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NEWEST UPDATES IN RHEUMATOLOGY

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Rheumatology

Volume 1

Dr. Wahid Ali Khan is an assistant professor in the Department of Clinical Biochemistry, College of Medicine, King Khalid University, Abha, Kingdom of Saudi Arabia. Dr. Khan has served as a member of the editorial board of more than six international journals and is a guest editor for two journals. His research interest includes the role of estrogen and its metabolites in various autoimmune diseases. He is also interested in the cloning of interferon alpha 2b and discovering its role in the pathogenesis of different types of autoimmune diseases. Dr. Khan has published more than 25 articles, four reviews, and three book chapters. He is also the editor of four books, which have been well recognized and documented by the international research community.

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Scope of the Series

Rheumatology is a branch of medicine dealing with different conditions that affect musculoskeletal tissues such as joints, bones, cartilage, tendons, ligament and muscles. It is devoted to the diagnosis and therapy of various rheumatic diseases. Despite vast recent developments in this field, we are still searching for better technologies that might prevent and cure these diseases. This book series includes all the latest updates in the field of rheumatology.

Contents

Preface

Rheumatology is a branch of science dealing mainly with rheumatic conditions and their therapies. Keeping in mind all these aspects, this book includes chapters that explain the basics and therapies of rheumatology. Although this book has limited chapters it is hoped that it will help those interested in the field of rheumatology.

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Section 1

Basic Rheumatology

Chapter 1

Introductory Chapter: Introduction to Rheumatology

Wahid Ali Khan

Additional information is available at the end of the chapter

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1. Introduction

Rheumatology is a branch of science dealing with the different conditions that affect musculoskeletal tissues such as joints, bones, cartilage, tendons, ligament, and muscles. Rheumatism refers to various painful conditions that affect these tissues. Rheumatic diseases are those groups of diseases showing pain followed by reduction in the range of motion and function of musculoskeletal tissues. Arthritis is also one type of rheumatic disease referring to joint inflammation whether for joint pain, stiffness, inflammation, or joints damage. Most common types of rheumatic disease are rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (**Figure 1**). The autoimmune diseases (such as RA, SLE, multiple sclerosis, and inflammatory bowel) have complex pathogenesis and multiple etiologies [1]. Studies have reviewed the role of various factors including genetic factors, epigenetic regulation, and environmental factors (such as cigarette smoking, crystalline silica, Epstein-Barr virus and reproductive hormones) in the pathogenesis of these autoimmune diseases [1]. There are many evidences showed that autoimmune diseases are multigenetic and their identification is associated with various types of genes [2]. There are a group of genes which induced the expression of proteins involved in various key pathophysiological pathways such as formation and clearance of immune complexes or apoptotic material, control of innate and adaptive immunity, production of immunological molecules like cytokines, chemokines, and adhesion molecules [3, 4]. Autoimmune patients have a great diversity in their genetic background and the nature of genes decides what kind of responsiveness is required to change the state of immune system [5]. There are various environmental factors that play an important role in these autoimmune diseases. These include infectious agents, ultraviolet (UV) light, and chemical or other compounds modifying immunological responses like environmental pollutants, drugs, or other behavioral habits such as smoking or diet [6, 7].

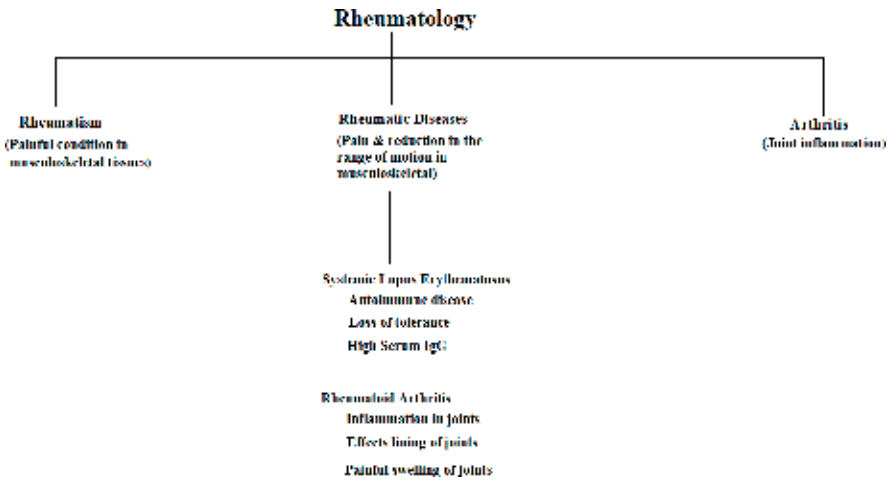

Figure 1. Different terms used in rheumatology.

2. Systemic lupus erythematosus (SLE)

SLE is a multisystemic and complex autoimmune disease/condition characterized by loss of tolerance to self-antigens and production of high-titers serum autoantibodies with multifactorial etiology, which mainly affect women. The exact etiology of SLE is still unknown but there are various factors that might contribute to the onset of the disease and disease flare [8]. These include a number of environmental factors such as cigarette smoking, alcohol, chemicals and biochemicals, UV light, hormones, and infections caused by viruses, bacteria, and vaccine; all these contribute to induced lupus and disease flares [9]. These environmental factors trigger SLE by altering the epigenetic mechanism [10]. Epigenetic mechanisms which might trigger SLE include histone modification and DNA methylation, in which the cytosine base of DNA undergoes methylation and modification of histone tails including deacetylation, ubiquination, and tri-methylation [11]. In SLE, hypomethylation of DNA from CD4 + T cells takes place and as a result, T cells can function as autoreactive in response to self-class MHC II molecules [12].

UV light causes the main symptoms of SLE and triggers its onset [13]. This ability to induce this disease is dose related, meaning that more radiation causes greater severity in the disease [14]. If UV dose is low, normal apoptosis takes place in keratinocytes, while in high or moderate concentration, fragmentation of DNA, elevation in the expression of IL-1α, and necrosis of keratinocytes take place [14]. In conclusion, intermediate and high dose of UV light causes pro-inflammatory apoptosis and necrosis followed by the discharge of autoantigen and pro-inflammatory cytokines, which might trigger various inflammatory responses [14]. Smoking is also linked with increased risk of SLE and discoid lupus [15]. In addition to the relation between the risk of development of SLE and smoking, smoking is also associated with skin flares in patients with SLE [15]. Smoking also decreases the efficacy of antimalarials, but induces cutaneous lupus erythematosus [16]. No clear link has yet been established

between the potential risk of SLE and alcohol consumption because the habit of smoking and alcohol often coexist, which interferes with the exact interpretation of the coexisting risk of development of SLE and alcohol habit [17]. One of the earlier studies has shown that neither past nor current alcohol consumption was associated with the development of SLE [18]. In an Internet-based study, it was found that current drinking habits are inversely associated with the development of SLE [19]. Certain medications are also known to induce lupus-like symptoms. Drug such as procainamide, which is an anti-arrhythmic drug, might induce lupus-like syndrome by acting as an inhibitor for DNA methyltransferase in human T cell lines [20]. Anti-TNF has been used in the treatment of inflammatory arthritis and is known to cause anti-TNF-induced lupus [21]. In addition, recent data suggest the role of estrogen and their metabolites in the pathogenesis of lupus [22–26].

3. Rheumatoid arthritis (RA)

Rheumatoid arthritis (RA) is a chronic inflammatory disorder in which inflammatory response develops especially in synovial joints. In this disease, the immune system mistakenly attacks our own body's tissues. It affects mainly the lining of the joints and results in painful swelling of the joints that in turn results in bone erosion and causes joint deformity. The inflammation associated with the joints can damage the other parts of the body as well. There are various disorders associated with RA [27]. These include depression, fatigue, malaise, and anorexia. In addition to that, there are various complications including cardiovascular and hematologic complications, neurological problems, respiratory system disorders, and various other complications associated with RA. RA is found worldwide and affects nearly about 1% of the world population [28]. Women are more likely to have this disease, and it generally occurs at older age. Rheumatoid arthritis generally occurs at an age in between 30 and 50 years of age and its incidence varies among different populations. The actual cause of this disease is unknown but it is assumed that it probably occurs if a genetically susceptible host gets exposed to an environmental antigen [29]. This might generate immune response leading to the formation of various types of immune complexes, which generate inflammation in the joints. Recently, one study showed how inflammation occurs in joints of these rheumatoid arthritis patients [30]. Synovial cells produced high concentration of 16α-hydroxyestrone in rheumatoid arthritis patients. Despite normal concentration of 16α-hydroxyestrone in serum and urine, there is an elevated 16α-hydroxyestrone found in the synovial tissues, where activated immune cells are present. Therefore, 16α-hydroxyestrone combined with histone resulted in the formation of 16α-hydroxyestrone-histone adduct that might generate autoantibodies against this antigen. As a result, these autoantibodies trigger inflammation in the joints of RA patients [30]. Some studies also showed that viral or bacterial infections could act as potential environmental culprits to cause RA. Immunization with type II collagen also caused RA in experimental animals. Some patients of RA have shown that autoantibodies directed against heat shock proteins might show cross-reactivity with the bacterial antigen. There are numerous signs and symptoms associated with RA [31]. The most commonly affected joints in RA include fingers, feet, wrists, elbows, ankles, and knees. Shoulder, hip, and cervical spines are among those joints

that are affected later. The inflammation affects the joints to such an extent that these joints have pain during movement, swelling, and stiffness that last for hours. RA might also cause cardiovascular complications such as the development of acute necrotizing arteritis, thrombosis of the blood vessel leading to myocardial infarction, stroke or mesenteric insufficiency. Because RA mainly afflicts the joints, patients with RA should be encouraged to remain active and avoid heavy work [31]. Some exercises are recommended to maintain normal function of the joints and anti-inflammatory drugs are given to relieve from pain and swelling in the joints. Disease-modifying anti-rheumatoid drugs (DMARDS) should be given if the disease goes beyond 2 months. For mild conditions, hydroxychloroquine/minocycline is given, while for moderate disease, methotrexate followed by tumor necrosis factor (TNF) inhibitors is given.

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Chapter 2

Molecular Imaging

Leszek Królicki and Adrian Michno

Additional information is available at the end of the chapter

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Abstract

Mechanisms responsible for the course of the rheumatic diseases have not been fully explained. Among the available tools that may help in studies of these mechanisms is molecular imaging—especially techniques emphasized by nuclear medicine. In contrast to CT, MRI or US examination that show only structural pathologies, radionuclide methods allow imaging of functional changes that occur in the course of the disease and usually are featured by a very high specificity. Recent advances in nuclear medicine allowed to develop target-specific agents making it possible to reveal molecular level disturbances that take place on the course of the ongoing disease. The fundamental radionuclide studies include PET, SPECT, and classic scintigraphy. Technological advances (especially hybrid modalities) allow obtaining images of much better resolution and allow combining both structural and functional data.

Keywords: PET, SPECT, markers of inflamation, radiotracers cordially

1. Introduction

Mechanisms responsible for the course of the rheumatic diseases have not been fully explained till today. Molecular imaging sits among the available tools that may help in studies of these mechanisms, particularly techniques emphasized by nuclear medicine.

In contrast to such imaging modalities as CT, US or MRI, that reveal only structural pathologies, radionuclide methods enable imaging of functional changes that occur in the course of the disease and more importantly are usually featured by a very high specificity. Radionuclide studies also make it possible to determine the changes in the molar concentration of specific chemical compounds up to 10^{-11} or even 10^{-3}–10^{-4} if an MRI is used. It means that radionuclide studies enable the performance of diagnosis of disorders in the molar concentration of the

specific chemical compounds that take place in the course of the disease. It is important to note that the functional character of disorders should be the basis for the choice of treatment.

Over the last several decades, the introduction of new biological agents has greatly improved the effectiveness of treatment. Those agents influence the activity of specific receptor and metabolic pathways [1]. Along with the application of new agents, it has become necessary to introduce new diagnostic methods, which allow evaluation of the activity of those biological processes. Unfortunately, neither clinical examination nor laboratory tests nor conventional X-ray does not provide such findings. A clinical evaluation can be highly subjective; laboratory tests are frequently non-specific and inconsistent, while conventional X-ray shows structural changes only at the advanced stage of the disease.

It seems that the introduction of molecular imaging techniques may lift those limitations. The fundamental methods of molecular imaging are radionuclide studies. Recent advances in nuclear medicine allowed to develop target-specific agents making it possible to reveal molecular level disturbances that take place on the course of the ongoing disease [2]. The fundamental radionuclide studies include a PET, SPECT, and classic scintigraphy. Moreover, hybrid devices are used more frequently in diagnostics these days—these consist of gamma camera and CR/MRI. Thanks to hybrid techniques, it is possible to perform CT/MRI combined with scintigraphy (for example PET-CT, PET-MRI, and SPECT-CT). It is also important to mention that the combination of those techniques allows applying attenuation correction of the absorbed radiation that is being emitted by radioisotope, as well as making it possible to determine the exact location of the abnormal uptake of the radiopharmaceutical. Finally, the images obtained are of very high quality.

Technological advances allow obtaining images of much better resolution and allow combining both structural and functional data. Vogel et al. noted in his study that hybrid imaging technique ^{18}F-FDG PET-CT, apart from providing the capability of assessing the degree of inflammation localized in the tarsus of a rheumatoid arthritis (RA) patient, also allows precise localization of the disease activity in particular joints [3]. Neither physical examination nor X-ray can provide such relevant data. Furthermore, ^{18}F-FDG PET/CT allows to visualize and diagnose metabolically active subcutaneous nodules, activated lymph nodes or other affected joints in the course of the disease (such as those of the knee or atlantoaxial joints). This method allows better discrimination between juxtaarticular disease and articular processes as well as delineation of tendon sheath and bursal inflammation. Furthermore, this method makes it possible to delineate inflammation of the tendon sheaths and bursae. Miese et al. [4] also assessed the high suitability of PET/MRI apparatus in his work about the diagnosis of RA, in which he showed increased uptake of ^{18}F-FDG in the corresponding metacarpophalangeal joints that were affected by synovitis and tenovaginitis as identified on contrast-enhanced MRI.

MRI is second modern imaging modality that is very useful in the diagnosis of rheumatic diseases. This method features a very high resolution and similarly good contrast between the soft tissues. Thanks to the new sequences and specific contrast agents, and modern MRI allows imaging of vast amounts of pathologies.

Both of these methods provide the information about molecular pathological mechanisms that accompany the disease; thus allowing a better understanding of the pathophysiology of rheumatic diseases. Moreover, they allow a search of the new forms of molecular treatment

that allow for an early and accurate prognosis as well as monitoring of therapy [5]. Beyond any doubts, another important feature of these methods is the capability of making the diagnosis at the subclinical phase of the disease, thanks to which a proper treatment can be started long before irreversible morphological changes occur.

Procedures offered by nuclear medicine meet the number of important expectations associated with the development of modern medical sciences, such as:

a. The capability of putting a considerably early diagnosis of pathological changes as well as the determination of their character, which is crucial to make an appropriate diagnosis before irreversible structural changes occur.

b. The ability of accurate determination of the degree of pathological changes.

c. The ability to predict the course of the disease and the subsequent introduction of personalized therapy.

d. The reduction of constantly increasing the costs of medical procedures by use of such evaluation tools that provide a good assessment of the effectiveness of the applied treatment.

e. The need of the determination of remission of the pathological changes as well as an early diagnosis of their relapse.

Taking these facts into account, it is clear that radionuclide studies and MRI have proven to be useful in solving all of the clinical issues. Moreover, it is important to note that it meets economic and pharmacological criteria for cost-effectiveness.

2. Radiopharmaceuticals

2.1. ^{18}F-FDG

The most commonly used radiopharmaceutical used in PET-CT studies is fluorodeoxyglucose (^{18}F-FDG), which is a structural glucose analog. Areas of increased tracer uptake indicate the intensification of metabolic processes associated with increased glucose demand. This feature can be found both in a tumor and inflammatory setting. Increased uptake of ^{18}F-FDG is associated with increased activity of the GLUT1 and GLUT3 transport mechanisms as well as the activity of hexokinase—these phenomena are typical for cells that undergo fast proliferation as well as other types of cells such as macrophages, neutrophils and young granulation tissue [6–8].

2.2. ^{11}C-choline

Choline is another tracer that features high sensitivity for proliferative processes that occur in the course of the disease. This tracer is uptaken by quickly dividing cells where it undergoes phosphorylation by choline kinase into phosphorylcholine. Choline is essential for phospholipids synthesis, especially phosphatidylcholine (also known as lecithin) which serves as the building block of the cell membranes. Increased uptake of choline depends on the

mechanisms responsible for active transport via the cell membrane as well as on congestion-dependent passive diffusion. It has been found that increased uptake can be seen even in the early phase of the inflammation. Once present, its grade of accumulation corresponds with both the extent of the contrast-enhanced signal in an MRI study (with Gadovist as a tracer) and the accumulation of ^{18}FDG [9].

2.3. ^{67}Ga-citrate

This radiopharmaceutical has been used in both the diagnosis of cancer as well as acute and chronic foci of inflammation for several years. Gallium (administered via i.v. infusion) acts as an iron analog that binds to transferrin, ferritin, and leukocytes (primarily neutrophils) [10]. These molecules accumulate within the foci of inflammation secondary to increased capillary permeability. At the time when ^{67}Ga-bound macromolecules reach the inflammatory interstitial space, the isotope undergoes the process of transchelation into lactoferrin, ferritin and bacterial siderophores (if present). Moreover, lactoferrin is then being secreted by neutrophils that are present in the inflammatory foci caused by the disorders of the connective tissue [11]. Recently, ^{67}Ga-citrate is rarely used because of the vast availability of other—frequently much better—markers as well as other factors such as the unfavorable energetic profile of the emitted radiation, the long-lasting radioactivity of the blood (due to high affinity to the white blood cells) and relatively long half-life time. This results in significantly increased absorbed radiation dose in comparison to other radioisotopes, not to mention low quality of the obtained images. Another disadvantage of ^{67}Ga-citrate is the lack of specificity for inflammation because it accumulates similarly in neoplasms.

2.4. ^{99m}Tc and ^{111}In HIG

Both 99m-technetium and 111-indium can be used for labeling of human (polyclonal) immunoglobulins (HIG). HIG accumulate in the inflamed tissues because its mechanism of accumulation is related to the increased permeability of the vascular capillaries as well as increased blood flow to the inflamed area. This technique features very high sensitivity, not to mention that the grade of its accumulation highly corresponds to the severity of the inflammation, hence making it a useful prognostic tool. On the other hand, one of the disadvantages of this radiopharmaceutical includes lack of specificity [12].

2.5. ^{99m}Tc-diphosphonates

Similarly to gallium-67, ^{99m}Tc-diphosphonates are non-specific tracers used in the diagnosis of the inflammation—its mechanism of uptake relies on the metabolism of the osseous cells—its degree of uptake depends on the activity of osteoblasts (hence its perfect for imaging of the bone turnover). Bone scintigraphy is readily available and cheap method of evaluation of bones [13, 14]. As the severity of the inflammation progress, the uptake increases. In the setting of inflammation evaluation, the bone scan comes in the form of a triphasic examination, which consists of vascular, parenchymal, and late (bony) phase (**Figure 1**). Each phase presents a specific aspect of the pathology:

- Phase I—hyperemia,
- Phase II—increased volume of the vascular bed,
- Phase III—metabolic turnover and remodeling of the osseous tissue as a result of the ongoing pathology (inflammation and destruction of the cartilage).

This method is highly sensitive but features low specificity, which can be improved by hybrid SPECT-CT technique if one is available.

It is important to note that the accumulation of described radiopharmaceuticals in tissues affected by inflammation is non-specific because these tracers accumulate similarly in neoplastic foci. Due to that fact, there is recently ongoing research that aims to develop markers specific for inflammation occurring in the course of the rheumatic diseases.

2.6. Labeled leukocytes

Since the inflammation features the migration of leukocytes, there was an idea to use that mechanism in the diagnosis and localization of the inflammatory foci. Nuclear medicine has

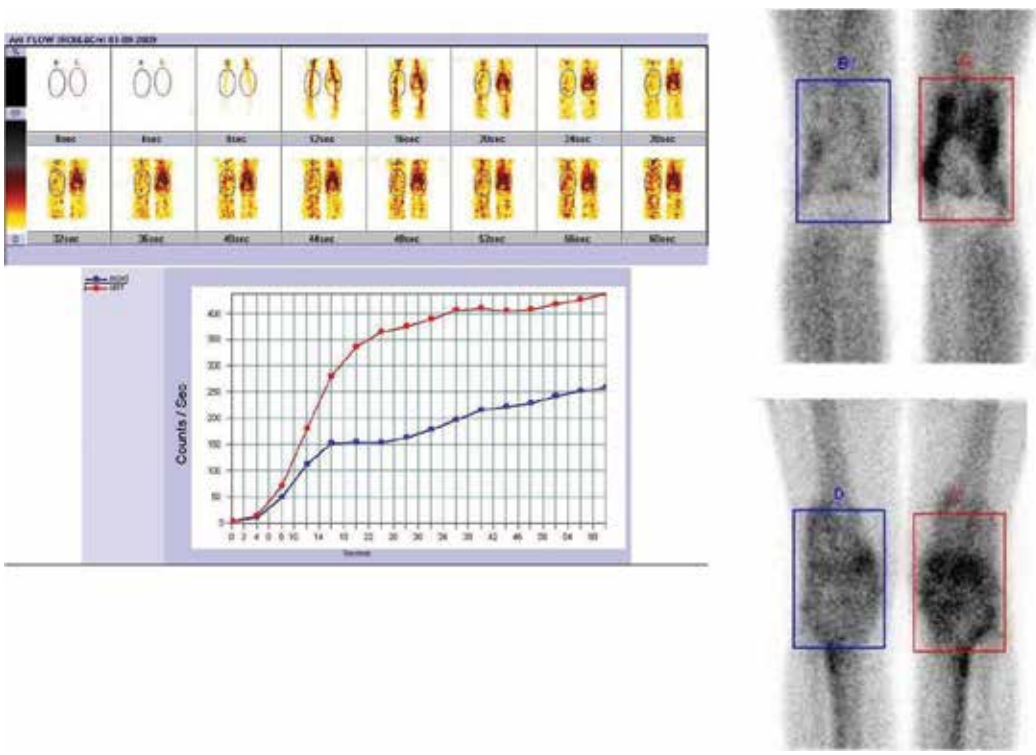

Figure 1. Triphasic bone scan after ^{99m}Tc-MDP administration in patient with RA shows increased blood flow (A), blood pool (B), and increased accumulation of the radiotracer in the bony phase (C) which indicates increased bony turnover in the affected left knee.

come to meet this need and has developed a radionuclide examination that makes it possible to label leukocytes of the patient (both in vitro and in vivo depending on the technique). In the setting of inflammation, neutrophils and monocytes are being recruited, and due to the phenomena of chemotaxis, they accumulate in the foci of inflammation. Thanks to this mechanism of accumulation, labeled leukocytes can be used in the diagnosis of RA as well as other inflammatory diseases. Labeled leukocytes emphasize two main techniques—in vivo and in vitro assay.

- *In vitro assay* is a technique that involves taking a blood sample, isolating a leukocyte suspension, labeling those leukocytes with ^{99m}Tc-HM-PAO or ^{111}In-oxine, and intravenous administration back to the patient. It is important to note that this procedure does not affect chemotaxis.
- *In vivo assay*, on the other hand, involves intravenous administration of ^{99m}Tc-labeled monoclonal antibodies against the specific NC-1 antigen that is present on the granulocyte cell membrane. In this procedure, the labeling process occurs directly in the blood of the patient. Moreland et al. [15] in his work have shown that there is a direct correlation between the foci of increased accumulation and the clinical picture in the course of rheumatoid arthritis. This examination may also be used as a tool to control anti-inflammatory treatment [15].

2.7. ^{11}C-PK11195

One of the proposals involved the introduction of ^{11}C-PK11195 isoquinoline carboxamide—this radiopharmaceutical binds to both monocytes and macrophages and serves as peripheral benzodiazepine receptor (PBR) antagonist also known as translocator protein (TSPO). This peptide is particularly active on the outer surface of the mitochondrial membrane of the activated macrophages, polymorphonuclear cells as well as nervous and lymphatic tissue [16–18]. It is responsible for the process of steroidogenesis, apoptosis, cell proliferation, and immune response. It has also been proved that this radiopharmaceutical is an effective marker in neuroinfection, due to the fact that peripheral benzodiazepine receptors can be typically found on activated glial cells.

2.8. ^{99m}Tc-J001X

Other noteworthy markers are those with the ability to label the macrophages. These kinds of cells are particularly active in inflammation based on the rheumatic disorders and play a major role in the inflammatory process. It was found that macrophages bind specifically to the proteoglycans of the bacteria. Hence a technique of proteoglycans labeling has been developed [19, 20].

Recently used tracer is called J001X—which is a poly-(1,3)-D-galactoside isolated from the cellular membrane of *Klebsiella pneumoniae*. This ^{99m}Tc labeled-substance allows tracing of the mononuclear phagocytes.

The effectiveness of this radiopharmaceutical in the diagnosis of RA-associated lung pathology appears to be promising, but until today there is no randomized study that would confirm its effectiveness. Lastly, this tracer was also used in imaging of sarcoidosis and scleroderma [21].

2.9. ^{99m}Tc-RP128

^{99m}Tc-RP128 is a peptide tracer used for imaging of leukocytes recruitment used for labeling of neutrophils and mononuclear phagocytes. ^{99m}Tc-RP128 is a pentapeptide tuftsin analog antagonist (TKPPR) that mediates the receptor-specific interaction and subsequently binds to tuftsin receptors. Tuftsin is an organic chemical compound consisting of amino acid residues such as threonine, lysine, proline, and arginine. It is produced by the spleen and its function is to stimulate macrophages and granulocytes to phagocytosis and chemotaxis. Tuftsin is derived from proteolytic cleavage of the Fc domain of the heavy chain of IgG. Tuftsin receptor's function is to mediate the immune functions; hence they represent important molecular targets. The mechanism of RP128 imaging is based on the upregulation of tuftsin receptors located in activated macrophages. Chaudhuri et al. [22] noted that the affinity of radiotracer is fourfold greater than their endogenous ligand. Despite that fact, this radiotracer was described only in a few works; thus there is a need for further research that would confirm its utility. Studies show that it accumulates in other organs to an only small extent (except kidneys). The grade of accumulation in healthy joints was moderate—in contrast to the affected joints, which featured a very high uptake. The sensitivity of the scan was 69% for swollen joints, 76% for painful joints, and 73% for joints with bone erosions [23].

