlated with angiographic plaque burden, it was suggested that CRP is stimulated not only by the extent of atherosclerosis but, importantly, by other factors. Indeed, they postulated that CRP is a measure of inflammed, unstable atherosclerotic plaque (both angiographically visible and occult), whereas angiography indicates the extent of visible stable and unstable occlusive plaque. The value of CRP in predicting future death or MI was apparent in all ranges of CAD severity. Those with extensive CAD were at relatively high risk of death or MI regardless of CRP levels. However, in the presence of a low to moderate risk angiogram (or even a normal angiogram), CRP became particularly useful in distinguishing patients at substantially lower versus higher risk for death or MI. A particularly high risk was observed in subjects with lower CAD scores but highly elevated CRP despite a lower prevalence of all traditional

risk factors in these patients with low/moderate CAD scores (i.e., lower cholesterol levels, absence of diabetes, hypertension). Because these patients may be considered to have insignificant CAD and hence to be at low risk for MI, less aggressive medical therapy may be offered to them in comparison to patients with more extensive CAD, thus possibly underestimating their cardiovascular risk (Pearson et al., 2003; Lowe, 2005; Beaglehole & Magnus, 2002; Greenland & O'Malley, 2005).

With the introduction of IVUS, new important data regarding CRP levels and CAD burden and, especially, plaque morphology have been provided, shedding some lights on CRP diagnostic and prognostic role in CAD patients. Sano et al. (Sano et al., 2003) investigated the relation between lesion morphology as seen under pre-intervention IVUS and CRP in 90 consecutive patients presenting with AMI. Patients were divided into an elevated CRP group (≥3 mg/L) or a normal CRP group on the basis of serum CRP levels. There were no differences in patient characteristics or angiographic findings. Interestingly, they observed significantly more plaque rupture in the elevated CRP group than in the normal CRP group (70% versus 43%, p=0.01). A multivariate logistic regression model revealed that the presence of ruptured plaque alone correlated with elevation of serum CRP (p=0.02; odds ratio, 3.35; 95% CI, 1.22 to 9.18). These data further suggest that in the setting of AMI, elevated CRP levels may reflect the inflammatory activity of a ruptured plaque. Additionally, Sano et al. (Sano et al., 2003) found that in the normal CRP group, 44% of analyzed lesions were non–rupture-type lesions. Indeed, several pathological reports showed that AMI may be caused not only by plaque rupture but also by plaque erosion, which is a major substrate for coronary thrombosis in AMI (van der Wal et al., 1994; Farb et al., 1996; Arbustini et al., 1999). A more recent IVUS study (Otake et al., 2008) investigated the relation between plasma CRP and adiponectin and coronary plaque components in patients with ACS. Ninety-three culprit plaques (ACS n=50, non-ACS n=43) and 56 nonculprit plaques (ACS n=28, non-ACS n=28) were analyzed using virtual histology (VH)-IVUS to examine relations among plasma CRP, adiponectin, and ratios of each coronary plaque component. Plasma adiponectin was significantly lower and plasma CRP was significantly higher in patients with than without ACS. Notably, culprit plaques in patients with ACS had greater amounts of necrotic core plaque than those in patients without ACS. There was an inverse relation between CRP and adiponectin with regard to necrotic core ratio in both culprit and nonculprit lesions in patients with ACS, but not those without ACS. Thus, once again, increased plasma CRP levels might be related to the progression of ACS. Zhang et al. (Zhang et al., 2006) performed IVUS examination in 152 patients with confirmed CAD before percutaneous coronary intervention and measured CRP levels in all patients. Unstable and ruptured plaque were found more frequently in patients with AMI and UA. The levels of plasma CRP were higher in ruptured plaque group. CRP >8.94 mg/L was used to predict ruptured plaque with a ROC curve area of 0.76 (95% confidence interval, 67.0%-85.8%), sensitivity of 71.8%, specificity of 77.0% and accuracy of 69.2% (p<0.01). Chen et al. (Chen et al., 2007) aimed at investigating whether combined IVUS and measurements of serum inflammatory biomarkers could predict coronary plaque ruptures in patients with angina pectoris. The study population consisted of 20 patients with stable angina and 40 patients with UA. IVUS was performed in the 2 groups to measure intima-media thickness, the plaque acoustic density of the common carotid arteries, and the flow-mediated dilation of the brachial arteries. Serum lipid profile and inflammatory biomarkers were measured in all patients. Of 139 coronary artery plaques identified by IVUS, 48

plaques (9 in stable angina and 39 in UA) developed ruptures. Among measured parameters, they found that the values of carotid intima-media thickness, coronary external elastic membrane area, plaque area, plaque burden, plaque eccentric index and remodeling index, and serum CRP were significantly higher in UA patients than in stable angina patients ($p<0.05$ to 0.01). Additionally, they found that soluble intercellular adhesion molecule-1, and soluble vascular cell adhesion molecule-1 were significantly higher in UA patients ($p<0.05$). However, of these parameters, carotid intima-media thickness, serum CRP, and the coronary remodeling index, only, were found to be significant predictors of coronary plaque rupture, with odds ratios of 9.51 (95% confidence interval 1.29 to 21.81), 3.02 (95% confidence interval 1.01 to 7.65), and 0.01 (95% confidence interval 0.00 to 0.34), respectively. Taken together, these data suggest the intriguing possibility that the association between CRP and risk of cardiovascular events is mediated by unstable plaque phenotype, raising the possibility that CRP is not only a marker of vascular inflammation but also of plaque disruption. However, Park et al. (Park et al., 2010) recently enrolled 188 patients who underwent 3-vessel VH-IVUS with peripheral blood sampling, including CRP levels measurements. VH-TCFA was defined as a necrotic core >10% of plaque area in the presence of >40% plaque burden. There were 38 patients with ruptured plaque and 150 patients without (107 patients with VH-TCFA, 43 patients without VH-TCFA) in culprit/target lesions. In the present study there were no significant differences in the CRP level between patients with and without VH-TCFA in the culprit/target lesions. However, it is worth mentioning that when lesions are analyzed post-rupture, after the necrotic core may have embolized, they most often appear "dark-green" and will be classified as fibrotic plaque rather than necrotic core in VH-IVUS analysis, and have a reduced calculated relative size of the necrotic core. Therefore, when the relationships between each plaque characteristic and plasma biomarker levels, including CRP, were evaluated in the study performed by Park et al. (Park et al., 2010), ruptured plaques were excluded to avoid the possibility of incorrect VH-IVUS interpretation and this may partially explain the different results between studies.

Takano et al. (Takano et al., 2005) have evaluated, using coronary angioscopy, changes of ruptured plaques in non culprit lesions in living patients and ability of CRP to predict disease activity of the plaque ruptures, also shown in other previous studies (Ishibashi et al., 2002). They have identified by angioscopy 48 thrombi in 50 ruptured coronary plaques in nonculprit lesions in 30 patients with mean angioscopic follow-up period was 13 +/- 9 months. They have shown that ruptured plaques in nonculprit lesions tend to heal slowly with a progression of angiographic stenosis and the serum CRP level in patients with healed plaques was lower than that in those without healed plaques, although in patients with healed plaques did not significantly decrease from baseline to follow-up. Of note, at univariate logistic regression, serum CRP levels at follow-up were predictors of healing in nonculprit ruptured plaques, while, a multivariate logistic regression analysis, the authors showed that serum CRP levels at follow-up were not independent predictors of plaque healing. Thus, the power of serum levels of CRP in predicting disease activity should also be confirmed. Furthermore, the same authors have previously reported that statin therapy reduces the serum CRP level and angioscopic complexity of the plaques (the existence of the thrombus and the irregularity of the plaque) in nonculprit lesions (Takano et al., 2003). In this study, data from univariate logistic regression analyses indicated that both statin therapy and serum CRP level at follow-up are considered predictors of healing in nonculprit ruptured plaques. However, a multivariate logistic regression analysis showed

that neither statin therapy nor serum CRP level at follow-up is an independent predictor of plaque healing. These findings, however, should be confirmed in larger study populations.