2.10. ^{99m}Tc- and ^{111}In anti-E-selectin

Adhesion is another mechanism used in the diagnosis of the inflammation. Molecules responsible for this phenomena cause leukocytes to bind to the activated endothelial vessels resulting in their transendothelial migration. One of such molecules that are used in the diagnosis of the inflammation is E-selectin labeled with ^{99m}Tc or ^{111}In. E-selectin (CD62E, ELAM1) is a transmembrane glycoprotein that is transiently expressed on the luminal surface of activated vascular endothelium during a normal inflammatory response. E-selectin mediates the initial tethering and rolling of granulocytes, monocytes, and some lymphocytes via specific interactions with its carbohydrate-based ligands. It is then activated by interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and bacterial LPS—it has to be noted that this may occur only in the setting of inflammation [24]. ^{111}In-1.2B6 monoclonal antibody (mAb) is another antibody that was used for imaging of selectin activity. Since this is a murine antibody, the administration of this radiopharmaceutical comes with a risk of developing human anti-mouse antibody (HAMA) response, a possibly life-threatening state that requires immediate medical attention. This fact limits the repetition of the study in patients in whom treatment control is necessary. Therefore, F(ab')2 fragments of 1.2B6 mAb—devoid of its Fc portions—were introduced [25].

2.11. Octreotide

Octreotide is a long-acting somatostatin analog that targets the activation of endothelium and macrophage recruitment at any of five identified G-protein-coupled somatostatin receptor subtypes (SST1–5). Hyper-expression of these receptors has been thoroughly documented in numerous pathological conditions hence it can be considered as a basis for octreotide imaging. The synovia of affected joints in RA patients features overexpression of somatostatin receptors—those that are targeted by octreotide. The SST2 expression on activated venule of endothelial cells and infiltrating mononuclear phagocytes have been identified in histological and immunochemistry examinations both in the affected synovia and fibroblast-like synovial

cells. It is important to note that patients with favorable treatment results also show significantly lower expression of the somatostatin receptors; thus somatostatin scintigraphy may also be used in the prediction of the effectiveness of the treatment [26].

Somatostatin receptor expression also features a good correlation with the clinical data—its sensitivity estimates 75%. Vanhagen et al. [27] also noted that no radiotracer accumulation was observed in the joints of healthy individuals. Numerous works point that somatostatin analogs may be used in RA treatment as well. It has also been found that the administration of somatostatin analogs reduced the symptoms of the disease—presumably due to the inhibition of IL-6 and IL-8 production as well as inhibited proliferation that occurs in the affected synovium. Therefore, somatostatin scintigraphy may be used as a tool in the prediction of the somatostatin analog treatment effects, although until today there has been no randomized study to confirm it. Therefore, more research is required before its utility may be introduced to the guidelines [28].

2.12. Other radiopharmaceuticals

2.12.1. Anti-CD-3

Since mature T lymphocytes play a role in the development of RA, some radionuclide studies make it possible to label monoclonal antibodies against CD3 and T lymphocyte migration into the affected synovium. Recent advances allow Muromonab to be labeled with ^{99m}Tc and use it for imaging of rheumatic disease. It is essential to note that this examination correlates well with physical examination and patient history [29]. Moreover, scintigraphy with this tracer can be used in differential diagnosis of juvenile idiopathic arthritis and RA as well as other rheumatic diseases.

2.12.2. ^{99m}Tc-anti-CD4 mAb

Anti-CD4 imaging is also used for the evaluation of T lymphocyte infiltration. CD4 is expressed on the cell surface of mature T helper cells, thymocytes, and dendritic cells [30]. ^{99m}Tc-MAX.16H5, murine IgG1 was used in patients and showed affected joints in all RA patients. Studies indicate that the sensitivity of the ^{99m}Tc-MAX.16H5 scan is better than the bone scan.

2.12.3. ^{99m}Tc-Anti-CD20 mAb

Almost 95% of circulating normal and malignant B lymphocytes expresses CD20 antigen [31]. Furthermore, its expression is exclusive to B lymphocytes simultaneously featuring the lack of expression in the hematopoietic stem cells. Due to this fact, Rituximab as a mouse/human monoclonal antibody can be labeled with ^{99m}Tc and used for B cell infiltration imaging [32].

2.12.4. TNF-alpha

TNF plays an essential role in the development of RA [33]. The promising results have been described in studies which involved the use of ^{123}I-anakinra, infliximab (a monoclonal antibody that binds to membrane-bound and soluble TNF [34]) and ^{99m}Tc-Adalimumab.

3. Clinical application of radionuclide studies in the setting of rheumatoid arthritis and other inflammatory diseases

3.1. Rheumatoid arthritis

3.1.1. Diagnosis and disease progression

In vitro studies of Matsui et al. [35] has shown that in the setting of a murine model of collagen-induced arthritis, the peak of ^{18}F-FDG uptake occurs both at the stage of pannus creation and during the destruction of the bone caused by inflammation caused by proliferating fibroblasts. Another prominent role of macrophages includes the fact that their inactivated form accumulates ^{18}F-FDG merely to a small extent, while glucose demands after their hypoxia-induced activation increases significantly.

The degree of ^{18}F-FDG uptake in correlation to the disorder severity:

- Moderate uptake can be seen in the initial period of the disease progression, at which interstitial inflammatory cells recruitment, synovial cells hyperplasia, and edema can be seen.
- Uptake of ^{18}F-FDG noticeably increases as the bony destruction and pannus creation progresses.

It has been shown that the highest grade of ^{18}F-FDG accumulation not only is related to the proliferation of fibroblasts but also to the neutrophils as well. On the other hand, resting macrophages feature moderate accumulation of ^{18}F-FDG. In the setting of hypoxia, the activity of various inflammatory cells changes, while the activity of proinflammatory cytokines (such as TNF-alpha) increases. It has been observed that in these conditions there is increased ^{18}F-FDG uptake by macrophages and fibroblasts, while in the case of neutrophils, it remains at the background level. T cells accumulate ^{18}F-FDG to a small extent regardless of the microenvironment.

Summarizing, the degree of ^{18}F-FDG uptake correlates with the activity of proliferating fibroblasts as well as macrophages activated by hypoxia; hence allowing the ^{18}F-FDG study to be used in the evaluation of the disease severity.

Beckers et al. [36] assessed that the sensitivity of this technique in the setting of rheumatoid arthritis equals approx. 90%. Some works indicate that the study allows the identification of lesions in the subclinical phase of the disease as well as at the stage of its clinical remission. This fact plays a particularly important role in the treatment [37].

^{18}F-FDG PET-CT study also allows the assessment of the disease severity in other maladies, such as spondyloarthritis, polymyalgia rheumatica, Still's disease, polychondritis, IgG4-related disease, polymyositis, and dermatomyositis [38].

Some of the studies aimed to assess the usefulness of ^{11}C-choline as a marker for both the diagnosis and the severity assessment tool in rheumatic diseases. These indicated that it might be a good marker for the proliferation progress, which occur not only in the setting of tumors but also in the rheumatic conditions as well.

Roivainen et al. [39] made a comparison between ^{11}C-choline PET-CT to ^{18}F-FDG and gadolinium-enhanced MRI. The authors have shown that there is very high compliance between the pharmacokinetics of ^{18}F-FDG and ^{11}C-choline at the site of the affected joints. Regardless of the clinical symptoms of the inflammatory process, the accumulation of both markers occurred in the same joints that featured a clear contrast enhancement in the MR study. Moreover, authors state that ^{11}C-choline may be a very promising tracer for quantitative imaging of proliferative arthritis changes. However, to characterize the relationship of PET-CT results with the clinical and functional measures of inflammation, a subsequent prospective study involving a larger number of patients is necessary [39]. Among other radiopharmaceuticals, ^{11}C-(R)-PK11195 isoquinoline carboxamide is also being used for both the diagnosis of RA as well as an assessment of the disease severity. It was noted that this tracer tends to accumulate primarily in the activated macrophages and its degree of uptake highly corresponds to the severity of synovitis priorly assessed by histopathological examination. This examination turned out to be highly sensitive in both localizations of acute phase inflammation spots and the assessment of initial phase of the disease. It is considered that increased PET signal in inflamed joints occurs as a result of specific PBR-mediated uptake of ^{11}C-(R)-PK11195 caused by activated macrophages.

Van der Laken et al. [40] performed one of the first studies in the setting of the rheumatic disease. The authors concluded in their work that ^{11}C-(R)-PK11195 uptake on the PET scans was significantly higher in severely inflamed joints in comparison to those with moderate or mild signs of inflammation. Additionally, tracer uptake in contralateral, unaffected by inflammation knee joints of RA patients was significantly higher than in joints of healthy individuals from the control group (with no history of inflammatory joint disease or the presence of any subclinical disease activity). PET tracer uptake in the affected joints is highly correlated with PBR staining of sub-lining of synovial tissue, which also proves its correlation to CD68 staining of macrophages.

It is believed that this tracer may allow imaging of the ongoing pathology prior to its clinical manifestation due to the fact that macrophages infiltration into synovial joints is a common feature of asymptomatic synovitis in early RA [41, 42]. Furthermore, the application of ^{11}C-(R)-PK11195 imaging to RA may prove to be relevant to patient management since the presence and the number of macrophages in rheumatoid synovium strictly correlates with the progression of joint erosions that can be seen in the X-ray. Another use of this trace encompasses diagnosis of subclinical synovitis in patients with arthralgia. Last but not least, increased uptake of the tracer correlates with the progression of the disease.

3.1.2. Treatment control

Numbers of works indicate that ^{18}F-FDG is an excellent marker in the assessment of treatment effectiveness. The utility of ^{18}F-FDG as the marker in this assessment was suggested by Palmer et al. back in 1995 [43]. The authors in their work studied the influence of prednisolone and subsequent methotrexate treatment on findings in MRI and ^{18}F-FDG PET-CT. Results showed that as the volume of the pannus seen on MRI decreased, so did the uptake of ^{18}F-FDG—not to mention the improvement of the clinical picture (such as reduction of pain sensation,

stiffness, and edema). However, none of the studied parameters correlated with the outcome of the treatment. Authors concluded that both morphological pictures visible in MRI study and the functional test of PET study allow the assessment of the quantitative effectiveness of the applied therapy.

Similar results were presented in 2004 by Beckers et al. who have shown a direct correlation between the clinical picture of a joint with the findings of an ultrasound examination and the degree of ^{18}F-FDG uptake in PET-CT [36]. Additionally, as it turned out, the degree of ^{18}F-FDG uptake in the affected joint highly correlates to the thickness of the synovial membrane priorly assessed in the ultrasound examination. Moreover, another correlation has been found between the number of joints with increased ^{18}F-FDG uptake (with consideration of their total uptake value) and the duration of the disease together with the degree of severity. In 2004, Brenner et al. indicated in his work that despite confirmed correlation between ^{18}F-FDG uptake and the effectiveness of the treatment, PET-CT is still not recommended as a routine evaluation tool due to its high costs and limited availability [44]. This technique would be more indicated if the findings could provide parameters that are not obtainable by other tests (such as MRI, bone scan or ultrasound examination). However, authors pinpoint few of such parameters, that is, the possibility of quantitative assessment of disease severity of each affected joint and the assessment of disease progression as well as monitoring of treatment effectiveness. Authors emphasize that further studies are necessary for the better determination of PET-CT study indications.

Beckers et al. [36] presented a noteworthy work which aimed to study the response to the anti-TNF-alpha treatment [44]. Authors in their work showed a significant correlation between the degree of ^{18}F-FDG uptake, MRI findings, synovial membrane thickness, and the concentration of matrix metalloproteinase (MMP)3 and CRP levels. Similarly, Elzinga et al. [45] showed in their work that decreased uptake of ^{18}F-FDG after application of anti-TNF therapy is an important prognostic factor that indicates the effectiveness of the treatment.

3.1.3. Prognosis

Elzinga et al. [45] concluded that decreased ^{18}F-FDG uptake in the metacarpophalangeal and wrist joints 2 weeks following the infliximab (anti-TNF-alpha) therapy allowed to predict the outcome of the treatment after 14 and 22 weeks. It was all possible to achieve that because of the presence of clear correlation between the fall of the tracer uptake and the severity of the disease, which has subsequently contributed to the development of disease activity score (DAS). However, this type of correlation was not found in later observations; thus it requires further research. Perhaps another radiopharmaceutical will turn out to be more useful in prognosis of anticipated outcomes of the given treatment.

3.2. Diagnosis of concomitant diseases

A PET-CT study is a useful tool in the diagnosis of concomitant neoplastic disorders in patients with lupus, systemic sclerosis, dermatomyositis/polymyositis or Sjögren syndrome. Epidemiological data in these groups of patients showed the occurrence is noticeably higher; especially lymphoma, pharyngeal, and pancreatic cancer [46–48].

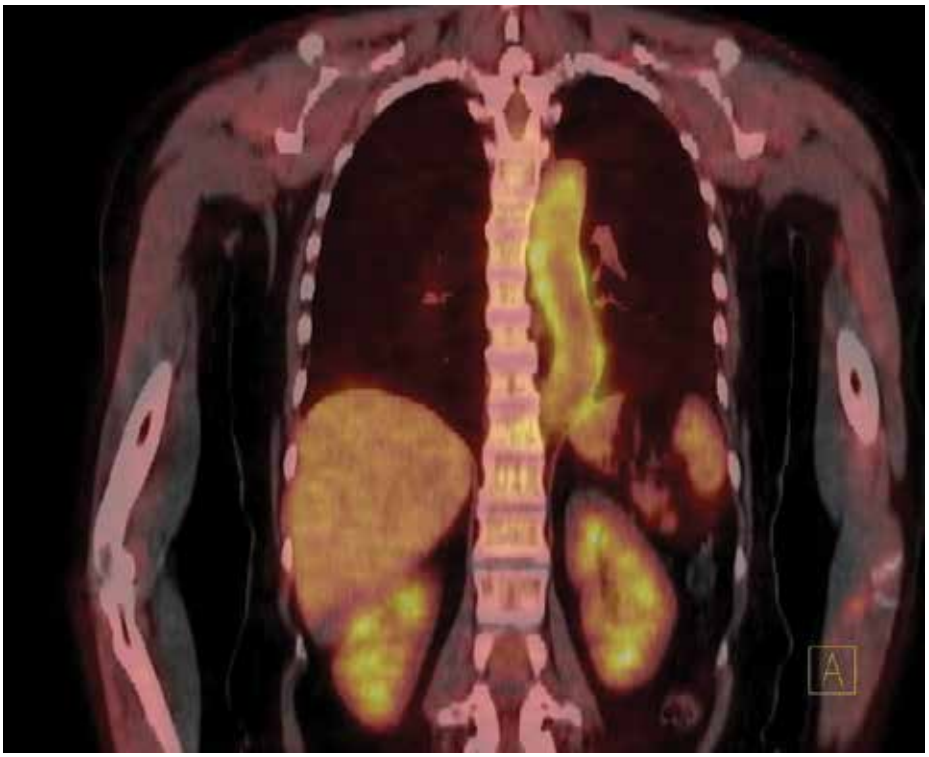

Figure 2. 18F-FDG PET-CT in patient with RA shows increased accumulation of the tracer in the wall of the aorta.

Autoimmune disease treatment involves the use of immunosuppressants, which aim to lower the immune response of the organism, simultaneously increasing the risk of infection (including a higher risk for relapse of tuberculosis) [49]. ^{18}F-FDG PET-CT study turns out to the most sensitive test for the diagnosis of infection. This examination can show abnormal focal uptake of the tracer in up to 90% of the patients suffering from autoimmune diseases; sensitivity and specificity in the diagnosis of neoplastic disorder equal 100 and 67%, respectively [50].

3.3. Fever of unknown origin

^{18}F-FDG PET-CT also showed to be promising in the diagnostic process in patients with the symptoms of FUO. In cases where other imaging studies showed to be inconclusive, ^{18}F-FDG PET-CT allows for the localization of pathological foci (either inflammation or a tumor based) in approx. 47% of the patients. Positive predictive value of this study has been assessed for 78% while negative predictive value—for 88% [51].

3.4. Vasculitis

Suspected large vascular vessels vasculitis is another indication for ^{18}F-FDG PET-CT. The term of vasculitis emphasizes the numbers of diseases, out of which Takayasu arteritis together with giant cell arteritis accounts for the most common types of vasculitis. Sensitivity and

specificity of ^{18}F-FDG PET-CT in this setting equal 90%. In a meta-analysis performed by Balink et al. [52], the authors point out that sensitivity of this modality is higher than in any other imaging method (**Figure 2**).

4. Conclusion

Indeed, we can say that recently used studies such as MRI, CT or US in the setting of rheumatology are the marriage of convenience while radionuclide studies may be considered as a marriage of love. Such complex disease processes that occur in rheumatic diseases require comprehensive data that can be obtained only by procedures from the field of nuclear medicine.

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Chapter 3

Raynaud's Phenomenon

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Additional information is available at the end of the chapter

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Abstract

Raynaud's phenomenon (RP) is a clinical consequence of recurrent vasospasm of the small arteries and arterioles of the fingers and toes provoked by cold and emotional stress. RP is classified into two categories, i.e., primary and secondary RP. Primary RP is an isolated finding in the absence of an underlying pathology, while secondary RP is a syndrome in the context of another disease. The patients with primary RP have a younger age at onset, sparing of the thumb, and benign course without development of digital ulcers. Contrarily, secondary RP is characterized with later age of onset above 30 years, thumb involvement, and more severe course with possible development of trophic changes. In these cases, a focused, complaint-directed history and physical examination aim to reveal clinical symptoms and findings that confirm the presence of an underlying disorder, e.g., connective tissue disease (CTD) or other pathology. Together with clinical examination, laboratory, immunological, and capillaroscopic assessments facilitate the internal differential diagnosis of secondary RP. The capillaroscopic examination should be performed in all patients with symptoms of RP even in those cases without signs of systemic rheumatic disease, because the abnormal capillaroscopic picture inherits a high positive predictive value for the development of CTD.

Keywords: Raynaud's phenomenon, primary, secondary, peripheral vascular syndromes, capillaroscopy

1. Definition

The term "Raynaud's phenomenon" (RP) originates from the name of the French physician Maurice Raynaud, who described transient digital ischemia provoked by cold exposure and hypothesized that the changes are associated with exaggerated response of the central nervous system. Currently, the term "RP" defines the clinical consequence of recurrent vasospasm of the small arteries, arterioles, and arteriovenous shunts of the fingers and toes provoked by

cold and emotional stress [1]. Other acral parts, e.g., the nose, lips, and ears, may be also affected. It manifests usually in three phases, ischemia, asphyxia, and reactive hyperemia, during which skin color changes occur consecutively from white to blue and red. A characteristic feature of RP is the clear demarcation between the affected and unaffected area.

2. Diagnosis of Raynaud's phenomenon

The diagnosis of RP is clinical and is based on direct observation of the vasospastic attacks [1]. Photographs that document the vasospastic attacks could also be used to confirm the history [2]. In routine clinical practice, it is not necessary to perform a cold provocation test to make a definite diagnosis of RP [1].

Observation of at least biphasic color changes is necessary for the diagnosis, as pallor and cyanosis are considered to be the most important signs. In a recent international consensus for the diagnosis of RP (Maverakis et al. [3]), a three-step approach for the diagnosis of RP has been suggested. The first two steps include asking more general questions: (I) a question about unusual sensitivity of the fingers to cold and (II) a question about "occurrence of biphasic color changes during the vasospastic episodes (white and blue)." Finally, during step III, the physician calculates the disease score by asking seven questions to the patient ((1) episodes are triggered by factors other than cold, i.e., emotional stress; (2) episodes involve both hands even if they are asynchronous or asymmetric; (3) numbness and paresthesias accompany vasospastic attacks; (4) well-demarcated border between the affected and unaffected skin; (5) photographs provided by the patient; (6) vasospastic episodes that affect other body parts such as the nose, ears, feet, and areolas; (7) occurrence of triphasic color changes during vasospastic attacks, e.g., white, blue, and red). If the score from step III is ≥3, the patient is diagnosed with RP [3].

3. Classification of Raynaud's phenomenon

RP is classified into two categories, i.e., primary and RP [1, 4, 5, 6].

Primary RP is an isolated finding in the absence of an underlying pathology, while secondary RP is a syndrome in the context of another disease. The patients with primary RP have a younger age of onset (below 30 years), sparing of the thumb, and benign course without the development of digital ulcers [4, 6, 7].

On the contrary, secondary RP is characterized with later age of onset above 30 years, thumb involvement, and a more severe course with possible development of trophic changes in some cases (digital ulcerations, digital necrosis) [4, 6, 7]. In these cases, a focused, complaint directed history and physical examination aim to reveal clinical symptoms and findings that confirm the presence of an underlying disease, e.g., connective tissue disease (CTD) or other disorders. The secondary RP is a characteristic feature in a number of rheumatic diseases. In systemic

sclerosis (SSc)/scleroderma, it is with the highest frequency of approximately 95% [8]. RP could also be a sign in a spectrum of nonrheumatic pathology that also should be recognized and properly differentiated by the rheumatologists in routine clinical practice (**Table 1**) [8–14].

Together with clinical examination, laboratory, immunological, and capillaroscopic assessments facilitate the internal differential diagnosis of secondary RP and reveal the definite final diagnosis.

Nailfold capillaroscopy is a noninvasive, easy-to-perform method for diagnosis and differential diagnosis of patients with primary and secondary RP in rheumatic diseases (particularly the scleroderma-spectrum disorders), which is of crucial importance because of the different severity, prognosis, and therapeutic approach. Normal capillaroscopic pattern is an established diagnostic criterion for the diagnosis of primary RP. In addition, it has been found that capillaroscopic pattern in healthy individuals is constant for long periods of time. During the follow-up of patients with RP, it has been found that the appearance of abnormal capillaroscopic findings inherits a positive predictive value of 47% for the development of CTD that is higher as compared with the predictive value of the positive antinuclear autoantibody (ANA) test (30%) [15]. The appearance of giant capillaries is the earliest capillaroscopic sign of microangiopathy and represents a local response to tissue hypoxia. In RP patients, nailfold capillaroscopic analysis should be performed every 6 months and more often if new alarming symptoms appear. The capillaroscopic examination should be performed in all patients with symptoms of RP even in those cases without clinical and laboratory signs of systemic rheumatic disease, because the abnormal capillaroscopic picture inherits a high positive predictive value for the development of CTD.

Systemic sclerosis
Mixed connective tissue disease
Undifferentiated connective tissue disease
Systemic lupus erythematosus
Dermatomyositis, polymyositis
Sjögren syndrome
Rheumatoid arthritis
Systemic vasculitides-Buerger disease, Takayasu arteritis, polyarteritis nodosa, granulomatosis with polyangiitis, etc.
Fibromyalgia
Cryoglobulinemia
Drug-induced Raynaud's phenomenon—beta-blockers, cytotoxic drugs, vinblastine, bleomycin, interferon, etc.
Paraneoplastic Raynaud's phenomenon—associated with solid tumors and hematological malignancies
Endocrine disorders—hypothyroidism
Neurologic disorders—carpal tunnel syndrome

Table 1. Differential diagnosis of secondary Raynaud's phenomenon in rheumatologic practice.

4. Pathogenesis of Raynaud's phenomenon

The vasospasm in primary RP is reversible, while the secondary Raynaud's phenomenon in systemic sclerosis is associated with endothelial injury and subsequent structural abnormalities that lead to tissue damage.

Adrenergic alpha-2 receptors that are more important than alpha-1 receptors in the control of vasoconstriction of digital arteries are suggested to be abnormal in RP. The receptor subtype alpha-2c is found to predominate in the vascular smooth muscle cells of distal cutaneous vessels [16, 17]. Alpha-2c adrenergic receptors are not active at room temperature. In response to cold exposure, they are activated as a result from translocation from the Golgi complex to the plasmatic membrane [18, 19]. Estrogens also activate alpha-2c adrenoreceptors that could explain the predominance of primary RP in females and the increased severity of symptoms between menarche and menopause [20]. An association between enhanced contractile response to alpha-2c agonists and increased protein tyrosine kinase activity and tyrosine phosphorylation has been found. Greater intracellular tyrosine phosphorylation in response to cooling (31°C) has been found in arterioles in both primary and secondary RP patients vs. controls using immunofluorescence and an antiphosphotyrosine antibody [21, 22].

The endothelium controls blood vessel tone via production of vasodilators (nitric oxide (NO), prostacyclin) and vasoconstrictors (endothelin-1, angiotensin). Endothelial damage in secondary RP in SSc leads to disbalance between vasodilators and vasoconstrictors [10, 23]. In SSc-related RP, increased level of asymmetric dimethylarginine (an endogenous NO synthesis inhibitor produced by endothelial cells) was observed. Elevated plasma level of endothelin-1 was found in SSc as compared with primary RP [24]. Increased expression of endothelin-1 in the skin of SSc patients was also detected [25]. Besides its properties of vasoconstrictor, it has been confirmed that endothelin-1 promotes fibrosis in scleroderma patients [26]. In SSc, endothelial injury represents a key pathogenic step that mediates the processes of inflammation, thrombus formation, and fibrosis [23]. The role of endothelin-1 in pathogenesis of primary RP has also been implicated in some studies, but the evidence is weaker in comparison with SSc [10, 27].

Calcitonin gene-related peptide (CGRP) is a neuropeptide and a potent vasodilator produced by peripheral sensory nerves. In RP (primary and secondary RP in SSc), especially the secondary forms in SSc, a reduction in the number of CGRP immunoreactive neurons in the skin was found [28].

5. Primary Raynaud's phenomenon

In a recent systematic literature review, the prevalence of primary RP is reported to vary from 1.6 to 7.2% (calculated pooled prevalence—4.85%) [29]. As mentioned above, the primary RP is characterized with early age of onset, mainly at puberty. This classic type of presentation predominates in female patients with family history. Despite the fact that the age of onset is considered to be a discriminating factor between primary and secondary RP, it should be interpreted individually as primary RP with late onset (above the age of 40) is also possible [30].