A study by Tanaka et al. (Tanaka et al., 2008) enrolled 43 consecutive ACS patients (with or without ST-segment elevation) undergoing OCT assessment and presenting with a ruptured plaque at the culprit site. Patients were divided into a rest group and an exertion group on the basis of their activities at the onset of ACS. Of interest, the thickness of the broken fibrous cap correlated positively with activity at the onset of ACS. The culprit plaque ruptured at the shoulder more frequently in the exertion group than in the rest group (rest 57% versus exertion 93%, $p=0.014$). The thickness of the broken fibrous cap in the exertion group was significantly higher than in the rest-onset group (rest onset: 50 μm [interquartile median 15 μm]; exertion: 90 μm [interquartile median 65 μm], $p<0.01$). These data suggest a thin-cap fibroatheroma (TCFA) is a lesion predisposed to rupture both at rest and during the patient's day-to day activity, and some plaque rupture may occur in thick fibrous caps depending on exertion levels. Moreover, this study demonstrated also an inverse relationship between fibrous cap thickness and serum levels of CRP ($r=-0.31$, $p<0.01$). Li et al. (Li et al., 2010) investigated in stable and unstable patients the relationship between plaque morphology assessed by OCT and serum levels of several inflammatory biomarkers, such as CRP, IL-18, TNF-α, white blood cell count. This study demonstrated that the plasma levels of inflammatory factors and white blood cell count were correlated inversely with fibrous cap thickness ($r=0.775$ for CRP, $r=-0.593$ for IL-18, $r=-0.60$ for TNF-α, and $r=-0.356$ for white blood cell count). Patients with TCFA (cap thickness less than 65 micron) had higher plasma levels of inflammatory factors as well as WBC counts than those with thicker fibrous caps. ROC curves for CRP, IL-18, TNF-α and white blood cell count, which displayed the capability of prediction about TCFA, showed the area under the curves were 0.95, 0.86, 0.79 and 0.70 ($p<0.05$), respectively. Meanwhile, in multivariate logistic regression analysis, CRP was the only significant independent predictor of TCFA. Therefore, although IL-18, TNF-α and white blood cell count could also show similar characteristics, their predictive value was weak compared with CRP.

Kashiwagi et al. (Kashiwagi et al., 2009) evaluated the relationship between coronary arterial remodelling assessed by IVUS, fibrous cap thickness assessed by OCT and CRP concentrations in patients with ACS. Positive remodelling was defined as remodelling>1.05, intermediate remodelling as remodelling=0.95–1.05, and negative remodelling as remodelling<0.95. On the basis of the IVUS findings, patients were divided into 2 groups (positive remodelling group, intermediate remodelling/negative remodelling group). Lipid-rich plaques and TCFA were more frequent in the positive remodelling group than in the other group and intermediate remodelling inversely correlated with the thickness of fibrous cap. Levels of CRP were higher in the positive remodelling group than in the other group and were higher in patients with a thin fibrous cap, suggesting that the inflammatory process may simultaneously contribute to both plaque growth and plaque instability.

Kitabata et al. (Kitabata et al., 2010) investigated in stable and ACS patients the relationship between the presence of microchannels in coronary plaque assessed by OCT and serum CRP levels. Microchannel was defined as a no-signal tubuloluminal structure on the cross-sectional OCT image. Microchannels were found in 24 (38%) of the 63 enrolled patients and patients were divided into 2 groups according to the presence or absence of microchannels. The frequency of plaque rupture tended to be greater in the microchannel group (50% vs

28%, p=0.11). The thickness of the fibrous cap (median 60 vs 100 micron, p=0.001) was significantly lower in the patients with microchannels, and significant differences were found in the frequency of thin-cap fibroatheroma (54% vs 21%, p=0.012) and positive remodeling (67% vs 36%, p=0.02) between the 2 groups. CRP levels in the microchannel group were significantly greater than those in the no-microchannel group (median 0.27 vs 0.13 mg/dl, p=0.015). Moreover, increased microchannel counts were associated with greater high-sensitivity CRP levels (p=0.01). Takarada et al. (Takarada et al., 2010) enrolled 82 Non-ST-elevation-ACS patients undergoing OCT assessment of culprit lesion at baseline and after 9 months, evaluating morphological changes of coronary plaque and changes in serum levels of CRP. Of interest, the change in fibrous cap thickness had a significant positive correlation with changes in CRP levels ($r=0.44$, $p<0.01$). A recently published study by Ferrante et al. (Ferrante et al., 2010) enrolled 25 consecutive ACS patients undergoing percutaneous coronary intervention and OCT assessment, evaluating the relationship between plaque morphology and serum levels of myeloperoxidase (MPO) and CRP. OCT classified the culprit lesion as ruptured in 18 (72%) or eroded in 7 patients (28%) and detected intraluminal thrombus in 89% of ruptured plaques and 100% of eroded plaques. CRP levels did not differ significantly between patients with an eroded plaque and those with a ruptured plaque (median, 11.3 mg/L; 25th to 75th percentile, 1.3 to 28.5 versus median, 3.9 mg/L; 25th to 75th percentile, 1.3 to 17.8; p=0.76, respectively). On the contrary, a study by Bouki et al. (Bouki et al., 2010) showed that higher levels of serum CRP and IL-18 were found in patients with plaque rupture vs. those with no plaque rupture (median value: 19.2mg/L vs. 1.6mg/L, $p<0.001$ and 219.5pg/ml versus 127.5pg/ml, p=0.001 respectively), and TCFA versus those without TCFA (median value: 15.2mg/L versus 1.6mg/L, p=0.004 and 209.0pg/ml versus 153.2pg/ml, p=0.03, respectively). Moreover, serum CRP was the only independent predictor of plaque rupture (p=0.02, odds ratio 1.1, 95% confidence interval 1.0 to 1.2), and a cut-off value of CRP>4.5mg/L could detect ruptured plaque with a sensitivity of 91.7% and a specificity of 77.8%. Taken together, these data show that the association of CRP levels with coronary atherosclerotic burden is still controversial, whereas its association with coronary instability has consistently been reported in several studies, by using different intracoronary imaging modalities. Although initially considered only a marker of inflammation, CRP itself has been shown to possess proinflammatory and pro-atherogenic properties. It stimulates endothelial cells to express adhesion molecules and secrete cytokines (Pasceri et al., 2000, 2001) and it decreases the expression of endothelial nitric oxide synthase (Verma et al., 2002; Venugopal et al, 2002). CRP accumulates in macrophage-rich regions of nascent atherosclerotic lesions and activates the macrophages to express cytokines and tissue factor, while enhancing macrophage uptake of LDL (Zwaka et al., 2001). It also amplifies pro-inflammatory effects of several other mediators including endotoxin (Nakogomi et al., 2000; Burke et al., 2002). These biological properties exerted by CRP may be held responsible for the detrimental role of this acute-phase reactant which may participate in the unstable patter of CAD.

To date, there are no widely accepted and established markers of inflammation for cardiovascular disease and no known therapeutic measures to modulate coronary inflammation. CRP is currently the best marker of inflammation, and in addition to weight loss, exercise, and smoking cessation, statins are the best therapeutic option to modulate inflammation. New avenues for diagnosing and treating coronary inflammation should be investigated in larger and randomized trials.

3.2 Fibrinogen

With improved understanding of the critical role of inflammation in atherothrombosis, attention has been focused on the circulating markers of inflammation, including fibrinogen (Ross, 1993; 1999; Lind, 2003). Evidence has been accumulated from several prospective studies that showed that high levels of fibrinogen were associated with an increased risk of coronary, cerebral and peripheral disease (Maresca et al., 1999; Danesh et al., 2004; Tamam et al., 2005). In a prospective study, Espinola-Klein et al. (Espinola-Klein et al. 2007) enrolled 720 patients preceding CAG. Patients were compared with regard to atherosclerotic burden and classified as follows: no clinically significant stenosis (n=57, 7.9%), CAD only (n=362, 50.3%), CAD with peripheral atherosclerosis (=multi-vascular atherosclerosis, n=301, 41.8%). They found a significant association between elevation of CRP and atherosclerotic burden (control: 2.6 (1.4–6.8) mg/l; CAD: 4.5 (2.1–12.1) mg/l; multi-vascular: 5.2 (2.0–15.6) mg/l, $p<0.001$). Results were similar with regard to IL-6 according to the extent of atherosclerosis (control: 7.8 (4.0–13.2) ng/ml; CAD: 11.4 (5.2–22.8) ng/ml; multi-vascular: 12.5 (5.8–24.5) ng/ml, $p<0.001$). The strongest association was registered, indeed, between elevation of fibrinogen and extent of atherosclerosis (control: 296.0 (256.0–326.5) mg/dl; CAD: 329.0 (281.8–399.3) mg/dl; multi-vascular: 351.0 (289.5–434.5) mg/dl, $p<0.0001$). Additionally, follow-up data after a median of 6.5 years were available in 719 patients (99.9%), and 75 patients (10.4%) died from cardiovascular causes. Presence of multi-vascular atherosclerosis, elevation of IL-18 and elevation of fibrinogen were independently related to cardiovascular death in a fully adjusted model Hazard ratio (95% confidence interval) 2.0 (1.2-3.5) for presence of multi-vascular atherosclerosis ($p<0.01$), 2.2 (1.2-3.9) for high fibrinogen ($p<0.01$) and 2.8 (1.6-4.9) for high IL-18 ($p<0.0001$). Fibrinogen was achieved as independent predictor for both, mortality and atherosclerotic burden, whereas IL-18 was not related to atherosclerotic burden. These data suggest that fibrinogen was the only inflammatory marker with an independent association to the extent of atherosclerosis in the arterial vessel three. Previous investigations found also a strong interdependence between high serum fibrinogen levels and severe peripheral occlusive disease, indicating advanced atherosclerosis (Wattanakit et al., 2005). Presumably, the predictive value of high fibrinogen level is caused by the association to high atherosclerotic burden which is predictive for a worse prognosis itself. Concordantly, Hoffmeister et al. (Hoffmeister et al., 2001) previously conducted a case-control study to assess the association between various markers of inflammation and the presence and severity of chronic stable CAD. They included 312 clinically stable patients with angiographically documented CAD, and the severity of CAD was evaluated by 3 coronary scoring systems: the clinical 1- to 3-vessel disease score, the American Heart Association extension score (1 to 15 segments), and the Gensini score. Fibrinogen levels were highly significantly elevated ($p<0.005$) in patients with stable CAD compared with controls. After multivariable adjustment by means of logistic regression analysis, the association between CAD and fibrinogen remained substantial. However, no association between fibrinogen levels and any of the coronary scores applied was found. Finally, Memon et al. (Memon et al., 2006) measured plasma fibrinogen levels in 138 patients with angiographically assessed CAD and in 183 healthy subjects matched according to age and gender. According to the number of significantly stenosed (>or=50%) vessels, the patients were classified in four groups: those without stenosis (0-vessel disease) and those with 1, 2 or 3-vessel disease. Fibrinogen levels were significantly higher in patients than in controls ($p<0.001$). Although fibrinogen levels tended to increase with the number of stenotic vessels, the differences were not statistical significant.

Taken together these data suggest that fibrinogen can be discussed as a serological marker of high atherosclerotic burden within arterial vessel tree and may be a screening marker to identify patients that should be evaluated carefully for atherosclerotic manifestations.

3.3 Eosinophil cationic protein

Leukocyte recruitment and expression of proinflammatory cytokines characterize all steps of atherothrombosis (Libby & Theroux, 2005). Recent observations suggest that eosinophils may play a role in coronary atherosclerosis. Indeed, prospective studies have consistently shown an association between eosinophil count and increased risk for future cardiovascular events (Prentice et al., 1982; Lee et al., 2001). Furthermore, eotaxin, an eosinophil-specific chemoattractant, is overexpressed in human atherosclerotic lesions (Haley et al., 2000) and patients with CAD show higher circulating levels of eotaxin as compared to healthy controls (Economou et al., 2001; Emanuele et al., 2006). Accordingly, a nonconservative polymorphism in the eotaxin gene (Zee et al., 2004) together with sequence variants affecting eosinophil count (Gudbjartsson et al., 2009) have recently been associated with an increased risk of myocardial infarction. Eosinophil cationic protein (ECP) is a zinc-containing, highly cationic protein, stored in the peroxidase-positive and negative eosinophil granules which is secreted through priming by various triggers, such as immunoglobulins and complement components (Venge et al., 1999). Several studies have shown that the measurement of ECP in most biological fluids may be used as a marker of eosinophil activity and turnover, and that increased ECP serum levels are related to the presence, activity and severity of asthma, atopic disorders, and other immune diseases, such as rheumatoid arthritis, psoriasis, and adult celiac disease (Hällgren et al., 1991). In this regard, the role of ECP in CAD has been recently assessed (Niccoli et al., 2010). One-hundred and ninety-eight consecutive anginal patients with angiographic evidence of CAD (stable angina or non-ST-elevation-ACS), or with angiographically normal coronary arteries were enrolled. The severity of CAD was graded according to Bogaty's score and coronary lesion morphology was defined as smooth or complex. Baseline ECP and high sensitivity CRP were measured in all patients. ECP levels were significantly higher in stable angina patients ($p<0.001$) and non-ST elevation-ACS ($p=0.016$) compared to patients with normal coronary arteries, without significant difference between stable and unstable patients ($p=0.45$). Additionally, CRP levels were significantly higher in unstable patients compared to stable patients ($p=0.03$) and normal coronary arteries patients ($p<0.001$), without significant difference between stable and normal coronary arteries patients ($p=0.20$). The addition of ECP to main cardiovascular risk factors improved the area under the curve from 0.88 to 0.92, $p=0.007$ for the angiographic diagnosis of CAD; further addition of CRP increased the area to 0.94, $p=0.014$. At multiple linear regression analysis, ECP levels independently predicted CAD severity ($p=0.001$), whereas CRP levels independently predicted lesion complexity ($p=0.01$). Taken together, these data suggest that ECP is a marker of CAD and that different inflammatory biomarkers reflect different phases of atherosclerotic plaque evolution (Niccoli et al., 2010). Further studies should address the role of ECP in CAD on larger study populations.

3.4 Myeloperoxidase

MPO is a member of the peroxidise superfamily that is predominantly found in neutrophils, monocytes and tissue macrophages and is released upon their activation as a response to

various stimuli (Nicholls et al., 2005). The enzyme is part of an innate host defence that acts on its substrate hydrogen peroxide of various sources augmenting its oxidative potential by producing potent oxidative species capable of modifying various cellular components by chlorination, nitration and cross-linking (Podrez et al., 2000; Heinecke et al., 2003). Demonstrations that MPO and its oxidation products are enriched within human atheroma (Daugherty et al., 2004) has increased interest in this enzyme in directions such as its participation in the promotion of atherosclerosis (Hazen et al., 1997), destabilization of atherosclerotic plaque (Sugiyama et al., 2001, 2004; Naruko et al., 2002) and in the possibility that MPO can serve as a biomarker to predict future adverse events in patients with CAD (Baldus et al., 2003; Brennan et al., 2003). Clinical studies have shown that plasma levels of MPO are increased in patients with stable CAD (Zhang et al., 2001) , ACS (Baldus et al., 2003; Brennan et al., 2003), and ST-segment elevation AMI (Mocatta et al., 2007; Khan et al., 2007). Other studies have proven that neutrophils, the main source of MPO release, are activated in ACS; however, their activation has not been linked with ischaemia/reperfusion per se (Biasucci et al., 1996). Furthermore, some observational studies have shown that baseline MPO plasma levels are predictive of future adverse coronary events in patients with acute chest pain (Brennan et al., 2003) or ACS (Baldus et al., 2003; Cavusoglu et al., 2007) regardless of troponin levels.

Ndrepepa et al. (Ndrepepa et al., 2008) recently conducted a case–control study in which 874 patients with angiographically proven CAD were included. Cases included 680 patients with CAD (382 patients with stable CAD, 107 patients with non-ST-segment elevation-ACS and 191 patients with ST-segment elevation AMI), while controls included 194 subjects with normal coronary angiograms. MPO was measured using an enzyme immunoassay before angiography and heparin administration. MPO levels were significantly higher in cases as compared to controls ($p<0.001$). MPO levels were significantly higher in patients with AMI as compared to patients with stable CAD and with non-ST-segment elevation-ACS ($p<0.0001$). Elevated MPO level was associated with ACS with an area under receiver operating characteristic curve of 0.731 (95% confidence interval 0.692–0.770; $p<0.001$). Independent correlates of MPO level were ACS ($p<0.001$), CRP ($p=0.007$), creatinine ($p=0.026$), left ventricular ejection fraction ($p=0.027$, negative association) and smoking ($p=0.028$). Taken together, these data suggest that MPO level is elevated in patients with CAD and higher levels of MPO may be found with progression of CAD from stable CAD to non-ST-segment elevation ACS and to AMI. In a recent study, Wainstein et al. (Wainstein et al., 2010) have demonstrated that, in stable CAD, there was no association between MPO polymorphism and CAD severity, although a relationship was observed between plasma MPO levels and extent of CAD. de Azevedo et al. (de Azevedo et al., 2011) recently tested the hypothesis that MPO levels are higher in ACS patients with a greater extent of angiographic coronary involvement, by performing a cross-sectional study, examining high risk ACS patients who underwent CAG within 72 hours of the onset of symptoms and measuring their plasma MPO levels after sheath insertion. Gensini score was used to evaluate angiographic severity of CAD in 48 patients. Spearman's correlation coefficient did not show a significant association between MPO levels and Gensini scores ($r=0.2$; $p=0.177$). There was no correlation between MPO and age, hypertension, diabetes, leukocyte count, troponin I, CK-MB, TIMI risk score C4 and Gensini score in the multivariate analysis. These findings indicate that MPO expression may not be associated with anatomical severity of