5.1. Diagnosis of primary Raynaud's phenomenon

Wide application in clinical practice have the diagnostic criteria of Le Roy and Medsger (1992) that encompass the following findings:

1. Vasospastic attacks precipitated by cold or emotional stress
2. Symmetric attacks
3. Absence of digital ulcerations or gangrenes
4. Normal erythrocyte sedimentation rate
5. Negative test for ANA
6. Normal capillaroscopic picture [31]

Maverakis et al. published an international consensus for diagnosis of primary RP, which includes the following criteria: (1) normal capillaroscopy; (2) negative physical examination for findings suggestive of secondary causes such as ulcerations, tissue necrosis or gangrene, sclerodactyly, calcinosis, or skin fibrosis; (3) no history of existing CTD; and (4) negative or low titer ANA as low titer is considered (1:40) by indirect immunofluorescence. In the newly accepted criteria of Maverakis et al., the presence of normal erythrocyte sedimentation rate is not included, and negative test for ANA is not required [3] (**Table 2**). Normal capillaroscopic pattern exists in both sets of criteria.

In primary RP, capillary morphology and capillary density are normal. Slightly enlarged capillary diameters could be found [32–34].

6. Secondary Raynaud's phenomenon

Older age of onset, thumb involvement, severe course with trophic alterations of the fingers (digital ulcers, digital necrotic lesions), clinical features suggestive of autoimmune disease, positive autoantibodies, and abnormal capillaroscopic findings are characteristic features of secondary RP in rheumatic diseases [4, 6, 7].

6.1. Raynaud's phenomenon in systemic sclerosis

RP is one of the most common symptoms in SSc with frequency of about 95% [8]. It is usually the initial clinical feature that appears years before other disease symptoms. SSc-related RP is severe and often complicates with development of digital ulcers. The capillaroscopic pattern in SSc is specific and is characterized with the presence of dilated and giant capillaries, hemorrhages, avascular areas, and neoangiogenic capillaries. It has been described by Maricq et al. and is termed "scleroderma" type capillaroscopic pattern, which is a reference capillaroscopic pattern in rheumatology. Maricq et al. also observed components of this pattern in diseases from the scope of scleroderma-spectrum disorders, e.g., mixed connective tissue disease (MCTD), undifferentiated connective tissue disease (UCTD), and dermatomyositis (DM)—the so-called "scleroderma-like" pattern [35–37].

Le Roy and Medsger [31]	Maverakis et al. [3]
1. Vasospastic attacks precipitated by cold or emotional stress	**1.** Normal capillaroscopy
2. Symmetric attacks	**2.** Negative physical examination for findings suggestive of secondary causes (e.g., ulcerations, tissue necrosis or gangrene, sclerodactyly, calcinosis, or skin fibrosis)
3. Absence of digital ulcerations or gangrenes	**3.** No history of existing CTD
4. Normal erythrocyte sedimentation rate	**4.** Negative or low titer ANA (e.g., 1:40 by indirect immunofluorescence)
5. Negative test for ANA	
6. Normal capillaroscopic picture	

Table 2. Diagnostic criteria for primary Raynaud's phenomenon (Le Roy and Medsger [31]; Maverakis et al. [3]).

Cutolo et al. [38] described three phases of capillaroscopic changes in SSc:

I. An "early" phase—appearance of few dilated and/or giant capillaries and few hemorrhages. In this phase, the distribution is relatively preserved without loss of capillaries.

II. An "active" phase—the changes in this phase include a higher number of giant capillaries and hemorrhages, a moderate loss of capillaries, slight derangement, and, in some cases, diffuse pericapillary edema (**Figure 1**).

III. A "late" phase—it is characterized with extensive avascular areas, severe capillary derangement, and bushy and ramified capillaries [38].

"Scleroderma" type capillaroscopic pattern is found in the majority of SSc patients (about 90%). Maricq et al. detected "scleroderma" pattern in 82–95% of SSc patients [36, 37]. In an own study, "scleroderma" pattern of the hands was detected in 97.2% (35/36) of SSc patients [34, 39, 40].

In addition, it has been found that a number of patients with a definite diagnosis of SSc (those with sclerodactyly, telangiectasia, subcutaneous calcinosis, esophageal dysmotility, etc.) do not fulfill the ACR (American College of Rheumatology) classification criteria for the disease (1980). An abnormal "scleroderma" type capillaroscopic pattern was found in these cases [41].

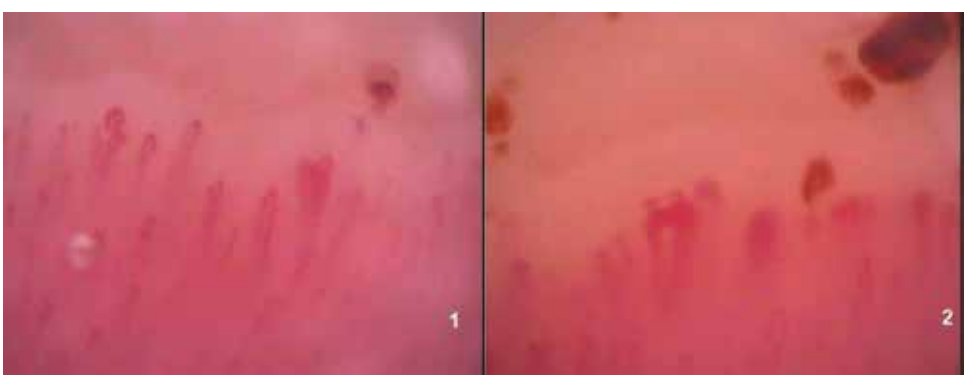

Figure 1. "Scleroderma type" capillaroscopic pattern in SSc (1) "early" phase, there is a single giant capillary loop and a single hemorrhage; (2) "active" phase, magnification 200×.

For establishing the diagnosis of SSc according to the ACR criteria (1980), the presence of the major criterion (skin thickening proximal to metacarpophalangeal joints of the hands) or two minor criteria ((1) sclerodactyly, (2) fingertip pitting scars, (3) bibasilar pulmonary fibrosis) is required [42]. These criteria have been proposed prior to the discovery of SSc-related autoantibodies and the characteristic capillaroscopic findings, which both had been found to improve the early diagnosis of SSc [43, 44]. Thus, Le Roy and Medsger suggested patients with RP and "scleroderma" type capillaroscopic changes or positive SSc-related autoantibodies to be diagnosed as "prescleroderma" *or limited SSc* even if other disease symptoms are absent [45]. The multicenter project VEDOSS (Very Early Diagnosis of Systemic Sclerosis) resulted in the new classification criteria for SSc (European League Against Rheumatism (EULAR)/ACR, 2013), which aim to detect SSc in early stages. In the new EULAR/ACR classification criteria, RP is scored with 3 points (1), the abnormal capillaroscopic pattern with 2 points (2), and presence of SSc-related autoantibodies (anticentromere, anti-topoisomerase I (anti-Scl-70), anti-RNA polymerase) with 3 points (maximum score for the immunologic criterion is 3) (3). The other criteria (overall 8 criteria) are as follows: telangiectasia (2 points) (4); fingertip lesions (digital tip ulcers - 2 points or fingertip pitting scars - 3 points), only the higher score is counted (5); pulmonary arterial hypertension and/or interstitial lung disease are scored with 2 points (6); skin thickening of the fingers (puffy fingers - score 2; sclerodactyly of the fingers - score 4), only the higher score is counted (7); skin thickening of both hands proximal to the metacarpophalangeal joints is scored with 9 points (8). A score ≥ 9 is necessary for a definite diagnosis of SSc [46, 47].

6.2. Raynaud's phenomenon in mixed connective tissue disease

MCTD combines symptoms of systemic lupus erythematosus (SLE), SSc, polymyositis (PM)/DM, arthritis, and the presence of a specific immunologic marker—anti-U1 RNP (an antibody against extractable nuclear antigen)—the latter being an obligatory classification criterion for the disease [48]. RP is met in 85% of patients with MCTD [8] and is the most common initial symptom [49]. Currently, the classification criteria for MCTD used in clinical practice are suggested by different author groups and not by professional organizations (criteria of Alarcon-Segovia, Kasukawa, Sharp, Kahn). RP is included in all of them because of its high frequency in MCTD [50]. Of note, the "SSc-like" capillaroscopic changes could be observed in a significantly lower proportion of MCTD patients in comparison with SSc. Maricq et al. have observed "SSc-like" pattern in 54% of cases in the examined group of 26 MCTD patients [36]. Bergman et al. detected "SSc-like" pattern in 50% (4/8) of the cases [51] and de Holanda Mafaldo Diógenes et al. in 65–71.5% [52]. Granier et al. observed a similar frequency of "scleroderma-like" capillaroscopic pattern—63.6% [53].

6.3. Raynaud's phenomenon in undifferentiated connective tissue disease

In UCTD patients, there are different symptoms of systemic rheumatic disease, but there is no full set of criteria of a well-defined rheumatic disorder. RP is a frequent symptom that may be observed in about 80% of the patients with UCTD. In evolution, a proportion of these patients (1/4–1/3) develop a distinct rheumatic disease, the most frequent being SSc, SLE, rheumatoid arthritis (RA), and Sjögren syndrome, but the majority of cases remain in a clinically

and laboratory stable condition in the scope of the term "UCTD." In UCTD, the frequency of "scleroderma-like pattern" is lower as compared with SSc (13.8%, Nagy et al. [41]; 38%, Lambova et al.) [34, 54]. The presence of "scleroderma-like" capillaroscopic pattern in patients with UCTD is considered to be a reliable predictive factor for the development of SSc [41].

6.4. Raynaud's phenomenon in systemic lupus erythematosus

The prevalence of RP in SLE is between 10 and 45% [8]. Although in a proportion of cases with SLE peripheral vessel is affected by vasculitis, RP usually tends to have a benign course without the development of digital ulcers or necrosis [9]. Microvascular capillaroscopic changes in SLE are less specific vs. those in scleroderma-spectrum disorders. However, several capillaroscopic features have been noted in SLE that are termed "SLE" type capillaroscopic pattern [51, 53, 55], which includes the presence of elongated [53, 56] and dilated capillary loops, tortuous and meandering capillaries [55], and prominent subpapillary plexus [53, 56]. "Scleroderma-like" capillaroscopic pattern is a rare finding in SLE with frequency varying between 2 and 15% [36, 41, 51, 56, 57]. An association between "scleroderma-like" capillaroscopic pattern and the presence of RP and anti-U1 RNP antibody has been observed that is suggested to be in the context of a possible subclinical overlap syndrome with SSc [57]. In an own study of 30 SLE patients, RP was found in 73% of cases, and "scleroderma-like" capillaroscopic pattern in 13.3% of the patients (4/30). Such changes were observed in patients with high immunological activity, two of whom were with active vasculitis of peripheral vessels and two with secondary RP. They did not exhibit signs of overlap with SSc or other CTD. Anti-RNP antibody was positive in a single case in a patient with symptoms of secondary RP without peripheral vessel vasculitis, while in the other three cases with "scleroderma-like" pattern, this immunological marker was negative [34, 54]. Thus, it could be concluded that "scleroderma-like" capillaroscopic pattern could be found in SLE patients with high immunological activity, both in cases with active vasculitis of peripheral vessels and only with symptoms of secondary RP but without evidence for overlap with SSc or other CTD.

6.5. Raynaud's phenomenon in dermatomyositis and polymyositis

RP occurs in about 20% of DM/PM patients [8]. Digital gangrenes of the hands are usually not observed. When such changes are evident, the presence of neoplasm should be suspected. RP is characteristic for the antisynthetase syndrome, which is a subset of myositis characterized with positive anti-Jo-1 antibodies (against histidyl-tRNA synthetase), interstitial lung disease, nonerosive symmetric polyarthritis in small joints, and cracking and fissuring of the skin of the fingers ("mechanic's hands") [58]. In a part of the cases with DM, "scleroderma-like "capillaroscopic findings could be observed [35]. Although the capillaroscopic changes in DM and SSc are usually indistinguishable, it is suggested that bushy and branching capillaries could be found more frequently in DM [59]. The frequency of "SSc-like" pattern detected by Bergman et al. in 11 patients with DM was 63.6% [51].

6.6. Raynaud's phenomenon in rheumatoid arthritis

The prevalence of RP in RA varies between 3 and 17% (2.7%, Carrol et al., North Australia [60]; 4.6%, Grassi et al., Italy [61]; and 17.2%, Saraux et al., France) [62]. In 31 RA patients, Redisch

et al. observed the following capillaroscopic changes: presence of elongated capillary loops, increased capillary tortuosity, and prominent subpapillary plexus. A "SSc-like" pattern was not detected in RA [55]. Nagy et al. also did not observe "SSc-like" pattern in a group of 14 patients with RA [41].

Of note, in an own study that included higher number of patients with RA (n = 62) with and without RP, a "SSc-like" pattern was observed in 14.5% (9/62; 2 males and 7 females). In one of the nine cases with such capillaroscopic changes, an overlap syndrome (RA overlap to SLE) with secondary RP and secondary vasculitis of peripheral vessels was evident. While in the rest eight out of nine patients, no overlap with other CTD was present. All RA patients with "SSc-like" capillaroscopic pattern (9/9) exhibited symptoms of secondary RP, and 2/9 a secondary vasculitis of peripheral vessels, respectively. Our results suggest that "SSc-like" capillaroscopic pattern could be observed in RA patients with secondary RP and with vasculitis of peripheral vessels although with low frequency. However, its presence is not mandatory in the context of overlap syndromes [34, 63].

6.7. Paraneoplastic Raynaud's phenomenon

RP may be a paraneoplastic symptom. When RP is newly appeared with late onset after the age of 60, it may also be an indicator for an underlying malignancy. Ischemia of the fingers has been reported in patients with carcinomas of the breast, the stomach, and the esophagus as well as in patients with oncohematologic disease (multiple myeloma, thrombocythemia, etc.). In a part of the cases, paraneoplastic RP is caused by secretion of vasoactive substances by the tumor cells and the respective immune response of the body, while in others like multiple myeloma and thrombocytopenia, it is associated with increased blood viscosity [64, 65]. We have not observed differences between microvascular changes in paraneoplastic rheumatic conditions in comparison with the respective idiopathic rheumatic diseases [66].

6.8. Raynaud's phenomenon in fibromyalgia

Fibromyalgia is an idiopathic, chronic, musculoskeletal, pain syndrome characterized with diffuse pain and presence of multiple tender points [67]. Primary fibromyalgia is an isolated disorder, while secondary fibromyalgia could be observed in different rheumatic diseases, e.g., RA, SLE, Sjögren syndrome, etc. [68]. RP is met in both primary and secondary fibromyalgia. The frequency of RP in fibromyalgia patients is about 17–30% [69, 70]. In an own study that included 26 patients with primary fibromyalgia, the most frequent capillaroscopic finding was the presence of dilated capillary loops analogous to primary RP. Capillaroscopic signs of microangiopathy were not detected [71].

7. Differential diagnosis of RP with other vascular acrosyndromes

Acrocyanosis represents painless bluish discoloration in the distal body parts, most commonly in the hands and feet and less frequently in the face that are affected symmetrically. In acrocyanosis, the bluish skin discoloration is a persistent finding that is aggravated by cold exposure and frequently is associated with local hyperhidrosis and edema of the hands and feet. In the

absence of an accompanying cause, acrocyanosis is considered primary (idiopathic, essential) that is suggested to be a benign condition and typically does not require specific treatment. It does not evolve into CTD or other diseases and may spontaneously resolve. secondary acrocyanosis is a manifestation of other major diseases. Both acrocyanosis and RP are influenced by cold exposure and emotional stress, but in acrocyanosis, there is relative persistence of skin color changes, symmetry, and absence of paroxysmal pallor. Of note, RP may occur concomitantly with acrocyanosis. In addition, the persistence of acrocyanosis is also relative, and it may also demonstrate improvement in the summer as well as in horizontal and elevated position of the hand vs. dependent position [72].

Perniosis (chilblain) is a localized cutaneous inflammatory reaction in response to acute or repetitive exposure to damp cold above the freezing point. The skin lesions are edematous plaques that may be purple or red and are often painful or pruritic. In severe cases, ulceration, superinfection, and scarring may occur. Fingers and toes are most commonly affected although other areas, e.g., nose, ears, buttocks, or thighs, could also be involved. Primary and secondary forms are recognized. Secondary perniosis could be associated with a variety of underlying pathological conditions such as hepatitis, autoimmune disease, and cryopathies [73].

Erythromelalgia is characterized with episodic, symmetric, and painful hyperthermia and erythema of hands and feet. Contrary to RP, erythromelalgia is provoked by exposure to heat or physical exercise. Primary and secondary forms are also recognized, primary being a rare hereditary disease that manifests in children and young people, while secondary is met in the context of myeloproliferative disorders, diabetes, and SLE and during drug treatment with calcium channel blockers (CCBs) [74].

8. Treatment

8.1. Primary RP

The mild forms of primary RP are controlled by non-pharmacological measures, e.g., patient education in avoiding exposure to cold, use of warm clothes and gloves, smoking cessation, using protective devices in working with vibration. Caffeine consumption, administration of vasoconstrictors such as beta-blockers, and use of interferons should be avoided when possible [9].

Dihydropyridine-type CCBs have been the treatment of choice for patients with both primary and secondary RP for many years. They have proven efficacy for reduction of the severity and frequency of ischemic attacks in primary RP and secondary RP in SSc. Diltiazem (benzothiazepine class CCBs) could also be used in RP. It is administered at a dose of 30–120 mg three times daily orally. Verapamil (diphenylalkylamine class of CCBs) does not possess therapeutic effect in RP patients [75–77]. Nifedipine is the best studied and the most often used drug from dihydropyridine class CCBs. It is used at a dose of 10–40 mg twice daily orally. Other dihydropyridines commonly used in patients with RP are felodipine (2.5–10 mg twice daily orally) and amlodipine (5–10 mg daily orally) [1, 9]. Dihydropyridine class CCBs are vasodilators with direct effect on vascular smooth muscles. They are indicated for the treatment of

arterial hypertension and stable angina, and some of them are also officially approved for vasospastic angina [78]. In RP patients, their use is off-label. Side effects of CCBs are common, e.g., flushing, hypotension, dizziness, headache, tachycardia, ankle edema, constipation, etc. Slow-release forms are better tolerated and preferred in clinical practice [1]. Pregnancy is a contraindication for administration of CCBs—a fact that deserves attention considering the high prevalence of primary RP in young women. This patient category requires specific instructions, and those women at childbearing age who plan conception or do not use effective contraception should not receive CCBs. Alternative, better-tolerated therapeutic options are often preferred in primary RP considering the milder clinical course and good prognosis of this condition as well as the side effects of CCBs.

Pentoxifylline inhibits phosphodiesterase and elevates cyclic adenosine monophosphate (cAMP) levels in polymorphonuclear leukocytes and other cells [79]. It exerts beneficial effects on microcirculation via improvement of blood fluidity, especially influencing erythrocyte flexibility [80]. Its maximal daily dose is 1200 mg (400 mg three times daily or 600 mg twice daily orally). It is not proven to be effective in severe forms of RP [1].

Therapeutic effect of *Ginkgo biloba* has been studied in patients with primary RP in double-blind, placebo-controlled trial. Significant reduction in the number of attacks has been observed in the group treated with high dose of *Ginkgo biloba* (360 mg daily) as compared with the placebo group (56 vs. 27%). It is suggested that *Ginkgo biloba* together with its vasodilator properties possesses also antiplatelet and radical scavenging effects [81].

8.2. Secondary RP in systemic sclerosis

EULAR recommends *dihydropyridine-type CCBs* as first-line therapy for RP in SSc [82]. A meta-analysis, including 8 randomized clinical trials (7 with nifedipine and 1 with nicardipine) with 109 SSc patients, indicates that dihydropyridine-type CCBs reduce the frequency and severity of ischemic attacks in SSc-related RP [77]. Apart from their effect to reduce the severity and frequency of vasospastic attacks, it has been demonstrated that CCBs lead to healing of digital ulcers [83]. In SSc with severe RP and/or those who do not respond satisfactorily to CCBs, *phosphodiesterase-5 enzyme inhibitors* are recommended (EULAR recommendation) [82]. NO is the main endothelium-derived vasodilator and an inhibitor of platelet activation and vascular smooth muscle proliferation. Its synthesis is regulated by the family of NO synthases, and its effect is mediated via cyclic guanosine monophosphate (cGMP). The intracellular concentration of cGMP is regulated by phosphodiesterases, which rapidly degrade cGMP in vivo [84]. A meta-analysis, including six randomized clinical trials (two with sildenafil, three with tadalafil, and one with vardenafil), demonstrated that phosphodiesterase-5 inhibitors have a significant effect on frequency and duration of RP attacks. Efficacy on healing of digital ulcers has been also reported. The therapeutic regimens used were as follows: sildenafil 50 mg twice daily or 200 mg once daily, tadalafil 20 mg daily or 20 mg on alternate days, and vardenafil 10 mg twice daily. The dosage regimens depend on half-lives of the different drugs that are 3–5 h for sildenafil and vardenafil (administered twice daily apart from modified-release sildenafil that is administered as a single dose) and about 18 h for tadalafil (administered once daily or on alternate days) [85]. Side effects during treatment with phosphodiesterase-5

inhibitors included different forms of vasomotor reactions, myalgias, allergic reaction, chest pain, dyspepsia, nasal stuffiness, and visual abnormalities. Considering long-term experience and good safety profile, EULAR experts recommend CCBs as first-line therapy for SSc-related RP and phosphodiesterase-5 inhibitors for SSc with severe RP and/or in cases with insufficient effect from the treatment with CCBs [82].

Intravenous iloprost possesses proven efficacy for reduction of the frequency and severity of SSc-related RP. Considering costs and feasibility, it is recommended after failure of oral therapies (CCBs and phosphodiesterase-5 inhibitors). Intravenous iloprost is also efficacious in the treatment of digital ulcers in SSc that is proved in randomized, placebo-controlled clinical trials [86, 87]. However, the need for hospitalization, the prolonged intravenous infusion (6 hours at the dose of 0.5–2 ng/kg/min), side effects, and high price are limiting factors for the administration of iloprost. Adverse effects include headache, nausea, vomiting, diarrhea, myalgia, arthralgia, chills, fever, arrhythmia, hypotension, chest pain (especially in patients with coronary heart disease), erythema, and pain at the infusion site. Thus, concomitant pathology should be assessed and hemodynamic parameters of the patients closely observed, e.g., blood pressure, heart rate, and pulse at the beginning and at every increase of the infusion rate. The risk for orthostatic hypotension should be also considered. Apart from its properties as a vasodilator, iloprost inhibits platelet aggregation, leukocyte chemotaxis, and adhesion to the endothelium. Iloprost also downregulates the expression of adhesion molecules on endothelial cells and phagocytes and enhances fibrinolytic activity [86–88].

In addition, in the most recent EULAR recommendation, *fluoxetine* (a serotonin-specific reuptake inhibitor and antidepressant) is suggested as an option in SSc-related RP despite the scarce published evidence [82]. In a small study that included 26 patients with primary RP and 27 with SSc-related RP, fluoxetine (20 mg daily) showed superior efficacy vs. nifedipine (40 mg daily). Observed side effects of fluoxetine were apathy, lethargy, and impaired concentration [89]. Despite the relatively low quality of published evidence, EULAR experts suggest fluoxetine as a useful alternative for treatment of SSc-related RP, especially in SSc patients who do not tolerate or fail to respond to vasodilators [82].

In SSc patients, in whom RP has complicated with digital ulcers, EULAR experts recommend *intravenous iloprost* and *phosphodiesterase-5 inhibitors* for treatment of digital ulcers and *bosentan (endothelin receptor antagonist)* for reduction of the number of new digital ulcers [82].

9. Prognosis

Being a first symptom in a number of CTD, the presence of RP requires regular follow-up that includes clinical, laboratory, immunological, and capillaroscopic assessment. The appearance of pathological capillaroscopic picture inherits a higher positive predictive value (47%) for the development of CTD vs. the predictive value of the positive ANA test (30%) [15]. Thus, nailfold capillaroscopy is the key investigation for monitoring RP patients. The interval of follow-up is 6 months, because a longer period of time is usually necessary for the development of morphological capillaroscopic changes, but this period may be shorter in cases with newly appeared alarming symptoms.

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Chapter 4

Longitudinal Growth in Rheumatologic Conditions: Current and Emerging Treatments of Growth Delay in Children with Chronic Autoimmune Diseases

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Additional information is available at the end of the chapter

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Abstract

Chronic rheumatologic, inflammatory diseases of childhood, such as juvenile idiopathic arthritis (JIA), Crohn's disease (CD), and systemic lupus erythematosus (SLE), affect both trabecular bone formation and remodeling and longitudinal bone growth resulting in short stature and causing bone developmental deformities. Inflammation alone or together with poor nutritional intake and chronic glucocorticoid therapy are major factors in growth retardation seen in children with chronic inflammatory diseases. When the growing process is continuous, acute or chronic inflammation causes dysregulation of both central endocrine and local paracrine secretion of the growth factors and hormones, impairing bone growth in children. In this chapter, we review major growth factors such as growth hormone that affect longitudinal growth and how they are affected by inflammation in childhood rheumatologic diseases. We also review a recently described growth factor, CNP, and its potential therapeutic role in chronic inflammatory diseases.

Keywords: juvenile idiopathic arthritis, natriuretic peptides, dwarfism, growth factor, atrial natriuretic peptide, B-type natriuretic peptide, C-type natriuretic peptide, natriuretic peptide receptor 1, −2, −3

1. Introduction

Longitudinal growth is a continuous process under the influence of multiple complex factors starting from prenatal life until end of puberty. Major factors that control longitudinal growth can be summarized as genetic background, nutrition, and endocrine growth factors. Major

endocrine factors that control longitudinal growth are growth hormone (GH) and insulin-like growth factor (IGF), thyroid stimulating hormone (TSH), parathyroid hormone (PTH), insulin, and sex steroids. There are also multiple growth factors in the growth plates and other organs that are involved in processing the growth factors in the regulation of longitudinal bone growth. Some of these growth factors are found and secreted by the growing cartilage and bone tissue and under the influence of the endocrine hormones. These are insulin-like growth factor 1 (IGF-1), insulin-like growth factor-binding protein 3 (IGFBP-3), fibroblast growth factors (FGF), Indian hedgehog (Ihh), parathyroid hormone-like receptor protein (Pthrp), and C-type natriuretic peptide (CNP).

1.1. How do growth hormones and factors affect longitudinal skeletal growth?

Skeletal growth and development follows two different pathways: chondrogenesis and osteogenesis (both endochondral and membranous bone growth). Longitudinal growth is mainly controlled by endochondral ossification which is orchestrated by a complex network of endocrine and paracrine growth hormones and factors that control growth plate cartilage and bone tissue.

While membranous bone such as those in our skull forms as a result of direct mechanism of mesenchymal cell differentiation into osteoblasts, endochondral osteogenesis follows an initial mesenchymal stem cell differentiation into chondrocytes, and the chondrogenesis process is later replaced by bone tissue [1–4]. During endochondral growth, mesenchymal cells (See **Figure 1**) first condense in the growth plate and then with interactions between cells via local transcription factors such as Sox9 and other extracellular molecules such as collagen II differentiate gradually into chondrocytes. Chondrocytes proliferate and organize in columns making stacks which are perpendicular to gravity. They gradually stop proliferating and become pre-hypertrophic with increased matrix synthesis (**Figure 1**) [5, 6]. Eventually, these cells stop proliferating and start terminally differentiating into hypertrophic chondrocytes. Finally, the hypertrophic zone of the growth plate becomes mineralized. Then the vascular system merges into this hypertrophic chondrocyte region and with more signaling the mineralized tissue is possibly resorbed by osteoclasts that originate from hematopoietic stem cells. Eventually, mineralized tissue is replaced by bone tissue which is made by osteoblasts that differentiate from mesenchymal cells. Thus, endochondral bone growth combines together chondrogenesis, extracellular matrix formation, mineralization, and osteogenesis process. These processes are synchronized by a series of systemic growth hormones such as growth hormone (GH), thyroid-stimulating hormone (TSH), glucocorticoids and local growth factors such as parathyroid hormone-related peptide (Pthrp) and members of the transforming growth factor β (TGF- β), fibroblast growth factors (FGF), Indian hedgehog (Ihh), and Wnt's [7, 8] (**Figure 1**). Intracellular pathways that are activated by the orchestra of factors are yet to be determined. Sox9 and Runx2, transcription factors, have been shown to regulate chondrogenesis and hypertrophic differentiation [9, 10].

During linear growth, endocrine hormones such as GH, IGF-1, glucocorticoids, and thyroid stimulating hormone first interact at the level of hypothalamus and pituitary [11, 12] and

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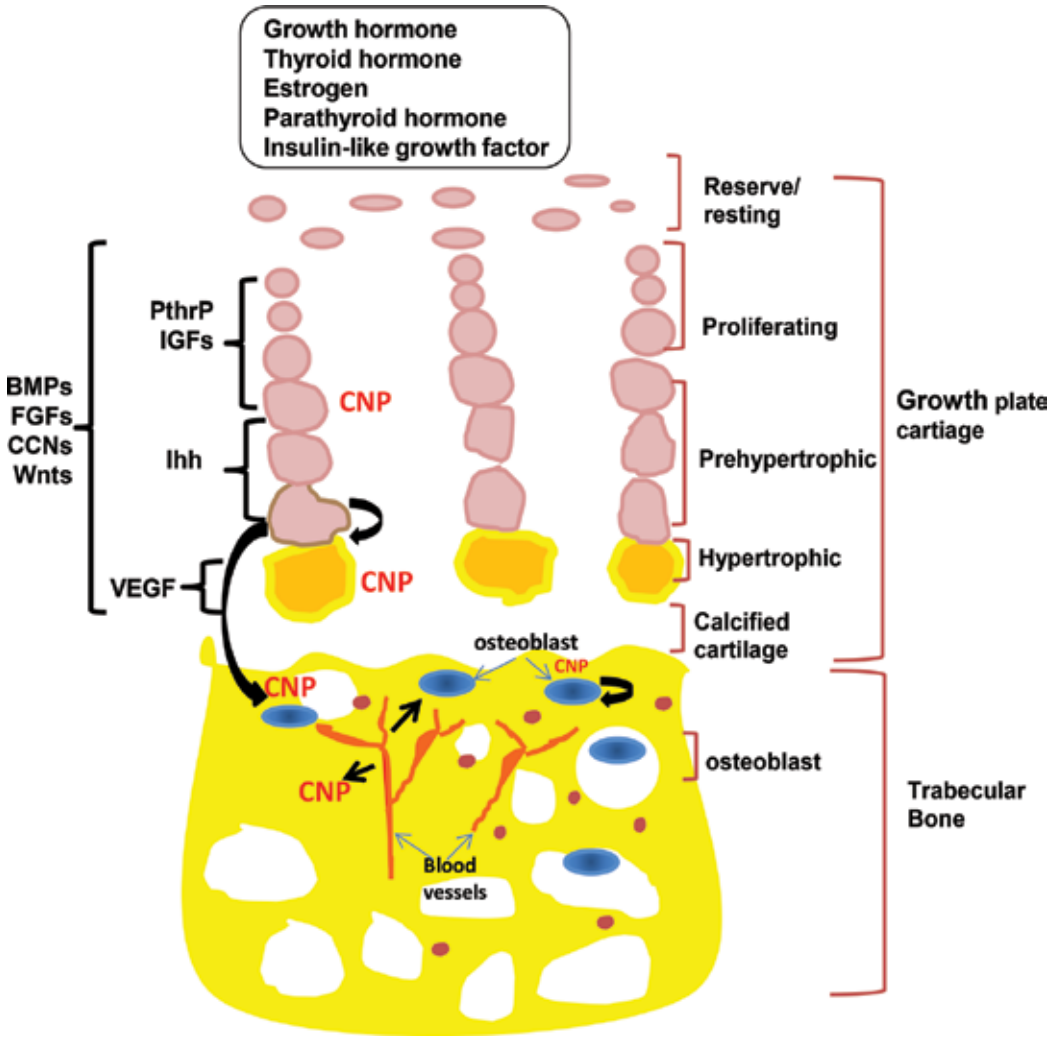

Figure 1. Longitudinal growth is regulated by multiple systemic (in bubble) and local growth factors expressed differentially in the proliferating, hypertrophic chondrocyte zones of the growth plate (black text on the left of the figure). CNP seems to be expressed and plays a role in the growth plate cartilage, in the osteoblasts and in the endothelial cells of vessel walls.

then, they act directly on peripheral target tissues, such as liver [13, 14], heart [15], kidney and growth plates [14, 16].

GH action regulates growth plates using both direct and indirect mechanisms. While GH directly stimulates chondrocyte proliferation on the growth plate [17], it indirectly stimulates the production of IGF-1 that promotes chondrocyte hypertrophy, which in turn exerts its effects directly on the growth plate.

Various danger signals or stimuli, such as TNF-α, LPS, or low-oxygen tension, increase the expression of IGF-1, vascular endothelial growth factor (VEGF), and FGF-2 with mechanisms

dependent on NF-κB activation and result in bone resorption, osteopenia [11]. Excess of TNF-α during systemic arthritis has been found to be responsible for periarticular osteopenia most probably due to the same mechanism. GH action has both direct and indirect effects on the growth plate. GH acts indirectly, stimulating the production of IGF-1 that promotes chondrocyte hypertrophy, which in turn exerts its effects on the growth plate. The direct effect of GH on the growth plate stimulates chondrocyte proliferation [18]. Most recently, nitric oxide (NO) and C-type natriuretic peptide (CNP) have been identified as new regulators of endochondral bone growth, as they both stimulate chondrogenesis and both act through a common mediator, cyclic guanosine monophosphate (cGMP) [19].

While it is important to study anabolic effects of growth factors and hormones that promote chondrogenic differentiation in the growth plate, it is also very important to recognize the effects of factors that play roles in remodeling such as factors that control osteoclastic differentiation or activity. Osteoclasts are major cells that degrade bones for remodeling. The balance between bone degradation and bone building is critical for physiological bone homeostasis. Factors such as NF-κB and cytokines that are controlled by this factor may cause an imbalance during systemic inflammatory diseases such as JIA [20, 21]. NF-κB activation is a relevant component for osteoclast development, differentiation, and survival, cooperating with other pro-inflammatory cytokines [22]. Loss of NF-κB signaling prevents osteoclastogenesis [23]. NF-κB knockout mice showed severe osteopetrosis [24].

2. Growth delay in chronic inflammatory diseases

In chronic inflammatory childhood diseases such as inflammatory bowel diseases, mainly Crohn's disease, juvenile idiopathic arthritis, systemic lupus erythematosus, or other diseases in which there are excess number of circulating cytokines, it is suggested that growth hormone signaling pathways are disrupted [25–27].

Out of all factors that affect growth during chronic inflammatory diseases, growth hormone GH/IGF axis has been studied the most. Multiple steps of the growth hormone and its effector IGF-1 axis may be interrupted; these are poor signal transduction of growth hormone in the liver and in the growth plate and diminished IGFBP concentrations, which directly affect the growth plate and suppress the sensitivity of IGF-1.

After the growth hormone is secreted by the pituitary gland, it stimulates the hepatic tissue to generate IGF-1 which increases the growth plate chondrocyte proliferation.

Pro-inflammatory cytokines play a critical role in the disruption of the IGF-1/GH axis. Many studies have shown that pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1β interact with the IGF-1/GH system. Transgenic mice for IL-6 develop low serum level IGF-1 and develop severe growth delay [25]. There is also evidence that suggests a role for suppressors of the cytokine signaling (SOCS) family proteins in IL-6 dysregulation [26]. The KxB/N transgenic mouse model for arthritis has also been described to have growth delay during our studies [27]. KxB/N mouse has increased serum levels of IL-1β and TNF-α and they develop systemic inflammatory

arthritis [28]. Thus, high levels of any pro-inflammatory cytokine are sufficient to arrest the growth process in developing organisms with open growth plates.

The IGF-1 signaling pathway is altered in chondrocytes during chronic inflammatory conditions by pro-inflammatory cytokine activities (TNF-α, IL-6, and IL-1). These pro-inflammatory mediators work via disruption of intracellular MAPK/extracellular signal-regulated kinases (ERKs) and phosphoinositide 3-kinase (PI3K) [29, 30].

Besides the inhibition of the MAPK pathway, there are also debates about the potential of disrupted miRNA effect on overexpression of proteins involved in the regulation of GH/IGF-1 axis. miRNA deregulation previously has been reported during childhood chronic inflammatory diseases such as IBD and JIA [21, 31].

In juvenile idiopathic arthritis (JIA), bone growth abnormalities are seen as either or both short stature and bone deformities. The prevalence of juvenile rheumatoid arthritis is as high as 20 per 100,000 people per year. Growth delay in generalized linear growth occurs predominantly in the systemic onset juvenile arthritis population and to a lesser degree in those with poly-articular onset JIA associated with RF positivity [32]. During active disease in JIA, elevated serum levels of cytokines may modify target cell's sensitivity by down-regulating the GH receptor (GHR) gene expression, leading to short stature as an adult [33]. Therefore, the shortcoming of GH function during JIA is explained more as resistance to growth hormones than deficiency in growth hormone secretion.

Growth hormone (GH) treatment by providing excess GH in the circulation can overcome growth hormone resistance and improve growth velocity and prevent development of short stature in children affected from JIA.

Recent studies suggest that early initiation of GH treatment helps in maintaining normal growth in children with JIA [34, 35]. Thus, recombinant growth hormone treatment has been the mainstream since no other medications that induce skeletal growth are available to be used in pediatrics [36, 37]. Nevertheless, even with GH treatment, catch-up growth is variable and is more dependent on the severity of the inflammatory state, duration, and additional corticosteroid treatment [34, 37–41].

Another childhood disease studied for its growth delay complication is Crohn's disease, an inflammatory bowel disease (IBD). Almost, one-third of the children affected by Crohn's disease (CD) develop longitudinal growth delay. Unlike JIA, Crohn's disease patients do not develop bony deformities since the major inflammatory target is not the joint cartilage but the intestinal system. Additional to the pro-inflammatory cytokine excess that directly affects the growth plate during active disease in Crohn's disease, other factors such as malnutrition, mal-absorption of the nutrients, and central nervous system were also blamed for longitudinal growth delay. Especially those patients affected more with jejunum inflammation have poor nutrition and severe deficiency in energy metabolism as well as a chronic inflammation state which contributes to the growth delay [42]. In Crohn's disease it has been suggested that chronic inflammation interferes with both central and peripheral growth hormone/factor secretion causing hormonal deficiency and/or resistance. While inflammatory

cytokines directly affect appetite centers, they also disrupt growth hormone signal transduction and proteolyze IGFBP-3 and inhibit the IGF-1 expression in the growth plate [43].

3. Other growth factors that affect longitudinal growth: C-type natriuretic peptide

C-type natriuretic peptide NP is anabolic in the growth plate, articular cartilage, and in bone tissue: We and others have shown that CNP is anabolic in the growth plate and that CNP/natriuretic peptide receptor-B (NPR-B)/cyclic guanosine monophosphate (cGMP) signaling regulates linear bone growth/endochondral bone formation through the cGMP-dependent protein kinase II (cGK-2). CNP induces chondrocyte proliferation, differentiation, and extracellular matrix (ECM) production.

We have recently shown that transgenic mice that overexpress CNP under the control of the type-II collagen promoter had increased endochondral bone growth with thick and matrix-rich articular joint cartilage. Most importantly, we have also shown that in an animal model of inflammatory arthritis, CNP overexpression in chondrocytes protects the articular cartilage integrity and prevents subchondral bone defects [27]. Our transgenic mice that overexpressed CNP on cartilage developed dense trabeculation under the subchondral bone supporting in vitro experiments showing increased matrix secretion by osteoblastic cells [44]. In addition, there is data about CNP enhancing ECM secretion in cultured articular chondrocytes seeded on a type-II collagen-coated scaffold [45]. CNP has a unique dual anabolic effect on chondrocytes and osteoblasts for matrix synthesis. Together, these findings suggest that CNP is an ideal growth factor to be used in TE for osteochondral defects since it may promote both cartilage and bone regeneration.

CNP improves vasculogenesis and graft survival: Vascular endothelial cells also express and secrete CNP, and CNP has a major role in embryonic vasculogenesis and graft vasculogenesis [46, 47].

Angiogenesis is essential for bone formation during embryonic life and after fracture, indicating a further role in bone fracture healing for CNP.

3.1. CNP signaling pathway

Natriuretic peptides are one of the main classes of cGMP inducers which are known as atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). Natriuretic peptides are secreted proteins that control cell behavior through activation of two major transmembrane receptors, natriuretic peptide receptor 1 and 2 (NPR1 and NPR2) [48–50]. Receptors NPR1 and NPR2 have guanylyl cyclase activity and synthesize cGMP in response to ligand binding. ANP and BNP signal mainly through NPR1/GC-A, while CNP predominantly activates NPR2/GC-B. All three ligands also bind to a third receptor, NPR3, which is known as the clearance receptor that does not have an intracellular signaling part from the molecule and limits ligand availability once attached and limits natriuretic peptide signaling.

Once CNP gene and its signaling were disrupted in mice, those mice suffered from postnatal dwarfism [51]. While CNP knock-out mice developed normal membranous ossification,

endochondral ossification was severely impaired and all long bones and vertebrae were significantly shorter. About 70% of null mice die in the first 100 days after birth. When crossed with transgenic mice that overexpressed CNP in cartilage, knock-out mice phenotype was completely rescued.

Further organ culture experiments (femur) confirmed CNP's effect as the potent stimulator of endochondral bone growth. Other mice models were present in which Npr2 was knocked out and similar dwarf phenotype was observed, thus further confirming the importance of CNP signaling pathways. A loss-of-function mutation in the cGMP-dependent protein kinase II (cGKII gene) has also recently been identified as the cause of dwarfism in mice which is a downstream effector of CNP. When Npr3 is knocked out, an opposite phenotype of skeletal over growth is observed [52, 53].

All natriuretic peptides (ANP, BNP, and CNP) have the ability to bind NPRA, NPRB, and NPRC, while CNP has the most affinity to bind NPRB which seems to control endochondral bone growth the most. Mice deficient for ANP, BNP, or NPR1 genes were reported not to develop dwarfism or abnormal skeletal phenotypes [54, 55], suggesting that these genes play only minor supportive roles during endochondral bone formation. Overexpression of BNP in transgenic mice developed skeletal overgrowth, but this was explained as overstimulation of NPR2 by excess levels of BNP [56, 57].

4. CNP's role in cartilage homeostasis

Transgenic mice that overexpress CNP (CNPcol2a1TG) in cartilage develop skeletal overgrowth and increased bone density: in order to further study the effects of CNP in skeletal growth and bone architecture in vivo, we generated transgenic mice by cloning human CNP cDNA (450 bp) into a construct that contained mouse collagen type II (Col2a1) promoter (GenBank #m65161) to specifically overexpress CNP in chondrocytes. Growth plates of CNPcol2a1TG mice showed increased numbers of proliferative chondrocytes measured by BrdU uptake ($p < 0.05$) and increased numbers of enlarged hypertrophic chondrocytes

Figure 2. 20 weeks old male CNPcol2a1TG mice with kyphosis and excess growth of longitudinal and vertebral bones.

with increased proteoglycan deposition as evidenced by strong Safranin-O staining [27]. CNPcol2a1TG mice developed increased endochondral bone growth (30%) and developed kyphosis due to vertebral overgrowth by 18 weeks (**Figure 2**). In the skeletal histomorphology of CNPcol2a1TG mice, the most intriguing finding was the increased trabecular bone formation in proximity to the growth plate cartilage, subchondral (juxta-articular) bones, and in vertebrae.

5. CNP's role in bone homeostasis

C-type natriuretic peptide (CNP) has dual anabolic effects on cartilage and bone tissues. We have recently shown that CNP is a major contributor to post-natal skeletal growth in humans by its effects on the growth plate cartilage [58]. CNP also has an anabolic effect on bone morphogenesis [59]. Previous reports of in vitro experiments suggest that the CNP signaling system is an autocrine/paracrine regulator of osteoblast growth and differentiation, and CNP plays a role in bone remodeling [60, 61].

Although it is one of the major regulators of endochondral bone growth with its impact on cartilage tissue and its homeostatic role in the growing bone tissue, CNP's role in adult bone is unclear. Recent research in ewes (adult female sheep) showed that when estrogen was given CNP, content was increased the most in the estrogen-responsive trabecular bones (vertebrae and iliac bones) more than the longitudinal bones (tibia). The same study suggested that dexamethasone injections to ewes did not change the content of CNP in bone tissue, while plasma CNP peptides and bone alkaline phosphatase levels were significantly decreased.

Dwarf mice, that is. Npr2 knockout or Nppc KO, have not been reported for the lack of their bone mineral content or bone mineral density. Our observation in the CNPcol2a1TG mice that overexpressed CNP, particularly in the cartilage tissue, was that both vertebral bones and the metaphysis/epiphysis of long bones, around the growth plates, develop significantly increased trabeculation and mineralization [59, 62]. This may be because Nppc overexpression was more significant in the growth plate cartilage and somewhat in the joint cartilage in our CNPcol2a1TG mice. Others using different promoters that caused increased production of CNP (SAP-CNP-Tg mice) in serum showed the effect of CNP on bone turnover microcomputed tomography (CT) analysis revealed increased trabeculation and dense bones lumber vertebrae in contrast to long bones such as femur. However, the fracture model showed that there is increased bone turnover and fracture healing in the SAP-CNP-Tg mice even in long bones with less trabecular ratio. Bone histomorphometric analysis of the tibiae from SAP-CNP-Tg mice showed that stabilized femoral fracture healing is advanced in SAP-CNP-Tg mice supporting the hypothesis that CNP regulates bone homeostasis and contributes to remodeling [63].

Osteoblastic cell culture experiments showed CNP's anabolic effect in osteoblastic activity.

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6. CNP's role in vascular homeostasis

One of the most important roles of CNP is in the venous system. CNP via its NPR2 a d NPR3 receptor signaling in the vascular wall regulates vasodilatation particularly on the venous wall. Enhanced osteoblastic and osteoclastic activities. In addition, serum levels of osteocalcin and tartrate-resistant acid phosphatase-5b, were elevated in the Tg mice. The same study showed that open and Vascular endothelial cells express and secrete CNP. CNP is suppressed by the VEGF secretion and is known to act as a vasodilator [64]. Also, CNP has been suggested to have a major role in angiogenesis [47, 65]. Once the growth plate is closed after puberty, the chondrogenic CNP will no longer available. Then CNP needed for trabecular bone remodeling will then have to be secreted partially by osteoblasts as a paracrine/autocrine factor and/or by vascular endothelium. Steady serum levels of NT-proCNP may also be regulating the cartilage and bone homeostasis and the main source of NT-proCNP might only be the vascular endothelium. Due to the vascular wall expression of CNP and its vasodilator effect, CNP's role in hypertension, vasculitis and myocardial infarction has been studied.

7. Lack of CNP signaling in growth plate causes short stature and dwarfism

Heterozygous carriers of a mutation in NPR-B, the receptor for CNP, have idiopathic short stature suggesting a quantitative effect of the CNP pathway on skeletal growth [66]. Serum levels of CNP's N-terminal pro-peptide (NT-proCNP), the inactive form of CNP, were found to be highest at birth, gradually decreased by puberty, and plateau after 18 years of age [67]. The levels of NT-proCNP correlate with levels of alkaline phosphatase (bone formation markers) in humans [67, 68]. More importantly, serum NT-proCNP levels are maintained at a level in adults.

Individuals with acromesomelic dysplasia-type Maroteaux (AMDM), a type of human dwarfism, develop periarticular osteopenia, loss of trabecular bone structure. AMDM is caused by loss of function mutations in the CNP's receptor, natriuretic peptide-B (Npr2 gene). AMDM patients have disproportional growth retardation and abnormal development of bone tissue and appear to have (juxta-articular) metaphyseal flaring and osteopenia but do not have any other health problems [58, 69]. The trabecular bone loss is more significant in the juxta-articular area resembling osteopenia of inflammatory arthritis.

8. CNP in skeletal overgrowth

Before CNP and it effectors can be used as a remedy for short stature and growth delay, its effects in humans need to be studied well. Evidence for complications of excess systemic CNP

came after the description of two novel mutations that resulted in gain of function in humans. The C-type natriuretic peptide (CNP), encoded by NPPC gene, is located on chromosome 2q37.1. Two independent studies have described three patients with a Marfan-like phenotype presenting a de novo balanced translocation involving the same chromosomal region 2q37.1 and overexpression of NPPC [70]. One study reported on two partially overlapping interstitial 2q37 deletions. These two patients showed opposite phenotypes characterized by short stature and skeletal overgrowth, respectively. The patient with short stature presented a 2q37 deletion causing the loss of one copy of the NPPC gene with normal CNP plasma concentration. The deletion identified in the patient with a Marfan-like phenotype interrupted the DIS3L2 gene without involving the NPPC gene. In addition, a strongly elevated CNP plasma concentration was found in this patient with Marfanoid features and a tall stature [71, 72].

9. CNP roles in energy metabolism

CNP was first isolated from porcine brain and was expected to be a neuropeptide [73], but the physiological significance of the CNP/GC-B system has been established in the vascular and skeletal systems [53, 58, 71, 72, 74–78]. It was reported that CNP and GC-B are expressed in the central [79–83] and peripheral nervous systems [84, 85]. It has been shown that the hypothalamus is an important center to control food intake and energy expenditure [86]. CNP mRNA was detected in the rat hypothalamus indicating this peptide's role in numerous

Figure 3. CNPcol2a1TG mice (on the right) are tall and slender as compared to the wild type littermates (on the left).

neuroendocrine regulation [87]. CNP is suggested to play important roles in central energy expenditure and food intake via its effects on the central nervous system (**Figure 3**).

10. CNP during inflammatory disease activity

Initial reports about CNP's effect on inflammatory disease activity came from osteoarthritis disease models. Since both nitric oxide and CNP are the two main activators of cGMP in cartilage they were checked for their role in development of osteoarthritis. The production of nitric oxide (NO) regulates host defense and inflammation. Nitric oxide has vasodilation, cytotoxicity, and it has a role in cytokine-dependent tissue injury. NO effects have been blamed in the tissue injury of a variety of rheumatologic conditions including systemic lupus erythematosus, rheumatoid arthritis, and osteoarthritis. Pro-inflammatory effects of nitric oxide include vasodilation, edema, cytotoxicity, and the mediation of cytokine-dependent processes that can lead to tissue destruction. In contrast to NO effect in the cartilage, NO secretion from vascular wall is protective against neutrophil adhesion and related vascular injury. Thus, nitric oxide has been found as a clear role player in osteoarthritis pathogenesis but not in microvasculature injury [88, 89]. Although CNP is known to induce articular chondrocyte hypertrophy, it was never blamed to be involved in osteoarthritis pathogenesis, while it was considered to contribute to the disease progression. On the other hand, we were able to show in an inflammatory arthritis murine model that cartilage overexpression of CNP increased the chondrocyte number, matrix synthesis, and maintained a thicker hypertrophic cartilage in the growth plate and in the joints and thus was protective against the cartilage degenerative effects of inflammatory arthritis. The mouse model we used was for a K/BxN rheumatoid arthritis mouse model that developed severe inflammatory arthritis which was evident from first synovitis, pannus formation, and then secondary cartilage deterioration [28, 90]. Our transgenic mouse under Col2a1 promoter overexpressed CNP in the cartilage tissue mainly in the growth plate and in the joint cartilage. CNP transgenic mice developed thick growth plates with enlarged chondrocytes and wider growth plates with proliferating chondrocytes that produced a rich matrix. Furthermore, when CNP overexpressing mice was crossed with K/BxN mouse and developed systemic arthritis, we observed that cartilage matrix integrity and cartilage structure was protected against the inflammation. CNP transgenic mice did not develop severe complications of arthritis (**Figure 4**) [27, 91].

Another evidence for CNP's systemic anti-inflammatory effect was reported in a rat model of hemorrhagic shock and resuscitation. CNP infusion to this model lowered the myeloperoxidase activity and decreased the expression of TNF-α, IL-6, and IL-1β in the kidneys. CNP treatment suppressed oxidative stress, ameliorated the inflammatory response, and caused acute kidney injury [92]. Investigators suggested that they demonstrated CNP infusion's inhibitory effect on the generation of reactive oxygen species (ROS) and pro-inflammatory cytokines after hemorrhagic shock induction and subsequently suppressed the activation, recruitment, and adherence of neutrophils in the kidney. Neutrophil recruitment is one of the fundamental pathways in hemorrhagic shock. It is suggested that neutrophil recruitment is also delayed after CNP treatment. In an earlier study, Chen et al. showed that CNP treatment

Figure 4. K/BxN+CNPcol2a1TG double transgenic mice do not develop growth retardation or severe complications of arthritis.

effectively attenuates lipopolysaccharide (LPS)-induced endothelial activation by eliminating intracellular ROS production, inhibiting the NF-κB and MAPK p38 signaling pathways and activating the PI3 K/Akt/HO-1 pathway in human umbilical vein endothelial cells (HUVECs), [93] suggesting an anti-inflammatory effect for CNP.