coronary lesions in ACS. However, in a previous study (Düzgünçinar et al., 2008), 48 stable CAD patients with angiographically documented coronary lesions were enrolled and the authors demonstrated a weak, but statistically significant association between MPO levels and Gensini score (r=0.228; p=0.04). Of note, they found a stronger association between MPO levels and calcium scores by multislice computed tomography in 30 patients (p=0.02). In another study, MPO levels were considered independent predictors of multivessel disease in 389 unselected stable and unstable patients referred for CAG (Cavusoglu et al., 2006). On the other hand, Kubala et al. (Kubala et al., 2008) evaluated 557 clinically stable patients submitted to CAG and did not find a significant difference in MPO levels between patients with documented CAD (at least 1 vessel with 50% stenosis) and patients without CAD. Finally, in a recently published cross-sectional study, after evaluating 118 stable CAD patients, a lack of association between MPO promoter polymorphism and angiographic severity of coronary atherosclerosis was observed (Wainstein et al., 2010). Nevertheless, as a secondary objective, that study revealed a trend toward greater angiographic severity of CAD among patients with plasma MPO levels in the upper range (Wainstein et al., 2010). The lack of association might be attributed to the fact that angiography-based Gensini scores provide information on anatomical severity, hindering a functional and morphological analysis of the atherosclerotic plaque. A recently published study by Ferrante et al. (Ferrante et al., 2010) enrolled 25 consecutive ACS patients undergoing PCI and OCT assessment, evaluating the relationship between plaque morphology and serum levels of myeloperoxidase (MPO) and CRP. OCT classified the culprit lesion as ruptured in 18 (72%) or eroded in 7 patients (28%) and detected intraluminal thrombus in 89% of ruptured plaques and 100% of eroded plaques. Baseline systemic levels of serum MPO were significantly higher in patients with an eroded plaque than in those with a ruptured plaque (median, 2500 ng/mL; 25th to 75th percentile, 1415 to 2920 versus median, 707 ng/mL; 25th to 75th percentile, 312 to 943; p=0.001), whereas CRP levels did not differ significantly (median, 11.3 mg/L; 25th to 75th percentile, 1.3 to 28.5 versus median, 3.9 mg/L; 25th to 75th percentile, 1.3 to 17.8; p=0.76, respectively). In addition, the same study showed that the density of MPO-positive cells within thrombi overlying plaques in postmortem coronary specimens retrieved from sudden coronary death victims was significantly higher in lesions with erosion (n=11) than ruptures (n=11) (median, 1584; 25th to 75th percentile, 1088 to 2135 cells/mm2 versus median, 579; 25th to 75th percentile, 442 to 760 cells/mm2; p=0.0012). Of importance, this study supports the concept that elevations in specific inflammatory biomarkers reflect different morphologies of complications of coronary atherosclerosis and highlights the heterogeneity of coronary mechanisms of ACS.

Taken together, these data suggest a controversial role of MPO for CAD burden, while an involvement in disease activity seems to be clear. However, this issue should be investigated in larger studies and, again, IVUS and OCT may play a crucial role in the accurate assessment of plaque vulnerability.

3.5 Matrix metalloproteinases

In human atherosclerosis, unstable atherosclerotic plaque is an important event that triggers ACS. Plaque rupture frequently correlates with loss of the extracellular matrix at certain locations, often in the shoulder areas of the plaque. Focal destruction of the extracellular matrix renders the plaque less resistant to mechanical stresses imposed

during systole and therefore vulnerable to rupture. Recent findings have revealed enhanced expression of matrix metalloproteinases (MMPs) in the vulnerable region of plaques and this contributes to the weakening of plaque caps by degrading the extracellular matrix.

MMPs are a family of zinc-containing endoproteinases that share structural domains but differ in substrate specificity, cellular sources, and inducibility. The list of MMPs has grown rapidly in the past several years, and by now >20 mammalian members have been cloned and identified. The members of the MMP family can degrade all of the components of the blood vessel wall and therefore play a major role in both physiologic and pathologic events that involve the degradation of extracellular matrix components.

In recent studies using knockout mice, electrical injury of femoral arteries in mice which stimulates intimal thickening, caused enhanced expression of MMP-2 and MMP-9 (Lijnen et al., 1998). In TIMP-1-deficient mice intimal thickening was significantly higher compared with that in wild-type controls (Lijnen et al., 1999) Together, these observations support a role for MMP involvement in intimal thickening, particularly in migration of vascular smooth muscle cells. Previous studies demonstrated that lipid-laden macrophages from human atherosclerotic plaque elaborate MMP-1 and MMP-3 (Galis et al., 1995) and culture of macrophages with fibrous caps of human atherosclerotic plaque induces MMP-dependent collagen breakdown (Shah et al., 1995). Other researchers have also detected the expression of several other MMPs including MMP-1, MMP-2, MMP-7, MMP-9, and MMP-12 in the shoulder areas of plaque (Shu et al., 1998). Brown et al. (Brown et al., 1995) reported that MMP-9 was commonly expressed in coronary atherectomy specimens from patients with recent plaque rupture. Kai et al. (Kai et al., 1998) reported that circulating MMP-2 and MMP-9 levels on admission were elevated in patients with AMI and UA. Inokubo et al. (Inokubo et al., 2001) also reported that plasma levels of MMP-9 were significantly increased in the coronary circulation in patients with AMI and UA compared with those in control subjects, suggesting a process of active plaque rupture in ACS. Hirohata et al. (Hirohata et al., 1997) also observed increased plasma MMP-1 and MMP-2 levels, respectively, in patients with AMI. Thus, increased MMP expression may modulate vascular and ventricular remodeling in ACS.

Wang et al. (Wang et al., 2008) evaluated the vulnerability of coronary artery plaque with CAG, IVUS and the levels of plasma inflammatory markers in 58 consecutive patients with lesion of a single blood vessel demonstrated by CAG. Patients were randomly divided into 3 groups based on the angiographic morphology of the lesions: type I lesion group (n=16), type II lesion group (n=25), type III lesion group (n=17). A control group of stable angina (n=17) was established. A subgroup of 28 patients (including 18 ACS patients and 10 stable angina control patients) who underwent IVUS study were analyzed. Then the plasma levels of MMP, including MMP-2 and MMP-9, CD40L and PAPP-A were measured with ELISA, along with CRP levels. The plasma levels of MMP-2, MMP-9 and PAPP-A in type II lesion group were significantly higher than the other groups ($p< 0.05, 0.05, 0.001$, respectively). In type II lesion group, linear correlation analysis manifested significantly positive correlation between levels of CRP and MMP-2 ($r=0.508$); MMP-2 and MMP-9, CD40L, PAPP-A ($r=0.647$, 0.704, 0.751, respectively); MMP-9 and CD40L, PAPP-A ($r=0.491$, 0.639, respectively); CD40L and PAPP-A ($r=0.896$). IVUS subgroup analysis showed that the area of plaques and plaques burden in culprit lesion, the incidence of high-risk plaques, remodeling index and positive remodeling percentage in ACS patients were significantly greater than those in the

control group (p=0.0001, 0.037, 0.028, 0.015, 0.040, respectively). Compared with the control group, the plasma levels of CRP, MMP-2, MMP-9 and PAPP-A were markedly elevated (p=0.033, 0.0001, 0.0001, 0.027, respectively).

Park et al. (Park et al., 2010) recently enrolled 188 patients who underwent 3-vessel VH-IVUS with peripheral blood sampling, including plasma levels of MMP-2,-9, tissue inhibitor of metalloproteinase-1, adiponectin, macrophage migration inhibitory factor, along with CRP levels measurements. Among the biomarkers, only the MMP-9 level was significantly higher in patients with ruptured plaque (p=0.002). In the subgroup without ruptured plaque, significant differences in the levels of several biomarkers were not observed between patients with and without VH-TCFA. In both culprit/target and nonculprit/non-target vessels, the MMP-9 level showed a weak correlation with the total number of ruptured plaques (r=0.231, p=0.002). Among the biomarkers tested in this study, the MMP-9 level was significantly higher in patients with ruptured plaque. However, measurement of several biomarkers, including MMP-9, was incapable of predicting the presence of VH-TCFA. This 3-vessel VH-IVUS study of 188 patients showed that the plasma level of MMP-9 might increase in patients with multiple ruptured plaques, as well as in patients with ruptured plaque in the culprit/target lesions. Both acute coronary syndrome and the MMP-9 level were independent predictors of ruptured plaque in the culprit/target lesions. However, the presence of VH-TCFA in the culprit/target lesions or multiple VH-TCFAs detected by 3-vessel VH-IVUS study were not be predictive with the use of several biomarker assays, including MMP-9, in this study. The clinical presentation of ACS, not the level of the biomarkers, was the only independent predictor of VH-TCFA in the culprit/target lesions. MMPs belong to a family of multidomain zinc-dependent endopeptidases that promote degradation of all protein and proteoglycan–core-protein components of the extracellular matrix (Galis et al., 2002). Among the family of MMPs, MMP-2 and MMP-9 are found in the macrophages and smooth muscle cells covering the shoulder region of atherosclerotic plaque (Galis et al., 1994). MMP-9 and MMP-2 are highly expressed in the vulnerable regions of atherosclerotic plaque and it has been suggested that they are causally involved in plaque rupture (Lijnen et al., 2003). Other studies have shown that the level of MMP-2 activity is higher in stable lesions of carotid artery plaque (Sluijter et al., 2006) and that the level of MMP-9 is increased in more unstable plaque (Sluijter et al., 2006, Lotus et al., 2000). Blakenberg and collegues reported that a higher level of plasma MMP-9 was a predictor of cardiovascular mortality: the patients in the highest quartile of MMP-9 level (>72 ng/ml) had the highest probability of cardiovascular death (Blankenberg et al., 2003). Taken together, these data suggest that MMP-9 has a more significant role in plaque vulnerability than MMP-2, but more clinical studies are required to evaluate the exact role of MMP-2 in plaque vulnerability. Thus, CAG and IVUS combined with the study on plasma levels of inflammation mediators were helpful in judging the vulnerability of coronary artery plaques.

Matrix metalloproteinases play a crucial role in initiating ACS by degrading extracellular matrix components, which leads to vulnerability of the plaque as well as formation of atherosclerotic lesions. Although the use of MMP inhibitors may have unforeseen adverse effects if used in the wrong setting, development of therapeutic drugs specifically targeted against MMPs may be useful in the prevention of atherosclerotic lesion development and cardiac events.

4. Lipidics

Inflammation, neovascularisation, imbalance between coagulation and anticoagulation system are interesting aspects (Ross et al.,1993; Katagiri et al., 2007; Glass et al., 2001), but another one is that of lipid system and coronary plaque.

4.1 Lipid profile

One of the major predisposing factors to atherosclerosis is an abnormal lipoprotein metabolism and it may be present in over 70% of patients with premature CAD (Genest et al., 2005).

The association between CAD and levels of total cholesterol, LDL and low HDL has been proven and widely accepted in diagnostic practice. As a consequence the National Cholesterol Education Program III (NCEP III) recommended the analysis of Tc in combination with LDL and HDL-c as a basis for the screening and treatment of patients with CAD. High concentration of LDL cholesterol and lipoprotein (a) or low levels of HDL cholesterol are able to promote atheroma formation and are recognised as particularly important risk factors for atherosclerosis and CAD (Rosenson, 1996; . Grundy et al., 1989; LaRosa et al., 1990; v et al., 1994). Of note, randomised trials showed that LDL reduction decreases mortality and coronary events both in a primary and in a secondary prevention (Shepherd et al., 1995, Sacks et al., 1996). It was found that the reduction in non-fatal MI and CAD death was strongly correlated with the small incremented serum HDL-cholesterol level (Robins et al., 2001). An independent association of triglycerides (TG) with atherosclerosis and/or CAD remains uncertain. Some studies suggest that TG level does not influence the CAD risk whereas others provide some proofs that it does (Avins & Neuhaus, 2000). Ten years ago it was suggested that there is an inverse association between HDL level and the number of diseased coronary vessels (Romm et al., 1991). However, some other studies manifested that there is no association of CAD severity with lipid concentration (Lekakis et al., 2000; Cerne et al., 2000). This conflicting data persuaded Tarchalski et al. (Tarchalski et al., 2003) to perform a prospective study for evaluation of the relationship between serum lipid levels and the extent of atherosclerosis within coronary arteries in patients with suspected CAD and no previous MI, and who were not treated with lipids lowering therapy before entering the study. The study was conducted in 141 patients (53.6 ± 7.8 years old; 32 female) who underwent a routine CAG for CAD diagnosis. A modified angiographic Gensini Score was used to reflect the extent of coronary atherosclerosis. Fasting serum lipid concentrations were determined using cholesterol esterase/peroxidase (CHOD/PAP) enzymatic method for total cholesterol and its fractions and lipase glycerol kinase (GPO/PAP) enzymatic method TG evaluation. Gensini score was positively correlated with total cholesterol ($r=0.404$; $p<0.001$), LDL cholesterol ($r=0.484$; $p<0.001$) and TG ($r=0.235$; $p=0.005$). There was a negative correlation between Gensini Score and HDL cholesterol ($r=-0.396$; $p<0.001$). This study clearly showed that in angina pectoris patients with no previous MI, the extent of coronary atherosclerosis is positively correlated with pro-atherogenic lipids, i.e. total cholesterol, LDL cholesterol and TG and negatively correlated with antiatherogenic HDL cholesterol. Cabin and Roberts (Cabin & Roberts, 1982) previously assessed the amount of cross-sectional area narrowing by atherosclerotic plaques histologically in each 5 mm segment of the entire lengths of the right, left main, left anterior descending, and left circumflex coronary arteries in 40 patients with fatal CAD and known fasting serum total cholesterol and TG levels. The number of 5 mm segments of coronary

artery narrowed severely (76 to 100% in cross-sectional area) by atherosclerotic plaques in each group was as follows: 172 of 505 (34%) 5 mm segments from group I; 242 of 353 (69%) segments from group II; 120 of 295 (41%) from group III and 425 of 884 (48%) segments from group IV. The mean percentage of 5 mm segments narrowed severely was significantly greater in group II than in group I ($p<0.005$) or group III ($p<0.01$). Additionally, the mean number of four coronary arteries per subject severely narrowed and the number of subjects with severe narrowing of the left main coronary artery were significantly greater in groups II and III than in group I. The percentages of 5 mm segments narrowed severely correlated significantly with the serum triglyceride level ($p<0.03$). Although it correlated with the number of severely narrowed coronary arteries per subject, the serum total cholesterol level, however, did not correlate with the percentage of 5 mm segments of coronary artery with severe narrowing. In 1994, Ladeia et al. (Ladeia et al., 1994) correlated lipid profile with CAD. One hundred patients with symptoms of CAD were studied by CAG. Coronary artery stenotic and normal proximal lumen were measured with a pachymeter, and the percent degree of obstruction calculated. CAD was documented in 74 patients, (56-75.6% men), with > or = 50% stenosis in 67 (90.5%), 54 (79.1%) men. The lesions were univessel in 24 (33.4%), bivessel in 29 (39.7%), and trivessel in 20 (27.4%). Seventy patients had total cholesterol > or =200 mg/dl, 29 (41.4%) > or =240 mg/dl); 69 (71.9%) LDL > or =130 mg/dl, 37 (38.5%) > or =160 mg/dl; 35 (36.5%) HDL <35 mg/dl and 10 TG > or =200 mg/dl. CAD patients had lower HDL values (38.8 +/- 10 mg/dl vs 48.2 +/- 13.6 mg/dl, $p=0.01$) and higher Castelli risk indexes (total cholesterol/HDL =5.9 +/- 1.7 vs 5.1 +/- 1.4 and LDL/HDL =4.1 +/- 1.5 vs 3.4 +/- 1.2, $p=0.04$). Patients with > or =50% stenosis and multivessel disease showed higher Castelli risk indexes ($p=0.01$ and $p=0.04$ for total cholesterol/HDL, and $p=0.01$ and $p=0.02$ for LDL/HDL, respectively). Twenty one (70%) of the 30 patients with total cholesterol<200 mg/dl had CAD (28% of the patients with CAD), in whom there was a high frequency of patients with a low HDL level (11/21, 52.4% vs 3/9, 33%, $p=0.06$). Thus, lower HDL and higher Castelli risk indexes values were associated with more severe and intensive CAD. Additionally, total cholesterol <200 mg/dl is compatible with CAD, especially if there is a low HDL level. These findings further strengthen the need of HDL measurement for CAD risk assessment.