Another study that showed CNP's effect in reducing the LPS-induced lung injury suggested that mechanism of action might involve downregulation of inflammatory cytokine expression in lung parenchyma and again downregulation of neutrophil migration in the lungs [94].

Finally, evidence for CNP and its derivate anti-inflammatory treatment potential were shown in a wounded cartilage explant model in steers. In this study, wounded explants were cultured with 0 or 10 ng/mL IL-1β and/or microcapsules loaded with or without CNP for a period of 48 h. The presence of CNP microcapsules had a concentration-dependent effect with significant inhibition of NO release in response to IL-1β at 2000 ($p < 0.01$), 10,000 ($p < 0.01$), and 50,000 microcapsules/well ($p < 0.001$) [45]. Others suggested that the effect of CNP on preventing the inflammatory effects of IL-1β in chondrocytes depends on local protein concentration. While low concentrations (pM) were shown to promote a proliferative response, high concentrations (μM) lead to anabolic effects such as matrix synthesis in chondrocytes [95, 96].

Acute inflammation and the inflammatory mediators seems to suppress the activity of CNP in growing organisms [97]. It is possible that in chronic inflammatory diseases, serum NT-proCNP levels are also low and contribute to the growth arrest during active disease in children.

11. Other conditions in which circulating CNP levels are affected

CNP regulates fat metabolism in adipogenic tissue, and adipogenic CNP transgenic mice is resistant to obesity when fed by high fat content. Natriuretic peptides regulate intracellular cGMP and phosphorylated vasodilator-stimulated phosphoprotein (VASP). Adipogenic CNP

transgenic mice showed a decrease in fat weight and adipocyte hypertrophy and increases in fatty acid β-oxidation, lipolysis-related gene expression, and energy expenditure during high fat diet (HFD)-induced obesity. Furthermore it seems like CNP overexpression diminished the inflammatory activity in adipogenic tissue. Adipogenic cell CNP transgenic mice were reported to have significantly decreased gene expression of TNF-α, interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and F4/80 in mature adipocytes [98]. Adipogenic CNP transgenic mice also developed better glucose tolerance and insulin sensitivity, which were found to be associated with enhanced insulin-stimulated Akt phosphorylation. It was suggested that CNP overexpression in adipocytes protects against adipocyte hypertrophy, excess lipid metabolism, inflammation, and decreased insulin sensitivity during HFD-induced obesity. An earlier report also suggested that overexpression of endothelial-specific CNP overexpression protects against visceral adipose tissue hypertrophy, systemic inflammation, and insulin resistance during the development of obesity due to the feeding of a high-fat diet (HFD) [99]. Overall current knowledge suggests that natriuretic peptides are new pathways controlling human adipose tissue lipolysis operating via a cGMP-dependent pathway.

One of the intriguing studies is one that examined serum levels of 53 patients with Behçet's disease and showed that all patients with active disease had lower levels of CNP indicating its suppression by inflammatory disease activity [100]. Thus, there is evidence that during chronic inflammatory diseases CNP serum levels might be suppressed which can impact the skeletal growth if the individuals affected have open growth plates and ongoing longitudinal growth process.

12. Current clinical use of CNP

Use of CNP and its analogues in achondroplasias: gain-of-function mutations in the FGFR3 gene result in achondroplasia. Achondroplasia is known as the most common form of dwarfism. In patients with achondroplasia there is impaired proliferation and differentiation of the chondrocytes in the growth plate cartilage that causes stunted longitudinal growth due to endochondral growth suppression and skull abnormalities due to membranous ossification disruption. In achondroplasia, FGFR3 mutations induce increased phosphorylation of the tyrosine kinase receptor FGFR3 and increase the mitogen-activated protein kinase (MAPK). It is known that C-type natriuretic peptide (CNP) suppresses FGFR3 downstream signaling by inhibiting the pathway of mitogen-activated protein kinase (MAPK) in vivo and in vitro. Mice overexpressing CNP rescues FGFR3 gain of mutation-related dwarfism. Exogenous administration of CNP has been challenging since it is rapidly cleared and degraded in vivo through receptor-mediated and proteolytic pathways such as proteolytic-neutral endopeptidase degradation. Therefore, multiple variants of CNP molecules have been tested for their efficacy. Recently, a variant of CNP called BMN111, neutral endopeptidase-resistant CNP analog, showed significant ability to stimulate signaling downstream of the CNP receptor, natriuretic peptide receptor B. Initial trial of continuous delivery of CNP through intravenous (IV) infusion in the form of BMN111 in 2014 showed normalization of dwarfism [101].

Since subcutaneous (SC) route of administration is preferred over continuous infusion in pediatric individuals, it is expected that BMN 111, a 39 amino acid CNP pharmacological analog, would be very effective in diseases where CNP signaling pathway is impaired. BMN 111 can be applied once daily via SC administration at physiological concentrations [101].

In the near future, CNP analogues might be used in other diseases where CNP signaling is impaired or blocked by chronic inflammation affecting cartilage and bone. Use of CNP analogues can be applicable to both adult and pediatric diseases.

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Chapter 5

Osteonecrosis of the Jaws

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Additional information is available at the end of the chapter

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Abstract

Osteonecrosis of the jaw is a very severe complication in patients using antiresorptive drugs, which have been widely applied for the last 10 years. It has prompted an increase in number of negative complications such as significantly restricted food intake, reduced quality of life with a negative impact on the general health status of the patient as a whole. The negative influence of antiresorptive drugs on jaw bones is still not precisely known and is the subject of research. More than 30% of patients with rheumatic diseases develop osteonecrotic lesions in the jaws due to a relation with bisphosphonates, corticosteroids or other antiangiogenic treatment administered orally or parenterally. The treatment is often protracted, variable and very complicated. The clinical symptoms and treatment possibilities are presented, and, based on the clinical results, compared with many investigative researches and multicenter studies all over the world. Preventive measures are often consistent with other studies, where precautions such as radical dental treatment were observed, especially before antiresorptive treatment initiation. Despite the clinical results, which widely differ, the best way to prevent the osteonecrosis of the jaw is a necessary interdisciplinary approach and further research.

Keywords: osteonecrosis of the jaw, antiresorptive drugs, interdisciplinary approach, quality of life, rheumatic diseases

1. Introduction

Medication-related osteonecrosis of the jaw (MRONJ) was first described in patients with multiple myeloma treated with intravenously administered bisphosphonates in 2003. At the time, the disease was classified as bisphosphonate-related osteonecrosis of the jaw (BRONJ). However, 15 years of development in diagnosing, treating and monitoring the course of the

disease revealed the correlation with drugs other than the bisphosphonates. The American Association of Maxillofacial Surgeons (AAOMS) standardized the diagnostic criteria for BRONJ in 2009, and updated the disease to MRONJ, eventually merging both diseases in 2014 [1].

Recently, there have been a growing number of cases with osteonecrosis of the jaw diagnosed in patients treated with cytostatics, hormonal preparations combined with corticosteroids and human monoclonal antibodies. This group of drugs is known as antiresorptive medicaments (ARM), with proven cytotoxic effect on mucous membrane of the oral cavity. Most often, however, following an invasive procedure in oral cavity that breaches its integrity and exposes the alveolar bone, while simultaneously failing to implement measures that promote wound healing, they lead to a necrosis. Osteonecrosis of the jaw is confirmed by the clinical picture of non-healing post-extraction wound (or dental trauma), which has not healed in 8 months without medical history of radiation therapy in head and neck region, edema, loose teeth, foetor ex ore, fistulas and, in advanced stages, pain.

It is possible to locate the affected area by employing the modern methods such as MRI, CBCT or through bone resorption biomarker findings. These diagnostic methods aid in determining the stage of the disease and subsequent method of treatment. The treatment is difficult and often ineffective, with recurrent complications. When the preventive measures are observed and the disease is diagnosed early, followed up by an adequate treatment, the disease can be cured, or at least in certain cases, the symptoms in the oral cavity can be alleviated. In the majority of cases, however, MRONJ patients who have been treated with antiresorptive drugs tend to also suffer from breast cancer, prostate cancer or multiple myeloma (**Figures 1** and **2**). We also encounter increasing number of patients with osteoporosis and rheumatoid arthritis [2]. Most cases of MRONJ arise after prolonged intravenous use of nitrogen-containing bisphosphonates. Orally administered nitrogen-containing bisphosphonates cause MRONJ less often [3].

The scope and course of the disease depends on the correlation between other drugs and the patient's overall health status. Antiresorptive treatment is a common treatment method, only excluding patients with cancer, rheumatoid arthritis, osteoporosis or the so-called skeletal-related

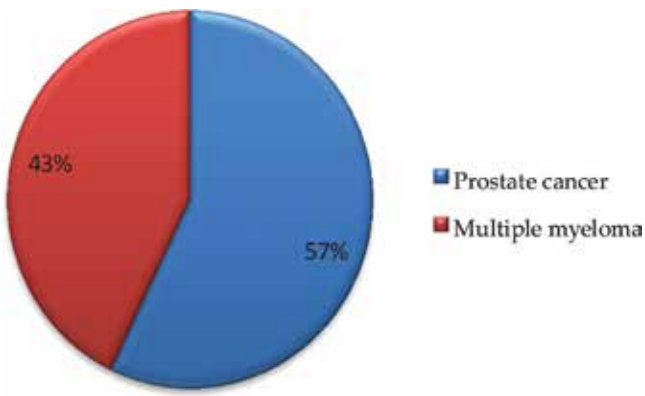

Figure 1. The basis of disease in men.

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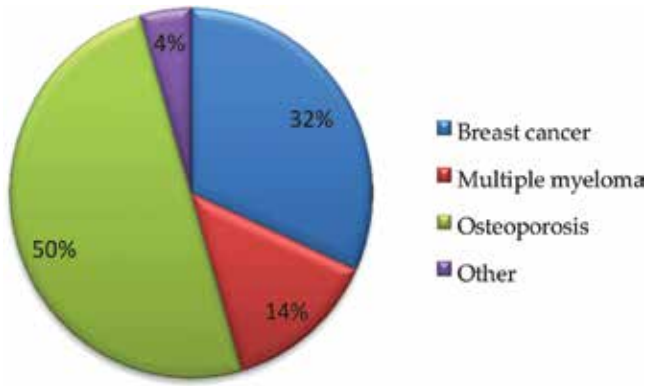

Figure 2. The basis of disease in women.

events (SREs). This term refers collectively to specific serious complications relating to the metastatic bone disease. SREs include pathological fractures of vertebrae and long bones, malignant spinal cord compression, hypercalcemia and cases requiring orthopedic and analgesic radiotherapy. This treatment improves the quality of life for such patients, and provides them with longer lifespan, with minimal undesirable effects.

2. Etiopathogenesis

There are a number of theories concerning the cause of this complication. They are mostly related to the formation of necrotic bone caused by the inhibition of bone remodeling with bisphosphonates and an antiangiogenic therapy. However, several studies on the development of the disease indicate that the bone remodeling inhibition itself cannot cause bone exposure. It is necessary for local risk factors to also be present.

The most significant theories about the possible cause are based on the following arguments:

1. The only barrier between the bone and the mouth is a thin mucosa that is easily damaged by mechanical irritation (chewing, prosthesis-related trauma).
2. Oral cavity contains a diverse microbial flora that may induce pulpo-periodontal diseases, when the area becomes pathologic.
3. A surgical procedure in the area exposes the alveolar bone to a high concentration of bacteria.
4. Bone remodeling rate of the jaw and mandible is very high, which leads to a greater accumulation of bisphosphonates in the mineralized bone tissue [4].

According to a theory, keratinocytes undergo bisphosphonate-induced apoptosis resulting in diminished mucous barrier in the oral cavity, which plays a role in the development of MRONJ. If an infection in the oral cavity is caused by the naturally occurring post-surgery

bacteria (*Actinomyces israelii*, *Escherichia coli*), they will cause the drop in the wound area pH, and will release bisphosphonates and calcium salts. The hypothesis is that high concentrations of bisphosphonates increase apoptosis of keratinocytes in the attached gingiva and consequently, allow the penetration of bacteria into deeper tissues. This hypothesis may explain why MRONJ occurs only in maxilla and mandible, but not in other bones of the skeleton.

Another theory considers the unique role of bone remodeling rate. Both maxilla and mandible are examples of a bone that is subject to an increased bone remodeling, mainly in the alveolar socket area and periodontal area, as a result of intensive mechanical stress acting on teeth during chewing and other movements of the teeth. It turns out that the bone turnover is constant during the life of an individual, regardless of their age [5].

The suppression of remodeling and decrease of bone turnover results from bisphosphonates directly affect osteoclasts and their function. Studies examining osteogenesis imperfecta in kids reveal that bisphosphonates do not always reduce the level of osteoclasts, but contrary to that, under certain conditions, they tend to increase their levels [6, 7]. Suppression of bone remodeling could therefore occur through other mechanisms such as intravenous bisphosphonate application.

Longitudinal animal studies with long-term application of bisphosphonates revealed increased number of multilocular phosphatase-positive cells in jaw and long bones. On the surface of the bone, however, the number of osteoclasts is decreased, while the number of osteoclasts in the woven bone is increased [8]. A traumatized alveolar compact bone with damaged periosteal and endosteal covering and diminished osteoprogenitor cells will activate osteoclasts and start the bone remodeling process. However, osteoclasts are unable to bind themselves to the bone surface and resorp the bone matrix as a result of incorporated bisphosphonates. Traumatized bone can hold the attempted osteoclast activation signal, and the osteoclasts then accumulate near the bone surface. The purpose of these accumulated unconnected osteoclasts in bone tissue is currently unclear [9].

2.1. Infectious agents

The oral cavity is colonized by a number of microorganisms that may become pathogenic even after the slightest superficial trauma to the oral mucosa, which then acts as a gateway for jaw bone infection. An organism treated with ARM has altered immune response and is unable to react efficiently against infectious agents and curb the spread of infection to surrounding tissues of the oral cavity and alveolar processes.

Various in vivo studies on rats describe a link between the periodontal infection and the osteonecrosis development. Young adult rats have been administered bisphosphonates for 15 weeks and had a circumdental wire applied to the first molar for 3 weTeks to induce an aggressive periodontitis. Osteonecrosis of the jaw diagnosed in this study had the identical course and histological finding to the human manifestations of the disease, with bone sequestration, numerous empty osteocyte lacunas and an expression of inflammation. Culture results proved that Fusobacterium nucleatum were present. After the subsequent ATB application, the signs of osteonecrosis have subsided; however, the healing ad integrum did not happen.

Numerous similar longitudinal studies confirm the significant role of infectious agents in the oral cavity with expressed osteonecrosis [10]. Surgical procedures conducted during a bisphosphonate therapy or a periodontal pathology indicating bone remodeling of the alveolar bone make it easier for bisphosphonates to accumulate in maxilla or mandible. After bisphosphonates in a bone reach the critical concentration, a trigger (tooth extraction) activates the bone remodeling, simultaneously releasing local deposits of bisphosphonates that inhibit the bone healing process. Necrotic osteomyelitis is induced by the slowed-down repair process, accompanied by a bone wound contamination by the Actinomyces bacteria.

Hence, efficient debridement, application of antimicrobial mouthwash and application of ATB directly on the bone defect and the wound play a very important role in the treatment of ONJ.

3. Clinical picture

In the past, osteonecrosis of the jaw proved to be a serious problem not only in the view of possible treatments, but also in the view of the diagnosis itself. Such lesions and conditions were usually considered to be osteitis, osteomyelitis or alveolitis, which were thought to have been the result of a preceding extraction.

Complications in the oral cavity in patients with MRONJ are usually diverse. The complications may emerge due to the progression of the disease, or as a result of medical procedures, which produce functional problems such as diminished chewing function, loss of teeth and limited rehabilitation of the chewing function. In addition, aesthetic obstructions may also emerge due to the loss of teeth, facial contour defects (owing to partial bone resections) or due to enduring oroantral fistulas. Patients experience sore mouth, impaired wound healing and drug-induced mucositis.

The most common clinical sign of MRONJ (up to 93.9%) is an exposed necrotic bone. The scope of bone exposure may vary greatly and is directly connected neither with the scope of the necrosis nor with the severity of the disease. Signs of infection such as swelling of soft tissues, intra/extra oral purulent discharge or abscesses may also be present. Patients may suffer from severe pain if the infection breaks out of the necrotic tissue, although this symptom is not a requirement—many patients do not report any pain. In severe cases, local infection may develop into abscesses in the deeper areas of the head and neck, resulting in life-threatening conditions. It may even lead to an abscess in brain tissues. Some rare cases of septic systemic infection have been documented.

Rare, although typical, symptom of MRONJ is the paresis of alveolar nerve, also known as the Vincent's symptom. It is interesting that it manifests itself in the earlier and in the advanced stages of MRONJ. Reduced sensitivity of nervus alveolaris inferior can also be a sign of metastatic infiltration. Histologic examination is recommended. Other symptoms associated with MRONJ include loss of teeth due to structural changes within the necrotic bone and bad breath due to bacterial inflammation.

Loss of teeth is the result of a progress of the necrotic damage to the alveolar bone. Bad breath as a symptom commonly occurs in patients suffering from MRONJ based on previous changes within the necrotic bone and the surrounding soft tissues. This can also be the result

of a bacterial colonization of the affected area, usually combined with a non-sterile infection of the bone and the surrounding soft tissue. This symptom occurs in 71–84% of MRONJ patients with periodontitis which form an inflammatory periodontal disease. Polymicrobial biofilm swab samples from oral cavity reveal specific bacteria such as Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia or Aggregatibacter actinomycetemcomitans.

Thanks to the adequate and effective management of these diseases and their various possible stages, it is now possible to correctly diagnose the patient and consequently, try and treat them. Though, the treatment itself usually does not bring neither adequate nor successful results, which is the reason why such an amount of studies and in vivo and in vitro experiments exist. The status of oral cavity in patients undergoing intravenous bisphosphonate therapy after primary prevention can be maintained to such an extent that the cancer treatment may continue without any negative impact on the quality of life of the patient, even when osteonecrotic defects and lesions are present. **Figures 3** and **4** describe the clinical picture of ONJ.

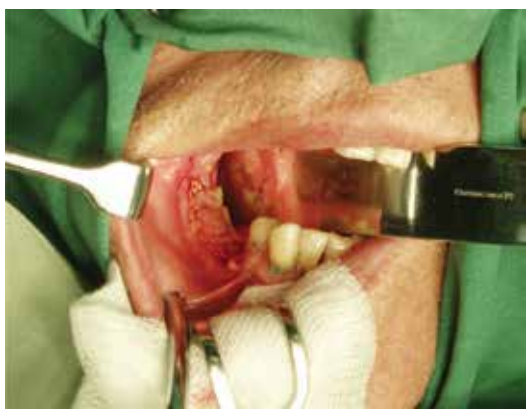

Figure 3. Osteonecrosis of the right mandible.

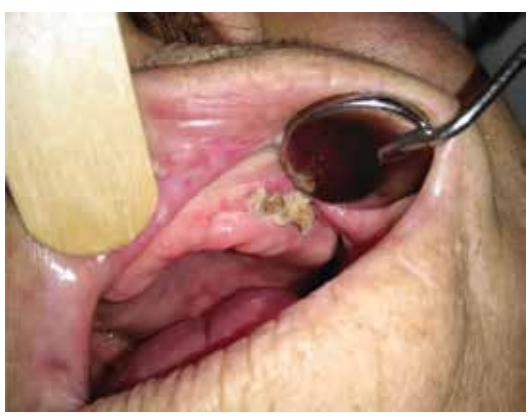

Figure 4. Osteonecrosis of the left maxilla.

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4. Prevention

Considering how complex the bisphosphonate-related osteonecrosis of the jaw therapy is, primary prevention may be the most important strategic approach to this complication.

Preventive measures are able to reduce, albeit not eliminate, the risk of formation of lesions. The rationale behind the primary prevention is the elimination of all focuses of infection in the jaw and total denture restoration lege artis.

The radical form of the therapy is comparable to denture restoration in patients before a radiation therapy in the head and neck region. All dentists should be familiar with the form of the therapy. Every patient should be subject to dental examination and panoramic dental X-ray before their planned antiresorptive therapy (**Figure 5**). In case surgical procedures in oral cavity (usually teeth extractions) are necessary as a part of the denture restoration procedure, it is recommended, if possible, to postpone the launch of ARM treatment by 2–3 weeks, or, preferably, until clear signs of bone healing show up on the skiagram. Other dental examinations and good oral hygiene are, of course, essential in the course of the ARM treatment.

In cancer patients taking intravenous bisphosphonates, the most conservative therapy possible is indicated for dental diseases. All invasive procedures involving jaw bones are strictly contraindicated (tooth extractions, periodontal-dentoalveolar surgery, implantology). It is recommended to refer the patients for whom these procedures are necessary to a specialized department of maxillofacial surgery. Such preventive measures are recommended to be followed not only in cancer patients who are subject to bisphosphonate treatment, but also in patients who use other drugs affecting bone metabolism or osteoclast function inhibitors.

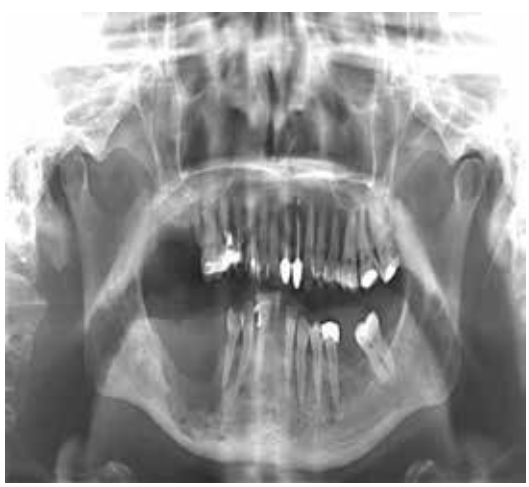

Figure 5. Panoramic X-ray with osteonecrosis of the right mandible.

The secondary prevention, in terms of ARM treatment interruption—the so-called drug holiday—is bit problematic. So far, there is no scientific evidence that the interruption of a therapy prior to surgery in the oral cavity reduced the risk of developing osteonecrosis of the jaw. According to AAOMS, suspending intravenous bisphosphonates has no significant short-term benefit in case the lesions are already present. Long-term treatment suspension, however, may stabilize the affected area, alleviate the clinical signs and also reduce the risk of new sites being affected. The priority still lies in the treatment of malignant diseases, and therefore, the suspension of bisphosphonates has to be thoroughly assessed.

The situation with monoclonal antibodies is different. Based on current knowledge about the effect of denosumab on bone remodeling, it is recommended to suspend the drug prior to any planned surgery in the oral cavity, in order to reduce the risk of developing osteonecrosis of the jaw. Suspending denosumab treatment seems to be appropriate, even in cases of an already developed osteonecrosis of the jaw, which can lead to heightened healing of the lesion. Some authors recommend suspending bevacizumab 6–8 weeks before surgery and resuming the medication 4 weeks after the procedure to prevent complications with wound healing.

5. Treatment

The primary goal of the treatment is to minimize the occurrence of MRONJ. Even though cases of spontaneous formation of MRONJ do exist, the majority of cases develop after a surgery.

In the first place, it is necessary to carry out a preservation treatment and consequent prosthetic and surgical treatment lege artis. This includes restorations of carious teeth, repairing of overhanging fillings, or extracting devitalized or destroyed teeth with extensive periapical findings. ARM treatment should initiate or resume only after the extraction wound in the socket has healed thoroughly. Prevention is important in terms of maintaining the functionality of healthy teeth.

Examination of the affected mucosa is necessary in patients with prosthetic replacement, since decubiti, traumatic lesions or fissural granuloma may emerge in the area. For these reasons, temporary restoration is contraindicated in many cases. Dentoalveolar procedure must be carried out in the gentlest manner, preferably at a maxillofacial surgery facility. It is necessary to inform the patient about the possible risks. Chlorhexidine mouth washes are indicated both before and after the dentoalveolar procedure. The surgery is performed under the influence of antibiotics, which continue to be employed after the procedure.

MRONJ treatment is very demanding in terms of time; therefore, AAOMS recommends a conservative approach, in an attempt to delay the surgical resection treatment, which is indicated in the advanced stages of the disease. Palliative conservative treatment is usually applied, since only a small percentage of patients will experience complete healing ad integrum. The conservative approach consists of equalization of sharp bone edges, sequestrectomy, necrotic area teeth extractions, and incisions and drainages under total antibiotic and topical treatment.

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Surgical treatment consists of complete removal of the necrotic foci, which serve as a fertile ground for infection, followed by wound closure with soft tissue that is finely vascularized, using layered suture. During the radical surgical resection, there are still concerns about the resulting wounds, difficulty in healing and progression of osteonecrotic foci.

Several studies point to the possibility of employing new treatment methods such as PRP, ozone or hyperbaric oxygen therapies. The benefit for cancer patients who are undergoing intravenous bisphosphonate treatment is bone pain relief and retreat of other bone complications. The basic rule is to preserve the quality of life for these patients, which includes a thorough oral health care, patient education, regular visits to the dentist, pain management and reports on health status, edemas, pain or bone exposure. It is also important to prevent the spread of new necrotic sockets by observing the proper prevention. Staging and management is described in **Table 1**.