Several mechanisms may explain these associations. The observed positive association of the extent of coronary atherosclerosis with total cholesterol seems to be caused by cholesterol present in LDL particles. Circulating LDL can enter cells via apo B/E receptors or through an unregulated scavenger receptor (Brown & Goldstein, 1986). The latest mechanism, present in smooth muscle cells and macrophages, can result in excess accumulation of intracellular cholesterol and the formation of foam cells. The unregulated scavenger receptors are able to uptake both native and modified LDL particles. The most common form of LDL modification is its oxidation, which can occur in any of the cells within the artery, including the endothelial cells, T lymphocytes, smooth muscle cells and macrophages (Hiltunen et al., 1998; Kloiche et al., 2000). Foam cells can rupture leading to the release of oxidized LDL, proteolytic enzymes and toxic oxygen derivatives that can altogether damage the vessel wall (Hiltunen et al., 1998; Kloiche et al., 2000, Dimmeler et al., 1997). Oxidized (Oxy) LDL particles can promote atherosclerotic changes by several mechanisms, such as acting as a monocyte chemoattractant, inducing endothelial cells dysfunction and apoptosis, and reducing nitric oxide release and endothelium-dependent vasodilatation (Li & Mehta, 2000; Mathew et al., 1997; Anderson et al., 1996). Oxy LDL may increase platelet aggregation and thromboxane release what causes vasoconstriction and

thrombus formation (Chen et al., 1996). Cytokines released from activated platelets and injured endothelial cells can stimulate smooth muscle proliferation and the formation of atherosclerotic plaque (Ross, 1993). Thus, the increased LDL cholesterol is related to the development of atherosclerotic changes within arteries. The inverse association of Gensini score with HDL cholesterol observed in the study performed by Tarchalski et al. (Tarchalski et al., 2003) can be explained by antiatherogenic properties of HDL particles. HDL is engaged in reverse cholesterol transport from cells and atherosclerotic plaques into the liver or to other tissues (Tall, 1990). The uptake of cellular cholesterol by HDL is controlled by apolipoprotein A-I and cholesterol efflux regulatory protein. Apolipoprotein A-I serves as a signal transduction protein to mobilize cholesterol esters from intracellular pools and also activates lecithin-cholesterol acyltransferase dependent cholesterol esterification. Cholesterol efflux regulatory protein promotes the transfer of intracellular cholesterol to the cell membrane and surface (Marcil et al., 1999). Further, cholesterol is removed from HDL particles by direct liver uptake or it is transferred to apolipoprotein B-containing lipoproteins, i.e. very low density lipoprotein, intermediate density lipoproteins and LDL (Tall, 1990; Marcil et al, 1989). In addition to reverse cholesterol transport, HDL has a variety of vascular actions that can counteract atherogenesis. HDL contains paraoxonase, an enzyme that protects lipoproteins from oxidative modification and it is able to destroy oxidized lipids present in oxy-LDL and to hydrolyze lipid peroxides in human atherosclerotic lesions. It was observed that lower serum paraoxonase is linked to more severe CAD (Aviram et al., 2000; James et al., 2000; Shih et al., 1998). HDL is thought to maintain endothelial function and a low blood viscosity through a permissive action on red cell deformability (Kuhn et al., 1991; Epand et al., 1994). It is speculated that HDL may downregulate thrombin generation and in this way limit its influence on atherosclerosis (Griffin et al., 1999). As regards TG serum levels and CAD burden, according to literature it remains uncertain if there is an independent association of TG with coronary atherosclerosis. Lipoproteins rich in TG, i.e. very-low density lipoprotein and intermediate density lipoprotein (lipoprotein remnants) have been identified in human atherosclerotic plaques (Rosenson, 2001). Moreover, hypertriglyceridemia is associated with other abnormalities predisposing to the development of atherosclerosis.
Taken together, these data suggest the role of lipid prophile both in coronary initiation, progression and instability.

4.2 Adiponectin

Obesity is one of the most common causes of cardiovascular morbidity and mortality (Matsuzawa et al., 1995). Abdominal visceral fat accumulation is accompanied by impaired glucose tolerance, dyslipidemia, and hypertension, and finally leads to atherosclerotic vascular disease. As important molecules linking these diseases and the inflammatory response, previous studies have shown the dynamic function of adipose tissue as an endocrine organ, releasing various cytokines, adipokines and inflammatory markers, which are involved in the development of atherosclerosis (Fortuno et al., 2003). Notably, adipose tissue secretes a variety of bioactive molecules that directly contribute to the development of cardiovascular disease (Matsuzawa et al., 1999). Adiponectin is an adipose-specific plasma protein, with antiatherogenic and anti-inflammatory properties (Ouchi et al., 1999; 2001). and low plasma adiponectin was observed in patients with CAD (Ouchi et al. 2001).
Additionally, several reports showed an inverse relation between serum CRP and adiponectin (Engeli et al., 2003; Yudkin et al., 1999). Otake et al. (Otake et al., 2008)

investigated the relation between plasma adiponectin and CRP and coronary plaque components in 93 patients with ACS, using VH-IVUS to examine relations among plasma CRP, adiponectin, and ratios of each coronary plaque component. They interestingly found that plasma adiponectin was significantly lower and plasma CRP was significantly higher in patients with than without ACS. There was an inverse relation between serum CRP and adiponectin with regard to necrotic core ratio in both culprit and nonculprit lesions in patients with ACS. The authors concluded that increased plasma CRP and hypoadiponectinemia might be related to the progression of ACS. Additionally, Sawada et al. (Sawada et al., 2010) enrolled 50 patients with stable CAD, showing that patients with TCFA had significantly lower plasma adiponectin levels than patients without TCFA ($p<0.0001$). Furthermore, the plasma adiponectin levels in patients with multi-vessel TCFA were significantly lower than those in patients with single-vessel TCFA ($p=0.049$). Multivariate logistic regression analysis revealed that plasma adiponectin was the strongest predictive factor of the presence of TCFA ($p=0.0007$). Of importance, these data suggest that in patients with stable CAD, adiponectin may be a useful biomarker to stratify "vulnerable patients" into risk categories. These findings suggest that adiponectin affected plaque components, and low plasma adiponectin, a well-known independent risk factor for CAD, might enhance the vulnerability of atherosclerotic plaques and vessels, which could lead to ACS.

5. Coagulative/fibrinolysis state

Coronary thrombosis occurs in most patients with AMI (De Wood et al., 1980) and there is also epidemiological evidence indicating the pathogenetic importance of haemostatic function in CAD (Meade et al., 1986). The precise role of haemostatic factors in the presence of coronary atherosclerosis, however, has not been fully elicited yet.

5.1 Platelets

Platelets are actively involved in the inflammatory cascade leading to vascular atherosclerosis (Libby & Simon, 2001;; Davi & Patrono, 2007). Platelets are enucleate cells of 1-2 μm length with average life span of 7–8 days, generated by bone-marrow derived megakaryocytes after cytoplasmic fragmentation, and play a pivotal role in the process of atherosclerosis. Large interest has been directed towards an improved understanding of platelet physiology, the assessment of platelet function and the development of improved antiplatelet treatment with faster and stronger activity. Patients with stable CAD have increased platelet reactivity and circulating monocyte-platelet aggregates (Furman et al., 1998), which also have been demonstrated early markers of AMI (Furmam et al, 2001). In addition, platelet reactivity is progressively increased as a function of the number of vascular districts involved by atherosclerosis (cerebral, cardiac, peripheral) (Keating et al., 2004). Although the platelet count does not appear to predict cardiovascular outcomes (Pizzulli et al., 1998) and a larger platelet size may correlate with CAD (Pizzulli et al., 1998) and MI. It has been observed a large variability in baseline platelet reactivity and effects of antiplatelet therapies, that may potentially due to the variability in platelet size. In fact, larger platelets have a greater mass and are both metabolically and enzymatically more active than smaller platelets. Indeed, they have a greater prothrombotic potential, with higher levels of intracellular thromboxane A2, and TG levels, as well as increased levels of procoagulant surface proteins (e.g. P selectin, GpIIb/IIIa). Hemostatically reactive platelets,

larger platelets, have more granules and adhesion receptors that have resulted in decreased bleeding time showing increased activation. Indeed, platelet aggregability has been directly related with systemic atherosclerotic disease (Keating et al., 2004). In addition, the most detrimental manifestation of coronary atherosclerotic disease (i.e., myocardial infarction) is mediated by platelet activation (Fitzgerald et al., 1986). On this basis, numerous therapeutic options, targeting platelet aggregability, have been proposed.

5.1.1 Mean platelet volume

Mean platelet volume (MPV) has been shown to be an indicator of platelet activation, that plays a pivotal role in the pathophysiology of atherosclerotic disease (Tsiara et al., 2003; Broadley et al., 2003). It has been reported that elevated values of MPV are associated with cardiovascular diseases (Pizzulli et al., 1998; Jagroop & Mikhailidis, 2003). Few and small reports have investigated so far the relationship between MPV and the extent of CAD, with contrasting results. Also an increase in MPV may be due to the usage of small platelets during acute ischemia (Pizzulli et al., 1998). On these grounds, MPV could be accepted as a parameter of platelet activity and has become a prognostic factor in CAD. Confirming the importance of MPV, several additional reports have demonstrated that MPV was associated with impaired myocardial perfusion and clinical outcome after primary angioplasty. De Luca et al. (De Luca et al., 2009) measured MPV in 1411 consecutive patients undergoing coronary angiography. Significant CAD was defined as stenosis >50% in at least 1 coronary vessel. They additionally measured carotid intima-media thickness in 359 patients. The relationship between MPV and platelet aggregation was valuated by PFA-100 in 50 consecutive patients who were not taken any antiplatelet therapy, and in a cohort of patients who were on aspirin by PFA-100 (n=161) and Multiplate (n=94). Patients were divided into three groups according to tertiles of MPV. Patients with higher MPV were slightly older ($p=0.038$), with larger prevalence of diabetes ($p<0.0001$), hypertension ($p=0.008$), previous CAD ($p=0.041$), less often with stable angina ($p=0.043$) and family history of CAD ($p=0.011$), more often on statins ($p=0.012$), and diuretics ($p=0.007$). MPV was associated with baseline glycaemia ($p<0.0001$) and red blood cell count ($p=0.056$), but inversely related to platelet count ($p<0.0001$). MPV was not associated with the extent of coronary artery disease ($p=0.71$) and carotid intima-media thickness ($p=0.9$). No relationship was found between MPV and platelet aggregation. This study showed that MPV was not related to platelet aggregation, the extent of CAD and carotid intima-media thickness. Thus, according to this study (De Luca et al., 2009) this parameter cannot be considered as a marker of platelet reactivity or a risk factor for CAD. This is the largest study so far conducted to investigate the relationship between MPV and CAD.

Several factors may contribute to explain these findings. The increase in MPV may be a process driven by increased production of bone-marrow derived larger circulating reticulated platelets within the blood stream (26). Indeed, the MPV has been shown to correlate with both megakaryocyte ploidy and with the percentage of circulating reticulated platelets (Smith et al., 2002). Furthermore, a positive correlation between thrombopoietin (a key thrombopoietic hormone) levels and MPV values has been demonstrated in CAD (Senaran et al., 2001). Thus, larger MPV may not imply higher platelet reactivity, that has been shown to be related to the extent and complexity of CAD (Korovesis S, et al., 2000) but may be associated with even reduced aggregation since larger platelets may be precursor and not fully mature platelets. In fact, De Luca et al. (De Luca et al., 2009) did not observe any impact of MPV on platelet aggregation or aspirin resistance. Supporting these data, van

der Planken et al. (van der Planken et al., 2000) did not find any relationship between platelet prothrombinase activity, a final pathway platelet procoagulant activity of type 1 diabetic platelets, and MPV. Furthermore, as shown by previous reports, MPV may be associated with other prognostic factors, such as smoking, diabetes, obesity, hypertension, that may primarily affect the extent of CAD and clinical outcome.

5.2 Coagulative and fibrinolysis markers

Previously clinical data support the proposition that activation of the coagulation and the platelet system is closely associated with myocardial ischaemia there is little information on the relation between the development of coronary atherosclerosis and the haemostatic system.

Nichols et al. (Nichols et al., 1982) did not detect increased concentrations of platelet factor 4, B thromboglobulin, and fibrinopeptide A in a group of patients with abnormal coronary angiograms without previous MI. Furthermore, Schmitz-Huebner et al. (Schmitz-Huebner et al., 1988) analysed blood samples for haemostatic assessment from 225 patients with angina pectoris who were admitted to hospital for CAG. Thromboglobulin, platelet factor 3, platelet factor 4, factor VII:C, factor VIII:C, von Willebrand factor antigen, activated partial thromboplastin time, fibrinogen, antithrombin III, protein C:Ag, plasminogen, and antiplasmin were measured before angiography. Of note, patients who had had a MI in the two months before the investigation were excluded from the study. Multiple linear regression analysis showed that none of the haemostatic variables contributed independently to the prediction of an angiographic score that indicated the extent of coronary atherosclerosis. There were some significant correlations between haemostatic variables and conventional risk factors for CAD. However, the importance of the coagulation/fibrinolytic system is highlighted by several autopsic studies that show a high prevalence of old plaque disruptions without infarctions. A transient shift in the coagulation and anticoagulation balance is likely to result in an acute event. The prolonged presence of residual thrombus over a disrupted or eroded plaque will induce smooth muscle migration and produce new intima, leading to plaque expansion (Hoffmesister et al., 1995). Autopsic studies show that plaque growth is induced by episodic plaque disruption and thrombus formation (Holvoet et al., 1997). Therefore, an active fibrinolytic system may be able to prevent luminal thrombosis in some cases of plaque disruption (Hoffmeister et al, 1999). t-PA, as a crucial factor in fibrinolytic system, plays an important role in the balance between coagulative system and fibrinolytic system, which is mainly responsible for the dissolution of fibrin clots in the circulation, by converting inactive plasminogen to active plasmin. A rapid decline in release of active t-PA is associated with an increasing plaque burden and vulnerability. The reduction in acute fibrinolytic capacity reflects impairment of acute t-PA release that is likely to involve endothelial cell injury (Munkvad et al., 1990; Gyongyosi et al., 2004; Hoffmeister et al., 1998). In the study performed by Wang et al. (Wang et al., 2008), the authors aimed at assessing the association between vulnerability of plaque assessed with IVUS and plasma levels of fibrinolytic biomarkers in patients with ACS. Eighty-nine patients with ACS were enrolled in the study. Blood was collected to measure t-PA levels by liquid phase bead flow cytometry. Eighty-nine non-bifurcated lesions (identified by coronary angiography) were investigated using IVUS before catheterization. The areas of plaque and media were calculated and lesions were classified into two groups: VH-IVUS derived VH-TCFA and non-VH-TCFA plaque. Plasma t-PA level in patients with TCFA was significantly lower than that with non-TCFA (1489 ± 715) pg/ml vs (2163 ± 1004) pg/ml).