Patients with aforementioned drugs in their medical history need to be treated as risk patients in view of invasive procedures in the oral cavity. Currently, the majority of osteonecrosis are of iatrogenic nature, caused by the incorrect choice of treatment for risk patients by the medical

Staging and Management of The ARM-Induced Osteonecrosis of The Jaw	
Stage	**Clinical Stages and Management**
Risk	**No evidence of necrotic bone. Patients who were, and still are treated with oral or intravenous antiresorptive drugs.** • Asymptomatic • Not requiring treatment • Patient education
0.	**No clinical records of necrotic bone, with non-specific finding and symptoms** • Systematic management • Analgesia
I.	**Asymptomatic patient with an exposed bone, without pain and infection of the surrounding tissues** • Daily use of an antibacterial mouthwash (chlorhexidine 0.12%) • Follow-up • X-RAY checks, analgesia
II.	**Osteonecrosis with signs of pain, inflammation and erythema** • Daily use of an antibacterial mouthwash (chlorhexidine 0.12%) • Analgesia, ATB p.o. based on the cultivation and sensitivity identification • Supportive treatment, polyvitaminosis (Tocopherol, Calcium)
III.	**Extensive osteonecrosis accompanied by pain, infection, fistula, osteolysis, extraoral fistula and pathological fracture. Exposed necrotic bone, or fistula that probes to bone in patients with pain and infection and at least one associated complication: exposed and necrotic bone extended beyond the area of the alveolar bone (i.e., the lower border of the ramus in mandible, or sinus and zygomatic bone area in maxilla), which leads to pathological fractures, extra and intra oral fistulas, oronasal and oroantral communication, or osteolysis extended to the lower border of the mandible** • Daily use of an antibacterial mouthwash (chlorhexidine 0.12%) • Surgical debridement/necrectomy • Analgesia, ATB p.o./i.v. based on the cultivation and sensitivity identification • Supportive treatment, polyvitaminosis (Tocopherol, Calcium)

Table 1. Staging and management.

staff. The cause of this unfavorable situation lies in the lack of communication between the specialist prescribing the high-risk drug and the treating dentist. The lack of awareness of the issue, both in patients and treating dentists, also plays its role. Medical specialist prescribing a high-risk drug is obligated to inform the patient about the risks and adverse effects of the planned treatment and to remind them to specifically inform their dentist about this fact. By disregarding this obligation on the part of the specialist (clinical oncologist, internist, rheumatologist, urologist, gynecologist, endocrinologist, orthopedist, etc.), the patient usually has no idea about the risk involved; however, the development of iatrogenic osteonecrosis may be prevented by the right approach by the treating dentist. They should not underestimate drug anamnesis prior to any invasive procedure in the oral cavity. Precise and targeted medical history can help identify at-risk patients and to choose the right treatment plan.

The incidence of MRONJ can be divided into two groups: patients with non-oncological disease (osteoporosis, rheumatoid arthritis) and patients with cancer who take high doses of intravenous bisphosphonates.

In the second group, the incidence after 36 months of treatment ranges from 1 to 12%. The majority of cases described are connected with the use of zoledronate and pamidronate in treatment of multiple myeloma and bone metastases. So far, the results and recommendations on potential treatment for these conditions refer to the multicenter studies conducted in the last 15 years.

The study named DEFEND (Denosumab Evaluation for Preserving Bone Density) was a double-blind, multicenter, placebo-controlled, third phase study on 332 postmenopausal women with osteopenia and respective T-scores in the range of 1.5–2.5 SD. Denosumab was applied in 6-month intervals at a dose of 60 mg subcut, in contrast to placebo. Both groups of patients took a calcium supplement (100 mg a day) and vitamin D. The primary objective was to observe the lumbar spine bone mineral density after 24 months of treatment.

The results of the study showed that, compared with placebo, denosumab significantly increased the value of BMD in lumbar spine (by 6.5%). Denosumab also increased the density in the proximal part of femur (3.4%) and in the distal end of radius (by 1.4%). In the placebo group, the BMD decreased in these areas.

In another study, titled DECIDE (Determining Efficacy: Comparison of Initiating Denosumab vs. Alendronate), the effectiveness of denosumab with the same dosage as in the study DEFEND was compared to alendronic acid with a dosage of 70 mg, once a week, in order to reduce the risk of osteoporotic fractures. The yearlong study enrolled 1189 postmenopausal, relatively older women with more serious osteopenia than in the DEFEND, with half the women having a fracture in their medical history. Calcium and vitamin D supplementation has been the norm throughout the study. The primary measured indicator was the change in the density of proximal femur. Moreover, bone densities of lumbar spine, femoral neck, trochanter and distal radius were also monitored.

The results showed that denosumab improved bone density in all the monitored areas markedly better than alendronic acid, as early as at the end of the first month. At the same time, resorption markers significantly decreased in the group treated with denosumab, compared to the alendronic acid group.

Large, randomized, placebo-controlled study called FREEDOM (Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months) studied the reduction of incidence of osteoporotic fractures. The authors monitored 7868 women aged 60–90, with an average BMD T-score of −2.5, but not lower than −4.0.

After 3 years, the incidence of new vertebral fractures identified on X-rays in women treated with denosumab was 2.3%, while the incidence in the control group was 7.2%. The treatment reduced the relative risk of vertebral fractures by 68%. The cumulative incidence of hip fractures was 0.7% in the treatment group and 1.2% in the control group (40% reduction in the risk of fractures). There were no recorded significant differences in incidence of side effects such as cardiovascular complications, infections, fracture healing time and hypercalcemia between the treated and the control group [11].

An important outcome of these studies was the discovery that denosumab significantly increases bone density, even in areas with prevalence of cortical bones. From these results, it can be concluded that alendronate has the longest half-life decay (10 years), while denosumab has a reversible effect because it does not deposit into bone tissue.

5.1. Combining antiresorptive drugs and hormonal therapy

Several studies refer to clinical cases of patients with antiresorptive drug-related osteonecrosis of the jaw that describe improvement in local findings and bone remodeling and an increase in patient's quality of life after switching bisphosphonates for hormonal therapy with recombinant parathyroid hormone teriparatide [12, 13].

Teriparatide was approved for the treatment of postmenopausal osteoporosis. Unlike the antiresorptive treatment, teriparatide has anabolic effect in bone which stimulates bone remodeling and bone tissue density. Intermittent administration (once a day) leads to a temporary increase of serum concentrations and preferential stimulation of the osteoblast activity, which leads to bone formation stimulation. The effect lies in the increase of bone mass and the number of osteoblasts, and the consequent strength of bones.

Some clinical trials document a positive effect of teriparatide and parathyroid hormone which reduces the risk of vertebral fractures while increasing the bone mineral density (BMD). The preparation is administered subcutaneously, one injection a day. Recommended treatment duration is 18 months. Side effects include cephalalgia, nausea and hypercalcemia. After its administration, osteal healing in mouth cavity was documented.

According to the trial results, hormonal preparations used after stimulating the activity of osteoblast and osteoclasts could be employed in the treatment of non-oncological osteonecrosis. However, current trials are very small, and there is no sufficient evidence, which calls for more studies. Treatment is difficult, and it is therefore available only for some patients.

Teriparatide should not be used in cancer patients due to an increased risk of osteosarcomas, which were found in preclinical trials on rats. The teriparatide therapy should not be indicated in patients who inject their bisphosphonates, zoledronic acid or pamidronic acid because of the increased incidence of necrosis and associated severe complications, in contrast to orally administered bisphosphonates.

6. Conclusion

The aforementioned clinical recommendations are based on relevant data, scientific evidence, available literature and the empirical experience of the authors. Despite the effort, neither standard nor recommended procedure may still be defined.

Clinical recommendation and subsequent treatment should be assessed by the treating physician based on the patient's status and their needs and preferences, which should alleviate their difficulties and improve the quality of life of the patient suffering from the osteonecrosis of the jaw. Compliance with preventive measures before and during the antiresorptive treatment seems beneficial; however, it is also considerably complicated.

The ARM-induced osteonecrosis of the jaw poses a current problem with a number of yet unanswered questions. In general, it is believed that the cause of the osteonecrosis is multifactorial, dependent not only on administered preparations, but also on the underlying oncological disease, type of chemotherapy, hormonal treatment, associated illnesses, age and addiction case history. It is therefore necessary to correctly set up and indicate the pharmacological and surgical treatment, monitor the bone antiresorptive therapy, with focus on prevention and complications.

Conflict of interest

No conflict of interest.

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Section 2

Therapy in Rheumatoid Arthritis

Chapter 6

Cardiovascular Safety of Anti-TNF and Non-TNF Biological Therapy in Patients with Rheumatoid Arthritis

Codrina Ancuta, Claudia Mihailov and Eugen Ancuta

Additional information is available at the end of the chapter

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Abstract

The association between cumulative inflammatory burden and increased cardiovascular (CV) risk in patients with immune-mediated inflammatory rheumatic disorders, particularly rheumatoid arthritis (RA), is widely recognized. Furthermore, the complex and dynamic interrelation between traditional cardiovascular risk factors, systemic inflammation, early accelerated atherosclerosis, and RA-related factors remains a challenge in routine practice. New European League Against Rheumatism (EULAR) 2016 recommendations have recently highlighted three key trends in cardiovascular risk assessment and management in patients with RA including optimal disease control (early diagnosis, treat-to-target strategy with the dynamic use of antirheumatic synthetic and biologic drugs) and non-pharmacological as well as pharmacological management of risk factors. The present chapter will emphasize excessive cardiovascular morbidity in RA, the optimal strategy to identify and stratify the cardiovascular risk profile, the prime selection of medication from the whole spectrum of non-biologic and biologic (TNF and non-TNF) drugs according to their cardiotoxicity.

Keywords: cardiovascular burden, rheumatoid arthritis, inflammation, TNF inhibitors, tocilizumab

1. Introduction

The association between cumulative inflammatory burden and increased cardiovascular (CV) risk in patients with immune-mediated inflammatory rheumatic disorders, particularly rheumatoid arthritis (RA), is widely recognized [1–5].

Furthermore, the complex and dynamic interrelation between traditional CV risk factors (such as hypertension, diabetes, obesity, abnormal lipid metabolism), chronic systemic inflammation, early accelerated atherosclerosis, and RA-related factors (e.g., C-reactive protein level, CRP, disease activity and severity, medication) taken together in a genetically predisposed background remains a challenge in routine practice [1–5].

European League Against Rheumatism (EULAR) 2015/2016 recommendations for cardiovascular disease management have recently highlighted three key trends in cardiovascular risk assessment and management in patients with RA and spondyloarthropathies (ankylosing spondylitis, psoriatic arthritis) including optimal disease control (early diagnosis, treat-to-target strategy with the dynamic use of antirheumatic synthetic and biologic drugs) and non-pharmacological as well as pharmacological management of risk factors [6, 7].

The present chapter will emphasize excessive cardiovascular morbidity in RA, the optimal strategy to identify and stratify the cardiovascular risk profile in different RA settings, the prime selection of medication from the whole spectrum of non-biologic (methotrexate and non-methotrexate drugs) and biologic (TNF and non-TNF) antirheumatic drugs according to their cardiotoxicity.

2. Cardiovascular risk in patients with RA

2.1. Cardiovascular burden in RA

Patients with systemic autoimmune conditions especially inflammatory joint disorders such as RA are at increased risk to develop cardiovascular comorbidity, in particular subclinical or clinically significant atherosclerotic coronary heart disease [1–5, 8].

Compared to general population, patients with RA experience excessive cardiovascular morbidity and, even, mortality, with an increased risk of about 50% [1, 2, 8, 9]. The rate of global cardiovascular as well as individual events comprising fatal and nonfatal myocardial infarction, congestive heart failure, stroke, and major adverse cardiac events (MACE) is increased in RA, the magnitude of cardiovascular risk in such patient being comparable with that related to diabetes [1, 2, 8].

A comprehensive overview of cardiovascular complications and safety issues indicates that RA develops twice myocardial infarction and features an increased risk (up to 87%) of heart failure as compared to general, non-RA population [1–3, 8–11]. Moreover, congestive heart failure in different RA subtypes has a worsen prognosis compared to non-RA patients; more than half of patients known with RA are prone to undergo diastolic heart failure, while diastolic ventricular failure and pulmonary arterial hypertension are broadly reported in long-standing RA [2, 8–11]. Myocarditis typically associates with active disease subtypes and is rare, clinically non-symptomatic, without influence on cardiac mortality [1, 2]. Conversely, atherothrombosis and clinically significant coronary heart disease are highly expressed (65% increase in cardiovascular risk) and account for premature death [2]. Finally, valvular disease, especially mitral regurgitation, is frequent (80%) but usually asymptomatic, while valve nodules, conduction anomalies, and arrhythmias are uncommon in such patients [2].

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Both early RA and established or long-standing RA are characterized by augmented CV risk, with a special emphasis on active status, irrespective of disease duration [1, 2, 4, 11].

Cardiovascular comorbidities of autoimmune disorders are the result of different contributing factors and their synergistic effects [1, 2, 4, 5, 8, 9, 11]. Although the prevalence and influence of traditional risk factors in RA is high, the excess of cardiovascular burden is only partially clarified [1–4, 6, 8, 9, 11]. A wide spectrum of nontraditional meaning RA-associated factors is already considered, including disease activity, severity, as well as antirheumatic drugs [1, 2, 4, 6, 8, 11–13].

2.2. Factors involved in cardiovascular risk in RA

The dynamic link between chronic systemic inflammation and atherosclerosis remains a key point in the pathobiology of cardiovascular involvement in various systemic autoimmune conditions, including inflammatory rheumatic disorders [1, 2, 10–13].

Cardiovascular risk in RA is multifactorial; since traditional risk factors fairly explain, specific issues related to disease activity and medication endorses the cardiovascular disease in RA [1–4, 10–13].

Smoking, diabetes, obesity, hypertension, as well as abnormal lipid pattern (dyslipidemia) are major metabolic risk factors for cardiovascular disease, while seropositivity (rheumatoid factor, RF, and anti-cyclic citrullinated peptide antibodies (ACPA)), systemic extra-articular features, anti-inflammatory (nonsteroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids), and disease-modifying antirheumatic drugs (DMARDs) are specific aspects with precise relevance for cardiovascular outcomes in RA patients (**Figure 1**) [1, 2, 4–6, 10–13].

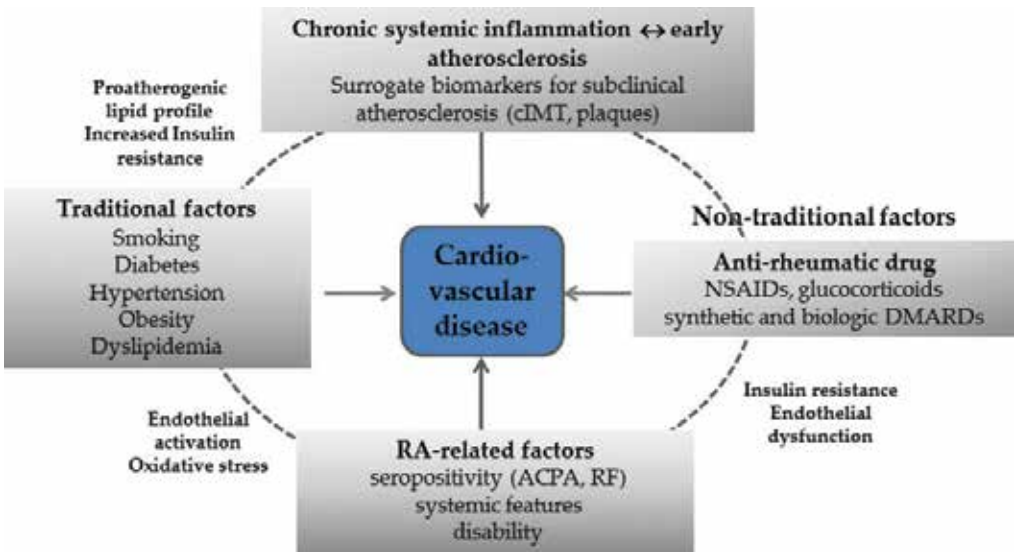

Figure 1. Traditional and non-traditional cardiovascular risk factors in RA (adapted from 8).

Furthermore, it is well established that chronic inflammation represents an independent risk factor for premature atherosclerosis, irrespective of other traditional factors [1, 3, 4], supporting severe, either subclinical or clinical coronary, and carotid disease in patients with RA [10–13].

Inflammation may alter the effect of existing risk or protective factors for cardiovascular disease, leading to an altered cardiovascular profile [1, 2, 6, 10–13].

Potent pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 and their signaling pathways play a fundamental role in persistent systemic inflammation and are the main determinants of cardiovascular risk in RA [1–4, 6]; in addition, they may interfere with classic factors resulting in altered endothelial function, insulin resistance, modified lipid spectrum, and the obesity paradox in RA [1, 2, 10–14].

There is a body of evidence showing the proatherogenic role of TNF-α and IL-1 as well as potential dual effect of IL-6 on atherogenesis [2, 3].

Thus, targeting inflammation to reduce the cardiovascular disease risk in inflammatory conditions seems to be a realistic project; additionally, an optimal control of rheumatoid inflammation through specific synthetic and/or biologic antirheumatic drugs always underwrites the decline of cardiovascular comorbidity [1–3, 6, 7].

The exclusive relationship of inflammation, endothelial dysfunction, and surrogate biomarkers of atherosclerosis with risks to cardiovascular disease among RA patients was extensively addressed [1–3, 10–13]. The pathophysiology of endothelial dysfunction is multifactorial, a complex network of interacting factors and mechanisms being emphasized. Not only different metabolic (dyslipidemia, hyperglycemia), non-metabolic (hypertension, smoking, oxidative stress) but also RA-specific factors (systemic inflammation) are associated with vascular dysfunction [1, 2, 9, 15–21].

Early intervention of irreversible vascular damage is strictly related to abnormal vascular tone, upregulation of fibrinolysis, and coagulation systems with subsequent procoagulant imbalance, subclinical and clinical atherosclerosis, and supporting cardiovascular disease development in RA (**Figure 2**) [1, 2, 6, 9–13].

2.2.1. Traditional risk factors

Although the relation between traditional cardiovascular risk factors and RA was largely addressed in the last decade, conflicting effects are still open [1–3, 9]. Obviously, classic factors mapping cardiovascular outcomes are more common among patients with autoimmune disorders [1, 2, 9].

A closer look to various traditional risk factors for cardiovascular disease in RA, their prevalence, and relations with disease activity and severity revealed the following aspects (**Table 1**).

2.2.1.1. Smoking

Recognized as a risk factor of both autoimmune diseases and accelerated atherosclerosis [1, 2, 15], tobacco smoking is able to modulate the immune system (e.g., induction of the inflammatory

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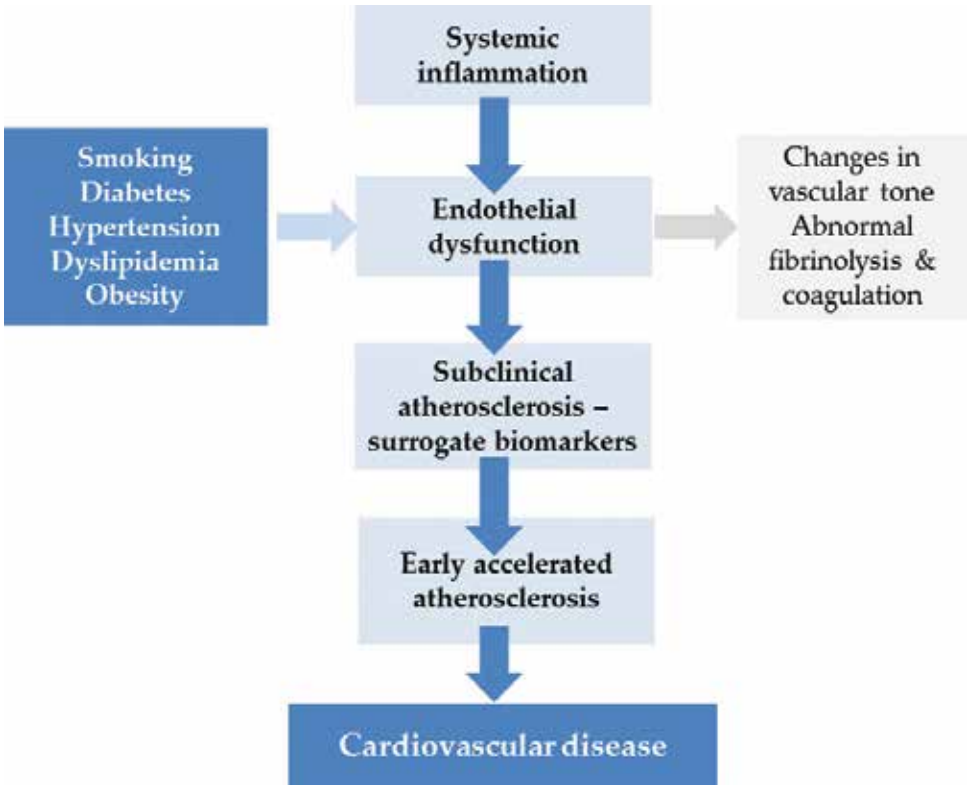

Figure 2. Inflammation, endothelial dysfunction, and atherosclerosis in cardiovascular disease.

responses and apoptosis, cytokine imbalance, DNA damage), to alter endothelial cells (e.g., overexpression of adhesion molecule, endothelial dysfunction), and to promote a procoagulant and inflammatory environment [1, 3, 15].

Smoking has a high prevalence in patients with RA and is classically associated with different predictive factors of the cardiovascular outcomes including seropositivity (for both RF and ACPA), disease severity, disability, and articular damage (erosions) [1, 2, 15]. Various studies have suggested that smoking and second-hand (passive) smoking may limit the therapeutic response in RA, promoting and perpetuating inflammatory aggressive effects on cardiovascular risk [1–3, 6, 15].

2.2.1.2. Hypertension

Among classical risk factors, hypertension is an important predictor of cardiovascular events not only in general population but also in patients with systemic autoimmune conditions, with the highest morbidity in RA (up to 40% in CORRONA) [1, 9, 16].

Since the pathobiology of increased blood pressure is multifactorial, blood pressure control in inflammatory autoimmune settings is largely related to chronic inflammation and immune-mediated mechanisms, along with classic mechanical injury of the arterial wall. Strong evidence

CV risk factors	Prevalence and particularities in RA	Relation with RA activity
Tobacco smoking	High prevalence in RA population String association with CV outcome Direct link with • seropositivity (ACPA, RF) • RA severity and activity • disability • articular damage	May impair therapeutic response, modulating inflammation effects on CV risk
Hypertension	High prevalence (COMORA 40%) Associated with NSAIDs and DMARDs increased vascular peripheral resistance interplay between inflammation and hypertension • TNFα results in endothelial vascular damage & oxidative stress • IL-6 alter the arterial tonus	Without evidence Adversely impact with NSAIDs and glucocorticoids
Insulin resistance	High prevalence in RA Promote low-grade chronic inflammation	Irrespective of RA activity
Body mass index & obesity	Paradoxical relation in RA: low body mass index associated with high CA risk Body mass index—independent CV risk predictor Relation obesity—inflammation via adipose tissue metabolism	Irrespective of RA activity
Dyslipidemia	Not only the quantity but also the quality, structure, and function of lipids 55–65% cases—abnormal lipid profile Lipid paradox (low lipids in inflammation associated with high CV risk) LDL-, HDL-, total cholesterol negatively correlates with inflammatory biomarkers Inflammation = consumption + decreased lipoprotein synthesis + defect of lipoprotein synthesis + structural and functional changes (altered HDL)	HDL cholesterol and atherogenic index improvement following non-biologic and biologic DMARDs

Table 1. Traditional cardiovascular risk factors in RA.

indicates the direct association between inflammation and hypertension; TNF-α can exert vascular endothelial damage and oxidative stress, while IL-6 enhances the arterial tonus. Not only blood pressure itself but also arterial inflammation is more prevalent in patients with RA and independently associated with both traditional cardiovascular risk factors and rheumatoid arthritis disease characteristics [1, 16].

There is no clear data suggesting the relation between hypertension and RA activity. However, the deleterious role of NSAIDs and glucocorticoids on hypertension is well established [1, 16].

2.2.1.3. Insulin resistance

Although underdiagnosed, especially in RA with longer disease duration, the prevalence of impaired fasting glucose, type 2 diabetes, as well as abnormal insulin resistance is largely increased among patients with autoimmune joint conditions compared to age- and gender-matched

controls [1, 13, 18–20]. In addition, the link between diabetes and chronic low-grade inflammation [1–3] is well known, supporting the hypothesis of the interaction between traditional CV risk factors and inflammatory burden in determining diabetes in RA [1, 2, 13, 18–20]. Furthermore, both classic risk factors (e.g., high blood pressure, high body mass index, high total cholesterol, metabolic syndrome) and novel cardiovascular risk factors (RA duration, exposure to glucocorticoids, radiographic damage, CRP levels) significantly correlated with glucose metabolism abnormalities and diabetes [1, 2, 13, 18–20].

2.2.1.4. Body mass index and obesity

While in general, non-RA population, body mass index is recognized as an independent predictor for cardiovascular disease, patients with RA are characterized by the paradoxical relation obesity (cardiovascular risk): low body mass index correlates with high cardiovascular events, unrelated to disease activity [1, 2, 6]. Moreover, the relation obesity-inflammation via adipose tissue metabolism is largely applicable in RA patients.

2.2.1.5. Dyslipidemia and lipid paradox in RA

Abnormal serum lipid pattern remains one of the main risk factors for cardiovascular morbidity and mortality in general population [1]; overall, modifications in high-density lipoprotein (HDL)-cholesterol as well as total/ HDL-cholesterol and triglyceride/HDL-cholesterol ratios are associated with adverse cardiovascular disease risk [1, 20].

Up to 65% of RA patients display an altered lipid profile [1, 2, 11, 12, 20], comprising modifications in absolute circulating lipid levels that widely reflect the interconnection between inflammatory and metabolic pathways [1, 2, 20].

The well-known lipid paradox, meaning low serum concentration of lipids associated with high cardiovascular burden in autoimmune inflammatory conditions, accounts for a negative correlation between total cholesterol, high-density and low-density lipoprotein (LDL)-cholesterol fractions, and inflammatory biomarkers.

Additionally, chronic inflammation is characterized by excessive consumption and deleterious synthesis of lipoproteins associated with structural (e.g., HDL associated with increased serum amyloid A with a potentially proatherogenic phenotype) as well as functional modifications (e.g., altered HDL phenotype) [1, 20].

Generally, both HDL-cholesterol and LDL-cholesterol are decreased in active RA and may increase with efficient control of inflammation with to non-biologic and biologic drugs [1, 2, 6, 20]. Moreover, small-dense LDL particles, known as more atherogenic than larger particles, are raised not only in metabolic disorders but also in RA [1, 20].

Interestingly, preclinical phase of RA accounts for raised inflammatory biomarkers (CRP) and dyslipidemia, with a negative correlation between the increased inflammation and decreased serum lipids [1, 2, 20]. This paradigm is applicable not only for chronic systemic autoimmune joint pathology but also in cardiovascular disease, postsurgery, or related to antineoplastic therapy [1, 2, 6, 20].

Tight control of inflammation in RA by non-biologic and/or biologic antirheumatic medication usually accounts for the improved lipid level [1, 2, 6, 7].

2.2.2. RA activity and cardiovascular risk

Individual inflammatory parameters (erythrocyte sedimentation rate, ESR, and CRP), composite scores reflecting disease activity (such as disease activity score (DAS28) and clinical disease activity index (CDAI)), and disease severity (extra-articular disease) are commonly associated with cardiovascular complications in RA [1, 2, 22–28].

The link between systemic inflammation and cardiovascular outcomes persists even after adjusting according to classical risk factors (obesity, hypertension, hyperlipidemia) [1, 2, 20–22].

Thus, elevated CRP levels at the baseline and the duration of uncontrolled disease correlate with cardiovascular burden in RA [1, 2, 22–28], while very high disease activity over time or high disease activity at RA onset also contributes to the cardiovascular risk [22, 23]. Moreover, it seems that uncontrolled high disease activity promotes the highest risk of developing cardiovascular disease [1, 2, 21–28]. CRP also correlated with acute myocardial infarction, one of the central complications of early accelerated atherosclerosis in RA [1, 22, 23].

Clearly, low disease activity (DAS28 < 3.2) and remission (DAS28 < 2.6) are fundamental in preventing a 10-years risk of cardiovascular events (particularly fatal or nonfatal cases of cardiovascular disease) in RA [1, 22, 23, 29]. In fact, it seems that low stable disease activity is able to reduce the cardiovascular burden and is appropriate to attain a protective effect against cardiovascular disease in RA [1, 22, 23]. Similarly, clinical remission or the absence of inflammation may be associated with a reduced risk of cardiovascular disease in RA [1, 22, 23]; apparently, remission has no additional protective effect against cardiovascular risk profile compared with low disease activity [1, 2, 22, 23].

Thus, it becomes clear that the manipulation of inflammatory response and tight disease control [22, 23, 28] are able to prevent cardiovascular events in RA [3, 26].

2.3. Cardiovascular risk assessment in RA

Different scores designed for the calculation of cardiovascular risk in general population (e.g., Framingham, SCORE, QRISK) underestimate the cardiovascular burden in patients with inflammatory rheumatic conditions, mainly RA [1, 2, 6, 29, 30]; thus, there is no standard or validated model for cardiovascular risk prediction in RA.

In EULAR 2009 has proposed to multiply by 1.5 points the SCORE risk for patients with RA if two out of three RA-related parameters are present: a disease duration more than 10 years, rheumatoid factor or ACPA positivity, and severe extra-articular manifestations [2, 6, 29, 30]. Despite good reliability, this score is not able to reclassify patients with RA in the adequate cardiovascular risk category [2, 6].

QRESEARCH Cardiovascular Risk Algorithm (QRISK) 2 is a prediction model including RA as a specific risk factor, multiplying with 1.4; it overestimates both nonfatal and fatal cardiovascular events [2, 6, 29, 30].

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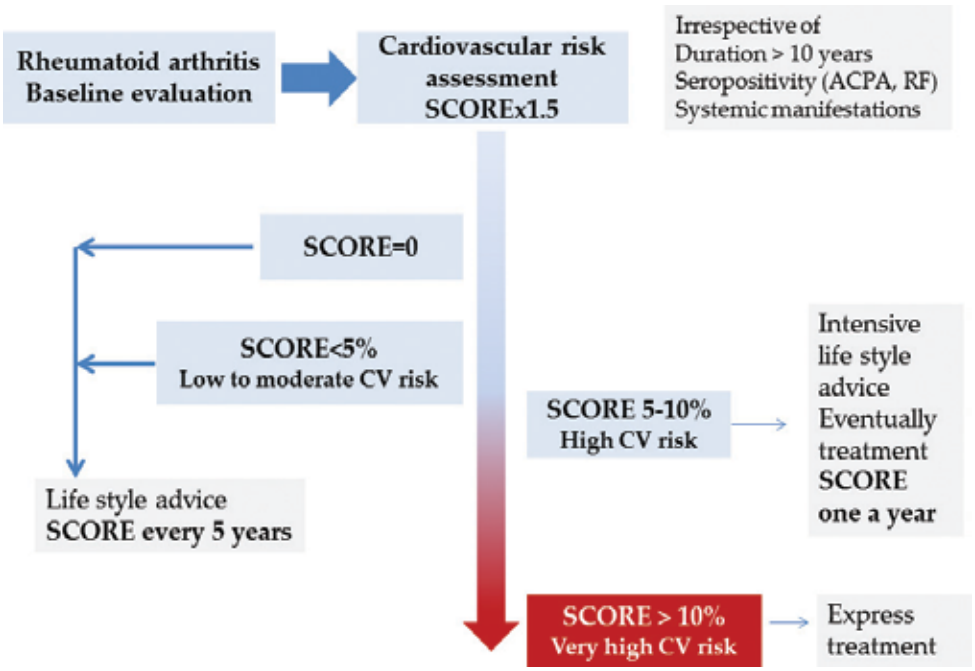

Figure 3. Algorithm for cardiovascular risk assessment in RA (adapted from Gualtierotti [4]).

EULAR 2016 has recommended the use of a modified SCORE adapted for patients with RA, meaning SCORE multiplied by 1.5 irrespective of disease duration, seropositivity, or the presence of systemic features [2, 6, 29, 30].

According to EULAR recommendations [6], the assessment of RA patients should be adapted based on CV risk stratification and several individual factors such as change of specific anti-rheumatic medication [2, 6, 29, 30]. Thus, risk monitoring is warranted once every 5 years if the risk is low to moderate or more often if the patient has an intermediate and high risk or when adapting the therapeutic protocol [2, 6]. An interesting algorithm was recently adapted/ proposed by Gualtierotti et al. (**Figure 3**) [2].

2.4. Recommendations for cardiovascular risk assessment in RA

Cardiovascular risk stratification and further monitoring based on the risk class is essential in RA [1, 2, 6, 29, 30]. EULAR 2009 guidelines have proposed the assessment of the cardiovascular disease on an annual basis, except for patients classified as low risk or low disease activity where the risk is typically monitored as 2–3 years [1–3, 6].

Updated EULAR 2016 guidelines released four new recommendations emphasizing the interval, scores, and protocol for cardiovascular disease screening [6]. Interestingly, cardiovascular risk assessment is no longer recommended annually but every 5 years or following a major change in the DMARD therapy (recommendation 2) [6]. Either local or national guidelines or SCORE are equally accepted for the calculation of CV risk (recommendation 3) [6]. Lipid profile comprising total cholesterol and its HDL fraction should be evaluated during stable

disease or remission (recommendation 4) [6]. Finally, surrogate biomarkers for subclinical atherosclerosis such as plaques on Doppler carotid ultrasound are recommended for the extensive screening of the cardiovascular disease in RA patients [6].

Recommendations for the management of CV risk in RA take into account three essential points [1, 2, 6, 14]: (i) control of disease activity meaning early diagnosis and dynamic choice of the antirheumatic drug starting with non-biologic DMARDs followed by biologic DMARDs, with or without glucocorticosteroids until reaching the therapeutic target (remission or low disease activity), (ii) non-pharmacological management of risk factors including smoking cessation, adequate level of physical activity, as well as healthy diet, (iii) pharmacological management of risk factors comprising statins for lipid abnormalities, antihypertensives, antidiabetics, etc.

Optimized disease control within the so-called treat-to-target (T2T) strategy consistently improves the cardiovascular risk in RA, as remission and low disease activity become realistic goals [1, 2, 4, 6, 7]. Recent advances in the pathobiology of the disease and the link between inflammation, RA disease activity, and cardiovascular issues have highlighted the following [1, 2, 6, 7]: (i) disease duration is not an independent cardiovascular risk factor; (ii) disease activity, the number, and duration of flares significantly alter the CV risk; (iii) reducing inflammation obviously improves the CV outcomes; and (iv) long-term DMARD therapy results in lowering the CV risk.

Not only chronic persistent systemic as well as local inflammation and RA disease activity endorse excessive cardiovascular burden in RA but also traditional CV risk factors, e.g., body mass index, lipids, gender, tobacco smoking, and hypertension [1–3, 6].

Aggressive T2T approach in daily clinical practice for patients with RA proved successful in negatively impacted systemic and synovial inflammation, impair cytokine release, and promote quantitative and qualitative lipid changes, significantly decreasing the cardiovascular disease in such patients [1, 2, 6, 7]. Conversely, anti-inflammatory drugs (e.g., not only NSAIDs and COX-2 inhibitors but also steroids) are commonly associated with high cardiovascular safety issues [1, 2, 6].

Regardless of substantial progresses in RA treatment, patients develop and die considerably earlier than the general population mainly related to cardiovascular comorbidities, generally in connection with accelerated atherosclerosis and cardio-metabolic complication [1, 2, 13, 18, 21].

2.5. 2016 EULAR recommendation for CV management

Since broad cardio-metabolic evaluation and optimal strategies for cardiovascular risk reduction are still poorly integrated in routine practice in autoimmune conditions [13, 18, 21], EULAR has recently updated guidelines for cardiovascular disease risk assessment and management in systemic inflammatory conditions including RA, psoriatic arthritis, and ankylosing spondylitis [6]. An extended EULAR task force has reconsidered the old guidelines and released new overarching principles and recommendations in accordance to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analysis) statement suggesting an appropriate algorithm for cardiovascular risk management in these patients [6].

Updated EULAR 2015/2016 guidelines for cardiovascular comorbidity in RA are listed below [6]:

1. Lowering the cardiovascular risk in RA largely relies on both early diagnosis and tight control of disease activity as well as the disease flares.
2. The cardiovascular risk reassessment is advised at least every 5 years or when major changes in DMARD therapy are specified.
3. EULAR endorses the application of risk assessment scores according to the general population such as systematic coronary risk evaluation (SCORE) or national guidelines for CVD risk evaluation.
4. A systematic evaluation of lipid profile including non-fasting total cholesterol and low- and high-density lipoprotein fractions is mandatory to further mitigate the cardiovascular risk; however, measurements should be achieved during stable RA or in remission disease.
5. A correct estimate of the increased risk of cardiovascular events in RA encompasses for a modified risk score multiplied by 1.5.
6. Surrogates of subclinical atherosclerosis and cardiovascular disease such as artery plaques identified by carotid ultrasound screening are useful to perceive the increased cardiovascular risk in RA.
7. Several protective measures, e.g., healthy diet, exercise on a daily basis, and smoking cessation, should underline the general approach of RA patients with raised cardiovascular risk.
8. New trends in treating hypertension and dyslipidemia with specific medication including antihypertensives and statins are widely recommended in patients with RA with such comorbidities.
9. Since both traditional nonselective NSAIDs and coxibs have an increased risk of developing cardiovascular toxicity in RA, these drugs are carefully proposed in such patients, especially in those with prior history of or at increased risk to develop further cardiovascular events (congestive heart failure, ischemic heart disease, peripheral arterial or cerebrovascular disease).
10. If mandatory, long-term corticosteroids are managed to minimize the total dosage, with tapering as soon as remission or low disease activity is reached.

3. Cardiovascular safety of non-biologic and biologic anti-rheumatic drugs

Evidence-based medicine provides key insights into the consequences of various classes of antirheumatic drugs on cardiovascular risk in RA, suggesting that TNF inhibitors (TNF-i) and methotrexate decrease the risk of such events, while corticosteroids and IL-6 receptor inhibition via tocilizumab exert a multifaceted intervention on cardiovascular outcomes [1, 2, 26, 31]. Data are summarized in **Table 2**.

Drug	Positive effects	Negative effects	Overall influence on CV safety
Methotrexate	Improved RA activity	High homocysteinemia	Protective
Other csDMARDs Leflunomide Antimalarials	Improved RA activity, improved lipid profile, reduced risk of diabetes	Arterial hypertension May be responsible of cardiomyopathy although rarely	Complex effect Protective
TNF inhibitors	Improved RA activity, reduced insulin resistance	Contraindicated in severe heart failure (class III/IV NYHA)	Protective
Non-TNF inhibitors Tocilizumab Rituximab Abatacept Tofacitinib	Improved RA activity Safe in heart failure Safe in heart failure Safe in heart failure	Abnormal lipids	Complex effect Complex effect Complex effect
Corticosteroids NSAIDs	Reduced inflammation	May increase CV events, BMI, dyslipidemia, insulin resistance, hypertension All NSAIDs increase CV risk (prothrombotic effect)	Complex effect

Table 2. Cardiovascular profile of anti-rheumatic drugs: non-biologic and biologic DMARDs, corticosteroids, NSAIDs.

3.1. Non-biologic DMARDs: methotrexate and non-methotrexate drugs (e.g., sulfasalazine, antimalarials, leflunomide) improve cardiovascular outcomes in RA

Recent meta-analysis as well as real-life data showed that traditional synthetic DMARDs are able to reduce the cardiovascular risk in RA. However, the precise cardioprotective mechanism of classic immunosupressants remains still under debate.

Undoubtedly, methotrexate and non-methotrexate agents efficiently control inflammation and disease activity, alter the lipid spectrum and concentration, and are able to reduce the arterial stiffness, improving cardiovascular risk in different RA scenarios [1–32].

Furthermore, methotrexate, the drug of choice as first-line treatment, is associated with a consistent reduction in mortality (70%), a decline in the rate of total cardiovascular events (around 28%), and up to 18% lower risk of myocardial infarction in patients with RA [31]. Unlike the anti-TNF agents, methotrexate is not related to a significant decrease in strokes and major adverse cardiac events; nevertheless, it seems that the risk of heart failure is also decreased [1, 2, 31, 32].

The interesting hypothesis of inflammatory origin of cardiovascular disease prompted the ongoing randomized Cardiovascular Inflammation Reduction Trial (CIRT) aiming to investigate whether low-dose methotrexate is able to decrease rates of heart attacks, strokes, and cardiovascular death among stable coronary artery disease patients with type 2 diabetes and/or metabolic syndrome, conditions associated with an enhanced pro-inflammatory response [3]. Although the study was designed to perceive a 25% cardiovascular risk decline within the

methotrexate group, failure to achieve this magnitude of risk reduction will not degrade the immune inflammatory background of cardiovascular pathology [3].

Data about other synthetic DMARDs are not consistent. Leflunomide has indirect positive effects on cardiovascular outcome through reducing disease activity, while its role in promoting hypertension is a potential limitation, particularly if high blood pressure is documented [1, 2]. Antimalarials, specifically hydroxychloroquine, are able to modulate lipid profile and to reduce the risk of diabetes, although rare cases of cardiomyopathy have been described [1, 2, 31, 33]. Finally, sulfasalazine seems to exert also protective effects, while further studies are required to clearly define its role [2].

3.2. Glucocorticoids enhance the cardiovascular risk in RA

The association between corticosteroids and the rate of total as well as individual cardiovascular events was systematically evaluated in patients with inflammatory rheumatic disorders, principally in RA and systemic lupus. It is widely accepted that corticosteroids are able to shape the cardiovascular risk by two competitive pathways [1, 2, 6, 31, 34]:

- The risk for future cardiovascular disease is increased due to not only metabolic effects, e.g., abnormal lipid metabolism, impaired glucose tolerance, insulin resistance, relative weight gain, or true obesity, but also glucocorticoid-induced hypertension.
- The cardiovascular risk is attenuated through rapid and salutary anti-inflammatory and anti-proliferative effects of corticosteroids.

Their cardiotoxicity as well as cardiovascular mortality is dose-dependent (actual and previous cumulative dose) [1, 2, 6]. Sustained administration of corticosteroids classically accounts not only for an increased risk of myocardial infarction, stroke, and congestive heart failure but also for all major adverse cardiac events in RA patients as suggested by different studies and meta-analyses [1, 2, 6, 31, 34].

The well-known trend in RA is to maintain the lowest dose of corticosteroids for the shortest period of time and tapering as soon as remission or low disease activity (recommendation 10, EULAR 2016) [6]; the benefits of further administration should be deliberated and reconsidered once the disease outcomes have been achieved [6].

Although NSAIDs no longer represent the mainstay of RA therapeutic protocol, both nonselective and COX2-selective medications are still used, even if intermittently, during RA flares and in short-time administration [1, 2, 22, 23, 31, 35]. NSAID safety profile should be emphasized, as their effect on cardiovascular outcomes in RA is challenging. Both nonselective NSAIDs and coxibs increase the cardiovascular risk in general population; however, not all of them exert the same deleterious cardiovascular effect, especially talking about patients with RA [2, 6, 31, 35].

It is widely recognized that long-term NSAIDs increased the risk of all cardiovascular events and stroke in general population, particularly if cardiovascular disease is documented (congestive heart failure, ischemic heart disease, peripheral arterial disease, or cerebrovascular

disease) [2, 6, 31]; only few studies have addressed the cardiovascular outcomes of COX2-selective and traditional NSAIDs specifically in RA [1, 2, 6, 31, 35].

3.3. Biologic DMARD: TNF inhibitors decrease the cardiovascular risk in RA

TNF-α, a pivot pro-inflammatory cytokine involved in both systemic and local (synovial microenvironment) persistent inflammations in RA, may also alter lipid metabolism, insulin resistance, and endorse endothelial dysfunction [1, 2, 6, 31, 36, 37]. Moreover, chronic inflammation and atherosclerosis share common pathways, tailoring the basis of amplified cardiovascular risk in inflammatory rheumatic conditions [1, 2].

On the other hand, TNF inhibitors provide multifaceted influences on cardiovascular safety in patients with RA comprising [1, 2, 6, 31, 37]:

- Rapid and effective control of chronic inflammation signaling with significant decrease in inflammatory parameters (serum CRP levels and ESR), RA activity, and delay articular damage articular
- Changes in lipid pattern with increase in HDL-cholesterol fraction, total cholesterol, triglycerides with or without influence on LDL-cholesterol fraction, and with no major impact on the atherogenic index (TC/HDL); in fact, the trend of rising serum lipids under anti-TNF therapy typically reflects the stabilization through the lipid levels before the RA onset
- Improvement of endothelial dysfunction and insulin sensitivity as well as the anti-oxidative response of HDL

Thus, it seems that TNF inhibitors are efficient in reducing cardiovascular comorbidities in RA, comprising not only all cardiovascular events but also some specific stroke, myocardial infarction, and even MACE [1–3, 31, 36, 37].

A meta-analysis of several observational studies and registries in RA patients treated with TNF inhibitors performed by Roubille et al. showed a significant reduction in the risk of all cardiovascular events (up to 30%), myocardial infarctions, strokes, and major adverse cardiac events with biologics vs. non-biologic DMARDs [1–3, 31, 36, 37]. However, no significant effect on heart failure was detected [2, 31]. Interestingly, the risk of future cardiovascular consequences under anti-TNF medication is twice lower that the risk with non-biologic non-methotrexate drugs [1–3, 31, 36, 37].

Besides, Ljung et al. recently published their study investigating the effects of response to anti-TNF agents on short-term (1- and 2-year follow-up) risk of acute coronary syndrome in individuals with active RA and no previous ischemic or congestive heart disease before starting TNF-i as the first biologic agent [37].

RA patients classified as good (but not moderate) EULAR responders (improvement of more than 1.2 points in disease activity score) to anti-TNF therapy showed a 50% risk reduction of acute myocardial infarction or unstable angina pectoris or acute myocardial infarction as the underlying cause of death in short-term follow-up, similarly to the risk of acute coronary events in general population [1, 2, 6, 31, 36, 37].

In fact, the magnitude of coronary event risk reduction was reported irrespective of the baseline risk stratification (by gender, age, cardiovascular risk factors, disease duration), drug exposure, as well as the risk window [2, 31, 36, 37]. Additionally, the ability to achieve optimal control of RA activity instead of simply improved disease control or TNF-i per se theoretically stabilizes the pattern of ischemic heart disease in RA [1–3, 31, 36, 37].

Still considered the "black box" of TNF biologic class, the link between TNF blockade and moderate to severe congestive heart failure (class III/class IV NYHA) remains controversial [1, 2, 31, 36]. Clearly, heart failure in general population is associated with high TNF-α, with consistent correlation between serum levels and clinical significance and severity of cardiac failure [1, 31, 36]. However, TNF inhibitors are able to improve ventricular dysfunction in experimental models of cardiac failure [1, 2, 6, 31, 36, 37].

Overall, TNF inhibitors significantly damper the cardiovascular comorbidity in RA, especially in patients declared responders to such therapies.

3.4. Non-TNF DMARDs: tocilizumab

Data about tocilizumab, a humanized monoclonal antibody acting against the IL-6 receptor (membrane-bound and soluble), and its cardiovascular toxicity are widely accessible from different clinical trials, including ADACTA, TOWARD, MEASURE, and ENTRACTE [38–41].

Although modest increases of LDL- and HDL-cholesterol as well as triglycerides were noticed in RA patients treated with tocilizumab in randomized controlled trials, only ENTRACTE was specifically designed to evaluate the cardiovascular safety and to compare net cardiovascular risk-benefit ratio of anti-IL-6 therapy with biologics with another mechanism of action, such as etanercept, in active seropositive RA [1, 2, 38–41].

As a potent inhibitor of IL-6 signaling, tocilizumab is associated with excellent clinical efficacy essentially related to patent decline in systemic inflammatory biomarkers; in addition, tocilizumab-associated altered lipid profile, mainly increased LDL-cholesterol, is broadly recognized and potentially connected with atherogenesis and atherothrombosis in patients with RA [1, 2, 3, 39–42].

Moderate elevations of LDL-cholesterol, HDL-cholesterol, and triglycerides were faced in RA patients under tocilizumab in phase II and phase III trials, but the atherogenic implications of these changes are still unsettled [1, 2, 38–41].

A summary of trials reflecting the cardiovascular safety profile of tocilizumab in RA comprises the following:

ADACTA, a randomized clinical trial to evaluate tocilizumab monotherapy vs. adalimumab monotherapy for the treatment of RA, suggested that tocilizumab meaningfully decreases systemic inflammation as supported by low ESR and CRP levels and disease activity (DAS28, CDAI). Changes are obviously more evident as compared to adalimumab, a totally humanized monoclonal anti-TNF antibody; however, tocilizumab induces higher LDL-cholesterol levels more than adalimumab do [39].

TOWARD trial, evaluating tocilizumab in combination with traditional DMARD therapy, revealed the role of IL-6 blockade not only in reducing articular and systemic inflammation but also in improving insulin resistance, along with its capacity to promote increased total cholesterol levels in up to one-fourth of cases [38].

Tocilizumab may induce particular changes in lipid pattern, with potential relevance for cardiovascular safety. Persistent increase in mean fasting plasma lipids, within the normal range, is commonly reported with tocilizumab, together with a consistent decrease in serum CRP, suggesting that abnormal lipid levels may, in part, be related to significant decline in inflammation [38].

An increase in total cholesterol was observed in one out of five RA included in the study, while high LDL and altered HDL status from a pro-inflammatory to a significantly low inflammatory status (12 and 15%, respectively) increases of 30% in the total cholesterol:HDL-cholesterol ratio (12% cases) and more frequent in the LDL-cholesterol:HDL-cholesterol ratio (20%) were also reported. Finally, increases in the mean apolipoprotein A-I and apolipoprotein B, within the normal range, without changes in the ApoB:ApoA-I ratio were also commonly described. Only a limited number of patients initiated statin therapy during the study, with positive influence on lipid modifications [38].

MEASURE trial of tocilizumab effects on surrogates of vascular risk in RA was powered to demonstrate the modulation of lipid and lipoprotein particle (LDL, HDL, VLDL) levels and composition (HDL-associated serum amyloid A), alongside other surrogates of vascular risk (markers of coagulation, thrombosis, and vascular function) with IL-6 receptor inhibition vs. placebo in active disease [40, 41].

McInnes et al. [41] not only reported a dramatic decrease in inflammation in such patients but also demonstrated quantitative and qualitative changes in lipid metabolism profile. Overall, tocilizumab prompted the increase in total cholesterol, LDL-cholesterol, and triglycerides by week 12 of administration, while no significant influence on proatherogenic small LDL particle concentration, oxidized (ox)LDL, or HDL-cholesterol levels, in addition to ApoB:ApoA-1 ratio. Furthermore, tocilizumab-based IL-6 signal blockade altered the HDL particle composition toward a less pro-inflammatory phenotype [40, 41].

Besides, HDL-associated serum amyloid A, secretory phospholipase A2-IIA, lipoprotein(a), fibrinogen, and D-dimers presented a sizeable decrease, while the antioxidant enzyme associated with HDL, paraoxonase, and level significantly increased under tocilizumab. Since a prothrombotic status heightened risk for cardiovascular events independently of established risk factors in general population, the reduction of thrombotic potential with tocilizumab in patients with active RA remains of considerable interest [40, 41].

However, the clear benefit of such modifications for cardiovascular risk is still debatable.

ENTRACTE, a randomized clinical trial aiming to evaluate cardiovascular events with either i. v. tocilizumab monthly or etanercept s.c weekly, was designed as a non-inferiority study comparing cardiovascular safety of tocilizumab vs. the TNF receptor, etanercept, in RA. Primary endpoint focused on major cardiovascular adverse events (MACE) (cardiovascular death, nonfatal myocardial infarction, nonfatal stroke), while secondary endpoints were cardiovascular and non-cardiovascular safety of both drugs [1–3].

ENTRACTE has demonstrated a relative increase in the incidence of cardiovascular events among severe active RA patients with a background of cardiovascular risk factors receiving tocilizumab, specifically the hazard of MACE. Additionally, the average level of LDL-cholesterol was consistently higher under tocilizumab as compared with etanercept. Nevertheless, the cardiovascular issues with tocilizumab vs. etanercept should be deliberated/interpreted in the context of its clinical efficacy and general, non-cardiovascular, safety profile.

3.5. Other non-biologic and targeted DMARDs: rituximab, abatacept, and tofacitinib

- **Rituximab**, a B-cell depletory agent, typically indicated as a second-line biologic therapy after failure of at least one anti-TNF agent, showed no significant differences as compared to placebo in terms of cardiovascular events. There is no cardiovascular safety concern related to rituximab; furthermore, it seems that rituximab is able to improve lipid metabolism, alter HDL-cholesterol to a low atherogenic profile, as well decrease prothrombotic biomarkers. Also, rituximab has no influence on arterial stiffness [1–3, 6, 7, 42].
- **Abatacept**, by blocking the T and B costimulation, commonly acts on lipid pattern resulting in high total cholesterol and its fractions (HDL-cholesterol and LDL-cholesterol), without a significant decrease in the atherogenic index; however, abatacept is known to alter the arterial tonus [1–3, 6, 7].
- **Tofacitinib**, a JAK inhibitor already approved for the management of RA, promotes similar changes in lipid profile as tocilizumab do, meaning an increase of both HDL-cholesterol and LDL-cholesterol, with a minimal impact on atherogenic index [1–3, 6, 7, 43].

4. Conclusions

Systemic autoimmune rheumatic conditions, specifically RA, are widely associated with excessive cardiovascular morbidity, with a magnitude similar to that related to traditional cardiovascular risk factors, particularly diabetes.