Decreased plasma levels of t-PA were associated with plaque vulnerability. Plasma levels of t-PA correlated negatively with plaque plus media and necrotic core in plaque in patients with acute coronary syndrome. Thus, t-PA may be considered an independent risk factor and a powerful predictor of vulnerable plaques. Decreased levels of t-PA may reflect instability of atherosclerotic plaques and might therefore serve as noninvasive determinants of those at high risk for consequent adverse events. Furthermore, in order to elucidate the causes of coronary instability in patients without systemic evidence of inflammation, Niccoli et al. (Niccoli et al., 2007) compared rate of episodic production of markers of thrombin generation [thrombin–antithrombin complexes (TAT)], of fibrinolysis [plasmin–antiplasmin complexes (PAP)], and angiographic severity and extent of coronary atherosclerosis in patients with severe UA and high or low systemic levels of CRP. They enrolled 40 consecutive patients (age 59.7±8.7, 76% males) admitted to coronary care unit with severe UA (Braunwald class IIIB. The authors assayed TAT and PAP using commercially available ELISA assays and CRP with high sensitivity nephelometry. The evaluation of atherosclerotic disease severity and extent was performed according to Bogaty's score. Patients were divided in two groups according to CRP levels: G1=CRP>3 mg/L and G2=CRP<3 mg/L. Number of diseased vessels and number of stenoses plus occlusion were similar between the two groups (1.8±0.9 in G1 vs 2.2±0.9 in G2, p=NS and 2.6±1.9 in G1 vs 2.7±1.3 in G2, p=NS, respectively), as well as extent score and index (8.4±4.5 in G1 vs 9.2±3.1 in G2, p=NS and 0.6±0.3 in G1 vs 0.6±0.27 in G2, p=NS, respectively). Episodic activation of thrombin generation, as assessed by TAT was more frequent in G1 than in G2 (85% vs 47%, p=0.03). Episodic activation of the fibrinolysis was more frequent in G1 than in G2 (80% vs 40%, p=0.01). This study demonstrated that patients with severe UA Who have on admission high serum levels of CRP are more prone to thrombin generation and fibrinolysis activation compared to patients with low admission CRP levels. Severity and extent of CAD was similar between patients with high or low CRP levels on admission. Furthermore, in this study performed by Niccoli et al. (Niccoli et al., 2007), in patients with systemic evidence of inflammation at baseline, episodes of thrombin and plasmin generation were observed more frequently than in patients without systemic evidence of inflammation. This is in keeping with results of studies (Levi et al., 2006) in the septic patients showing that severe systemic inflammation triggers blood coagulation. However, the authors (Niccoli et al., 2007) failed to find evidence of a hypercoagulable state or of a more severe coronary atherosclerosis in unstable patients without systemic evidence of inflammation. Future studies are warranted to investigate other mechanisms of destabilization in patients without systemic evidence of inflammation as for instance, mechanical rupture of a thin fibrous cap caused by greater mechanical stress.

5.3 Tissue factor

Atherosclerotic plaque rupture or fissuring is a key event in the pathogenesis of UA and MI (Fuster et al., 1992a,b). The exposure of blood to a procoagulant surface triggers thrombin generation, platelet aggregation, and fibrin deposition and leads to thrombus formation that can precipitate an acute coronary event. However, atherosclerotic plaques may rupture without triggering thrombosis. Necropsy data show that between 9% and 16% of people who die suddenly of non-cardiac causes have fissured plaques without thrombosis in their coronary arteries, but patients who die of cardiac causes have both thrombosed and non-thrombosed ruptured plaques (Falk, 1985; Davies et al., 1989). The reasons why some ruptured plaques develop thrombosis and others do not are still not known. Tissue factor is

a small transmembrane cell surface receptor that mediates cellular initiation of the coagulation serine protease cascades (Edgington et al., 1991). In vitro studies have shown that tissue factor expression was observed on various cultured and stimulated cell types such as monocytes (Jude et al., 1994; Leatham et al., 1995; Barstad et al., 1995). smooth muscle cells (Taubman et al., 1993; Maynard et al., 1977), endothelial cells and fibroblasts (Green et al., 1971). Tissue factor in the media or the adventitial layer of human normal arteries is localized to aid the body in the prevention of blood loss as an initiator of blood coagulation (Weiss et al., 1989; Drake et al., 1989; Fleck et al., 1990; Wilcox et al., 1989) Tissue factor has recently been shown to be expressed in human coronary atherosclerotic plaque from directional atherectomy specimens from patients with stable and unstable coronary syndromes (Annex et al., 1995). Therefore, when tissue factor expressed in the coronary atherosclerotic plaques is exposed to the blood by the plaque disruption, it may lead to the thrombus formation and the occlusion of the related coronary artery. The plaque rupture is closely associated with the soft extracellular lipids (Berliner et al., 1995), macrophages (van der Wal et al., 1994) matrix-degrading proteases (Libby, 1995) such as interstitial collagenase (MMP-1), which degrades two major plaque structural proteins, type I and III collagen, and activated mast cells in the shoulder region of the atherosclerotic plaques. Of note, Van der Wal et al. (van der Wal et al., 1994) have reported that the macrophages were the predominant cells at the immediate site of either rupture or superficial erosion of the fibrous cap that contained few smooth muscle cells. However, it is not clear whether the macrophages express tissue factor in vulnerable human coronary atherosclerotic plaques in patients with unstable angina.

Kaikita et al. (Kaikita et al., 1997) determined whether macrophages express tissue factor in human coronary atherosclerotic plaques. They examined directional coronary atherectomy specimens from 24 patients with UA and 23 with stable exertional angina. In these specimens, macrophages were detected in 22 (92%) of 24 patients with unstable angina versus 12 (52%) of 23 with stable exertional angina ($p=0.003$). The percentage of macrophage infiltration area was significantly larger in patients with unstable angina than in those with stable exertional angina (17±3% versus 6±2%, $p=0.008$). The immunohistochemical double staining revealed the expression of tissue factor on macrophages in 18 (75%) of 24 patients with unstable angina versus 3 (13%) of 23 with stable exertional angina ($p<0.0001$). Thrombus was identified in 20 (83%) of 24 patients with unstable angina versus 12 (52%) of 23 with stable exertional angina ($p=0.02$). Fibrin deposition was mainly observed around macrophages expressing tissue factor in the patients with unstable angina. Thus, in this study, the authors demonstrated that tissue factor expression on macrophages was more frequent in coronary atherosclerotic plaques in patients with UA. Tissue factor expressed on macrophages may play an important role in the thrombogenicity in coronary atherosclerotic plaques of these patients.

6. Conclusions and future perspectives

Cardiovascular disease remains one of the leading causes of morbidity and mortality in the developed countries, thus the development of novel therapeutic strategies to reduce cardiovascular burden and risk further than is currently possible is mandatory. Early detection of CAD is of paramount importance for initiating aggressive control of risk factors (smoking cessation, lipid lowering therapy, weight reduction therapy, aggressive therapy for hypertension) and establishing of pharmacologic therapy in order to reduce occurrence

of life threatening acute cardiovascular events (anti platelet agents, statins). The use of vascular imaging, combined with soluble molecular markers of disease burden, progression and activity, can provide crucial information that may help developing new pharmaceutical approaches. It is evident from the discussions now ongoing between industry, government regulators, and academia that there is a shared recognition of the need for the application of new tools in drug development. This general philosophy, applied to atherosclerosis treatment, is critical to addressing the epidemic of CAD. In this regard, the association between plaque burden, morphology and activity, as assessed by imaging modalities, and levels of circulating biomarkers, assessing lipid metabolism, inflammation, platelets and white blood cells activation, and endothelial activation, is an intriguing field. New imaging modalities in combination with the development of new biomarkers (bioimaging) may significantly improve our understanding and management of patients at risk of coronary artery disease and its harmful complications, proving valuable insights into atherosclerotic disease progression and the relation to disease activity. In the last decades, an ongoing and intense research by different groups and investigators trying to delineate the role of different biologically active substances in the pathogenesis of progression of atherosclerotic CAD has provided data of paramount importance. Indeed, cardiovascular biomarker research efforts have resulted in the identification of new risk factors and novel drug targets, as well as the establishment of treatment guidelines, thus recognizing the importance of biomarkers in advancing therapies. However, given the complex pathophysiology of cardiovascular disease, no single biomarker will likely prove able to provide a universal surrogate whereby change observed independently predicts benefit, increased risk, or no effect across all drugs and mechanistic targets but the integration of different biomarkers looking at different phases of the coronary atherosclerotic disease should be the way forward.

7. References

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In the intervening 10 years tremendous advances in the field of cardiac computed tomography have occurred. We now can legitimately claim that computed tomography angiography (CTA) of the coronary arteries is available. In the evaluation of patients with suspected coronary artery disease (CAD), many guidelines today consider CTA an alternative to stress testing. The use of CTA in primary prevention patients is more controversial in considering diagnostic test interpretation in populations with a low prevalence to disease. However the nuclear technique most frequently used by cardiologists is myocardial perfusion imaging (MPI). The combination of a nuclear camera with CTA allows for the attainment of coronary anatomic, cardiac function and MPI from one piece of equipment. PET/SPECT cameras can now assess perfusion, function, and metabolism. Assessing cardiac viability is now fairly routine with these enhancements to cardiac imaging. This issue is full of important information that every cardiologist needs to now.

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