A multifaceted dynamic interplay between chronic systemic inflammation, RA-specific issues, early accelerated atherosclerosis, and classic cardiovascular risk factors typically highlights the cardiovascular burden in various RA settings.

The optimal strategy to identify patients at increased risk to develop cardiovascular disease as well as the correct assignment of different risk categories is mandatory in routine practice in every RA case. Furthermore, the selection of suitable medication (non-biological, TNF inhibitor, or non-TNF biological antirheumatic drug) according to cardiovascular toxicity is warranted so as to improve cardiovascular outcomes in RA.

Conflict of interest

No conflict of interest declared.

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Chapter 7

Efficacy of SSEA-3 Positive Cells Derived from Synovial Tissue in Rheumatoid Arthritis

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Additional information is available at the end of the chapter

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Abstract

Rheumatoid arthritis (RA) is a refractory systemic autoimmune disease with chronic synovial inflammation. Sustained synovial inflammation leads to progressive destruction of bone and cartilage. Treatment to restore joints that have been destroyed irreversibly is not to be established yet even with the recent development of antirheumatic drugs and biological agents. Stage-specific embryonic antigen-3 (SSEA-3), a marker of human embryonic stem (ES) cell, acts as stem cells in the blood. SSEA-3 positive cells derived from RA synovial tissue have higher differentiating abilities than that of SSEA-3 negative cells and inhibitory effects on arthritis in collagen antibody-induced arthritis mice study. SSEA-3 positive cells derived from RA synovial tissue might have the inhibitory effect on arthritis and would be one of the cell sources for new RA treatment. The present manuscript is a brief review of mesenchymal stem cells in RA and described with the potential of RA cell therapy by SSEA-3 positive cells based on our research.

Keywords: rheumatoid arthritis, synovial tissue, SSEA-3

1. Introduction

Rheumatoid arthritis (RA) is a refractory systemic autoimmune disease with chronic synovial inflammation. Sustained synovial inflammation leads to progressive destruction of bone and cartilage. In the pathogenesis of RA, activated T cells and antigen-presenting cells such as monocytes and macrophages produce inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-2, IL-6, and interferon-γ (INF-γ). They promote release of inflammatory mediators, infiltration of inflammatory cells, production

of autoantibody, proliferation of the synovial cells, and activation of the osteoclasts, resulting in the bone and the cartilage destruction [1–3].

In the last few years, development of disease-modifying antirheumatic drugs (DMARDs) and biological agents targeting inflammatory cytokines has been major advances in the treatment of RA. Biological agents targeting inflammatory cytokines such as TNF-α, which has been shown to be a key factor in the pathology of RA, are effective for RA. These are known to improve disease activity and inhibit the progress of joint destruction. The best possible treatment goal for patients is clinical remission and consistently stopping continuing joint damage through erosions.

However, treatment to restore joints that have been destroyed irreversibly is not to be established yet. Also, DMARDs or biological agents can have serious side effects affecting the blood, liver, or kidneys rarely. Therefore, a novel RA treatment that enables restoration of destroyed joints is needed. Use of mesenchymal stem cells (MSCs) derived from bone marrow as a biological method for repairing articular cartilage defects have been investigated [4–8]. We think that the treatment by thus autologous cells can overcome these problems.

2. Mesenchymal stem cells in RA synovial tissue

2.1. Multipotency

MSCs are self-renewing, multipotent progenitor cells with multilineage potential to differentiate into various types of cells including chondrocytes, osteoblasts, and adipocytes [9–15]. While MSCs are most commonly isolated from bone marrow [13] and proliferate rapidly in vitro, they are also isolated from other tissues including adipose tissue [16], placenta [17], and umbilical cord blood [18]. Due to their accessibility and convenient expansion protocols, ethical dilemmas and risk of tumor formation, such as in ES cells and iPS cells, can also be avoided and therefore MSCs are easy to use in clinical application and have been recognized as promising candidates for cell therapy.

We investigated earlier the potential of chondrogenic differentiation of MSCs derived from bone marrow and synovial fluid in human osteoarthritis (OA) [19, 20]. Our study concluded that both bone marrow MSCs (BMMSCs) and synovial fluid MSCs (SFMSCs) had a potential of cell proliferation and chondrogenic differentiation. Both cells were fibroblast-like cells and had similar cell surface antigen in flow cytometry analysis, namely positive for CD13, CD44, and CD105 and negative for CD10, CD14, and CD45. However, aggrecan (AGG) mRNA expression in SFMSCs, which are traditionally associated with chondrogenic commitment, was a significant high compared to BMMSCs in vitro. According to other researches, SFMSCs are considered the same as synovial MSCs [21, 22]. Study of Sekiya et al. [23] reported that synovial MSCs are a candidate cell source for regenerative medicine of cartilage due to their high chondrogenic ability. They demonstrated that chondrogenic potentials of synovial MSCs between RA and OA patients were similar, as the weight of the pellet is a quantitative indicator of the ability of MSCs to produce chondrogenesis in vitro. Therefore, autologous synovial MSCs can be expected in cartilage regeneration for RA patients. According to previous reports [24], there was a negative relationship between chondrogenic potential of synovial MSCs and magnitude of synovitis in RA, and some properties of synovial MSCs vary dependent on the

diseases patients have. Also, it was reported that chondrogenic potential in RA patients was inferior to that in OA patients. However, Jones et al. [24] reported that effective suppression of joint inflammation is necessary for the development of autologous MSC treatments aimed at cartilage regeneration in RA and synovial MSCs can be expected for RA patients with the inflammation well controlled as well as OA patients.

2.2. Immunosuppresive effect

Previous reports have suggested that synovial MSCs harvested from RA were capable of immunosuppression in vitro [24]. However, other reports have suggested that the immunomodulatory function of synovial MSCs seems to be disturbed and causes an inefficacy due to various factors within RA microenvironment and as a result of a direct contact with inflammatory cells and cytokines [25]. In RA synovial tissue, synovial MSCs appear to play an important role in controlling the inflammation and immune hemostasis.

3. Stage-specific embryonic antigen-3 (SSEA-3) positive cells in RA synovial tissue

3.1. SSEA-3

Multilineage differentiating stress enduring (Muse) cells are a novel type of pluripotent stem cells and recently reported as adult human MSCs without introducing exogenous genes. They are present in various organs such as pancreas, dermis, umbilical cord, fat, liver, trachea, bone marrow, spleen [26–31] and are contained at a proportion of several percent in cultured mesenchymal stem cells [26], 4–9% in human adipose tissue [27] and 1–2% in human skin fibroblasts [26]. Muse cells are able to differentiate into cells from all three embryonic germ layers both spontaneously and under media-specific induction. Also, Muse cells have a low tumor-forming ability compared with embryonic stem (ES) cells and a high efficiency of change to iPS cells by Yamanaka gene introduction [32]. They can migrate to damaged tissues by intravenous injection in vivo, spontaneously differentiate into cells compatible with the targeted tissue, and contribute to tissue repair. Thus, Muse cells will be expected to play an important role in regenerative therapy by further studies. SSEA-3 is a marker of human embryonic stem cell. Muse cells are able to be isolated as SSEA-3 positive cells from cultured mesenchymal cells.

SSEA-3 positive cells are autologous cells and act as stem cells in the blood and also possess immunosuppression effects [28–31]. Therefore, they could be one of novel cell sources as cell therapy in RA. We studied the possibility of SSEA-3 positive cells derived from RA synovial tissue.

3.2. SSEA-3 as cell therapy in RA

3.2.1. SSEA-3 positive cells in RA synovial tissue

We used synovial tissue harvested from 13 RA patients at the time of joint surgery in our hospital (**Table 1**) [33]. Diagnosis of RA for all patients was based on the American College of Rheumatology (ACR) criteria in 1987 [34] or the ACR/European League Against Rheumatism

Pts.	Age	M/F	Stage	Class	R/L	Surgery
1	73	M	IV	III	R	total knee arthroplasty
2	45	F	I	II	L	arthroscopic synovectomy
3	78	M	III	I	R	total knee arthroplasty
4	82	F	IV	II	R	total knee arthroplasty
5	77	M	IV	II	L	total knee arthroplasty
6	84	F	IV	II	R	total knee arthroplasty
7	69	F	III	II	R	total elbow arthroplasty
8	77	F	III	II	L	total elbow arthroplasty
9	66	F	IV	II	R	2nd, 4th PIP arthrodesis
10	69	F	IV	III	L	1st IP arthrodesis
11	61	F	IV	II	R	wrist arthroplasty
12	62	F	IV	II	R	1st IP arthrodesis
13	66	F	III	I	L	total hip arthroplasty

Pts, patients; M/F, male or female; R/L, right or left; PIP, proximal interphalangeal joint; IP, interphalangeal joint.

Table 1. Clinical data of patients with RA (n = 13) for this study.

(EULAR) classification criteria in 2010 [35]. Approval for this study was obtained from the Ethics of Human Experiments Committee at Hirosaki University Graduate School of Medicine, Hirosaki, Japan. Informed consent was obtained from all patients.

Immunohistochemical staining was performed to investigate the localization of SSEA-3 positive cells in RA synovial tissue. Harvested synovial tissue was immediately fixed in 4% paraformaldehyde/PBS and embedded in paraffin in a usual manner. Rat monoclonal antibody specific for human SSEA-3 (Merck Millipore, Darmstadt, Germany) was used as a primary antibody. Immunoreactivity was detected by incubation with a biotinylated anti-rat IgG antibody (Vectastain ABC kit; Vector Laboratories, Burlingame, CA, USA), followed by streptavidin-biotin reaction (Vectastain ABC kit). Immunohistochemical staining for SSEA-3 showed a few positive cells in RA synovial tissue (**Figure 1a–c**).

Harvested synovial tissue was minced, digested with 3 mg/mL collagenase Type V (Wako Pure Chemical Industries: Osaka, Japan) for 3 hours at 37°C, and cultured in the αMEM (Sigma-Aldrich: Tokyo, Japan) containing 10% fetal bovine serum (FBS) (Thermo Fisher Scientific: Waltham, MA, USA) and antibiotics (100 units/mL penicillin G and 100 μg/mL streptomycin) (Thermo Fisher Scientific) at 37°C in a 5% CO_2 incubator. SSEA-3 positive cells were sorted by suspending 1×10^6 synovial cells at passage 2 in 100 ml FACS buffer containing 1 ml of EDTA, 5 ml of BSA, and 44 ml of FluoroBrite DMEM (Thermo Fisher Scientific, Waltham, MA, USA). Cells were collected by using antibody specific for SSEA-3, approximately 1% in cultured cells (**Figure 1d**).

SSEA-3 positive cells strongly expressed CD44, CD90, and CD105 and lacked CD34 (**Figure 2**) in flow cytometry assay. SSEA-3 negative cells were similar to positive cells in immunophenotype, but they weakly expressed CD105.

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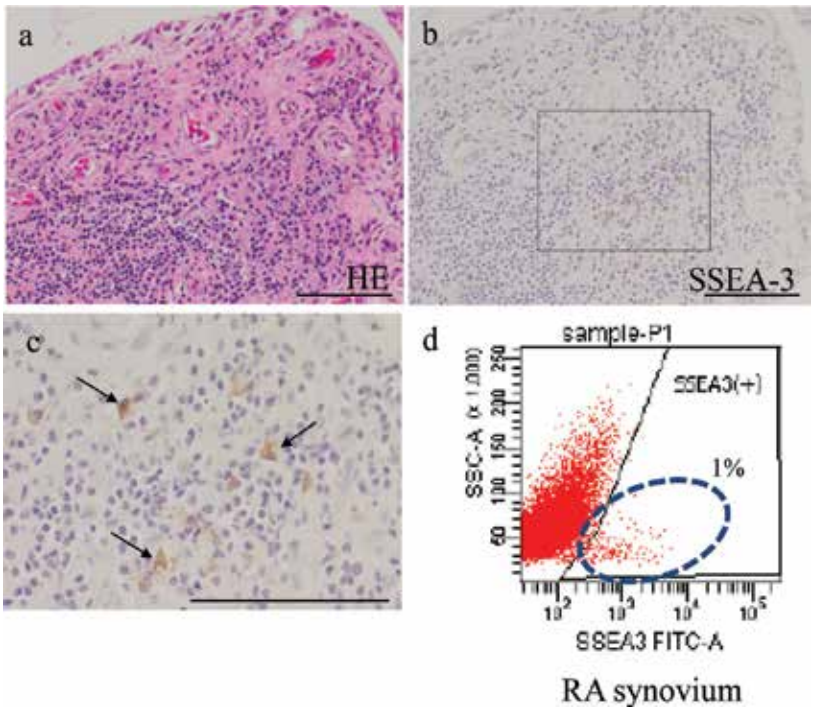

Figure 1. (a) RA synovial tissue. Hematoxylin and eosin (HE) staining. (b) Immunohistochemical staining specific for SSEA-3 in RA synovial tissue. (c) Magnified feature of (b). Bar = 100 **mm**. (d) Ratio of SSEA-3 positive cells in cultured cells derived from RA synovial tissue.

Histological staining after differentiation induction culture showed differentiation into osteoblasts, adipocytes, and chondrocytes from the cultured SSEA-3 positive cells (**Figure 3**). In all mRNA expression of alkaline phosphatase (ALP) and bone morphogenetic protein 2 (BMP2) for osteogenic differentiation, peroxisome proliferator-activated receptor gamma (PPARγ) for adipogenic differentiation and type II collagen (COL2A1), sex determining region Y (SRY)-Box 9 (SOX9) and aggrecan (AGG) for chondrogenic differentiation, SSEA-3 positive cells showed higher gene expression level than SSEA-3 negative cells although there were individual differences (**Figure 4**). These results indicate possibility of higher differentiation ability of SSEA-3 positive cells.

3.2.2. Inhibitory effect on arthritis

Collagen antibody-induced arthritis (CAIA) mice were established as the animal model for RA [36]. Induction of CAIA mice was performed on *scid/scid* mice, 7 weeks old (CLEA Japan), in which they were injected with 1.5 mg of 5-clone cocktail (arthrogen-CIA arthrogenic monoclonal antibody (mAb), Chondrex, Redmond, WA) by intraperitoneal (IP) injection at Day 0. Fifty micrograms of lipopolysaccharide (LPS) (Chondrex) was injected by IP injection at Day 3. 3×10^4. SSEA-3 positive cells labeled with cell tracker green (CTG) (Thermo Fisher Scientific) were suspended in PBS, filtered, then intravenously injected via the tail vein after the injection of LPS at Day 3. SSEA-3 negative cells labeled with CTG were used in the same procedure as control. Mice were scored for clinical arthritis; paws were assessed for signs of redness and swelling.

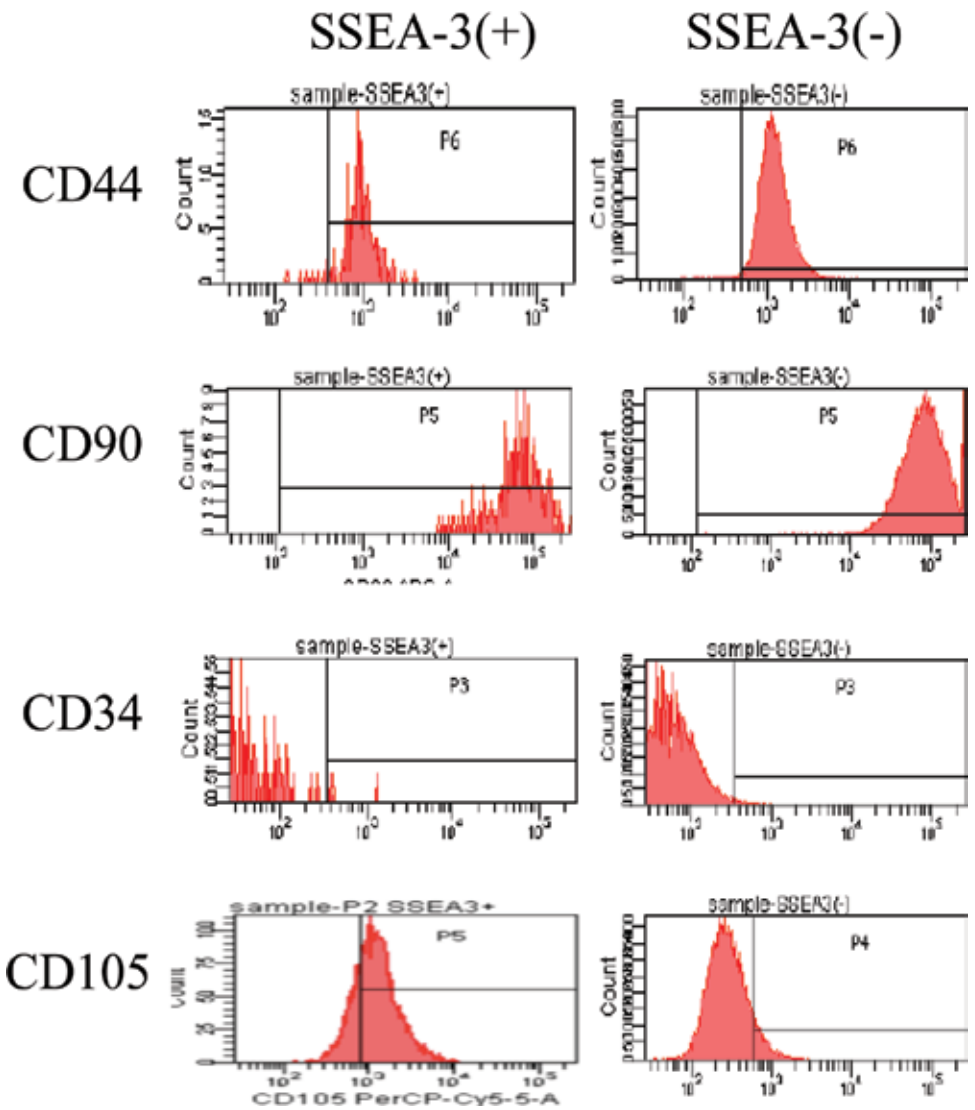

Figure 2. Representative flow cytometry analysis data.

Each paw was given a score of 0–4, giving a total maximum score of 16. (0, normal paw; 1, mild but definite redness and swelling in each joint of the digit or wrist/ankle; 2, moderate redness and swelling in two joints of the wrist/ankle with digit involvement; 3, severe redness and swelling in whole paw; 4, maximum inflammation within the wrist/ankle with many digits involved) [37]. **Figure 5a** displays the arthritis score of CAIA mice in the both transplanted groups after mAb injection. The group transplanted with SSEA-3 positive cells (n = 3) consisted of mice with intravenously transplanted SSEA-3 positive cells labeled with cell tracker green (CTG) seen in **Figure 5b**, while the group transplanted with SSEA-3 negative cells (n = 3) consisted mice with the transplanted SSEA-3 negative cells in the same procedure. Arthritis in the SSEA-3 negative cells group remained for 28 days, while arthritis score in the SSEA-3 positive cells group improved faster after peak inflammation (**Figure 5a**). There was a significant

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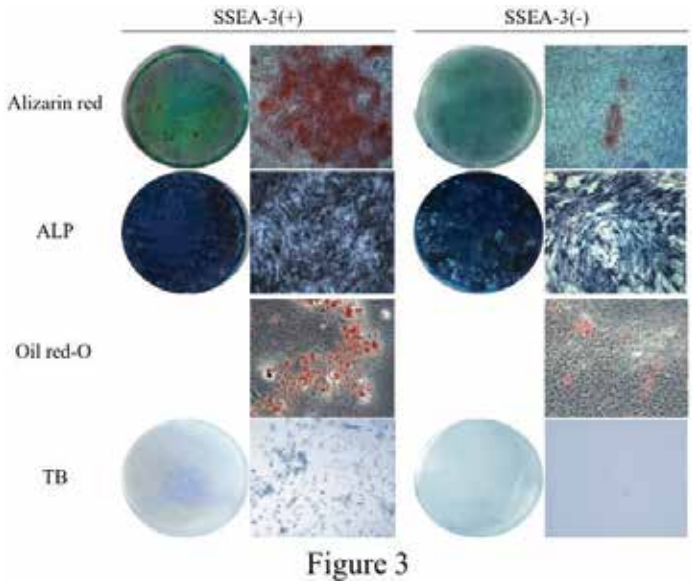

Figure 3. Multipotency of SSEA-3 positive cells derived from RA synovial cells. Osteogenesis was shown by alizarin red and alkaline phosphatase (ALP) staining. Adipogenesis was shown by oil red-O staining and chondrogenesis was shown by toluidine blue (TB) staining.

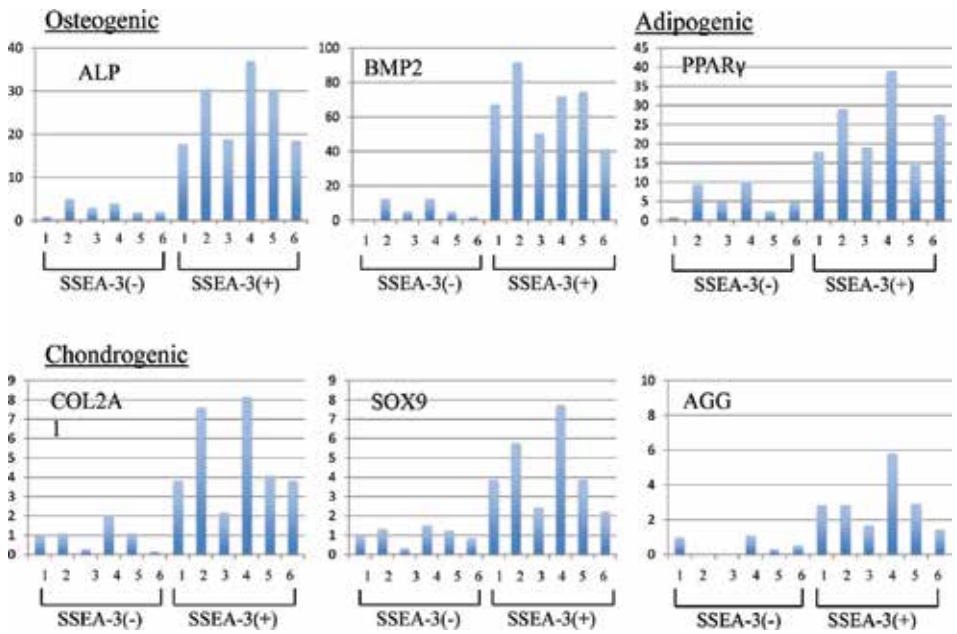

Figure 4. Real-time polymerase chain reaction (PCR) analysis. Results are reported as the mean of three independent experiments and the messenger RNA (mRNA) value of glyceraldehydes-3-phosphate-dehydrogenase (GAPDH) was set as an internal control. Osteogenesis was shown by the expression of ALP mRNA and BMP-2 mRNA, adipogenesis was shown by the expression of PPARγ mRNA and chondrogenesis was shown by the expression of COL2A1 mRNA, SOX9 and AGG. Each data represents an average of three times. Note that the x-axis numbers represent patients in **Table 1**. The mRNA values of y-axis were arbitrary set.

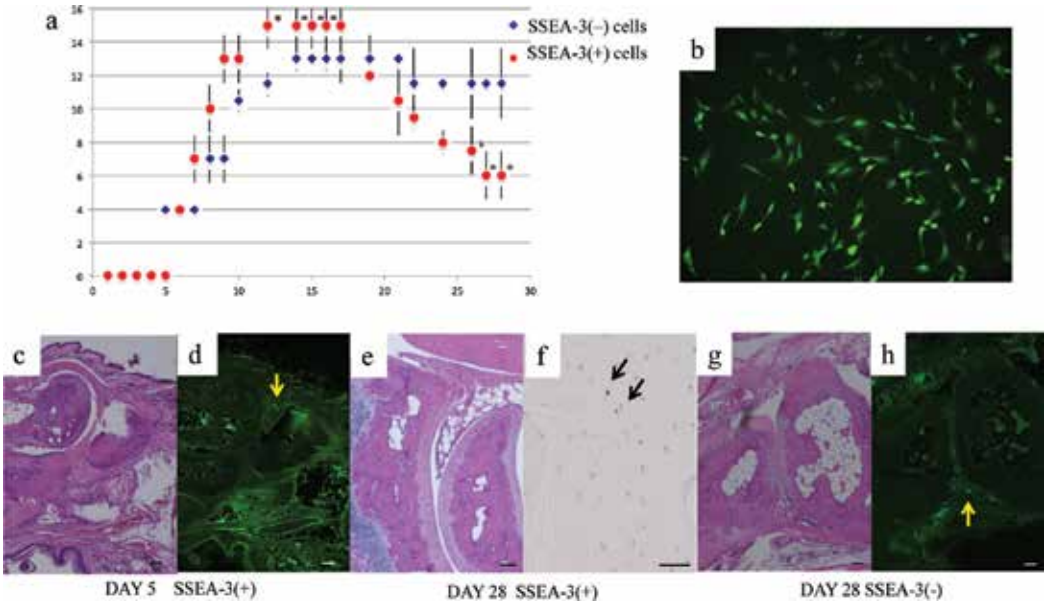

Figure 5. (a) Arthritis score of CAIA mice after mAb injection. SSEA-3 (−) cells group consisted of mice transplanted with SSEA-3 negative cells. SSEA-3 (+) cells group consisted of mice transplanted with SSEA-3 positive cells. Both groups contained three mice. Each score shows average values of three mice and * means $p < 0.05$. The X-axis means the day from day 0 when the monoclonal antibody cocktail was administered. (b) Cell tracker green (CTG)-labeled SSEA-3 positive cells (10X). (c, d and e, f) histology of joint of CAIA mouse after CTG-labeled SSEA-3 positive cells injections on day 5 and 28, respectively. Arrows shows CTG-labeled cells and positive cells for SSEA-3 in immunohistochemical staining. Bar = 100 **mm.** (g and h) representative histology of joint of CAIA mouse after CTG-labeled SSEA-3 negative cells injections on day 28. Bar = 100 **mm**.

improvement in arthritis in the SSEA-3 positive cells group. On Day 5, CTG-labeled cells were detected in the synovial tissue (**Figure 5c** and **d**) and were still present on Day 28 (**Figure 5e** and **f**) in the group transplanted with SSEA-3 positive cells. **Figure 5e** shows representative joint in the group transplanted with SSEA-3 positive cells on Day 28 and **Figure 5f** shows SSEA-3 positive cells in synovial tissue in the immunohistochemical staining of **Figure 5e**. **Figure 5g** and **h** show the progression of joint destruction in the group transplanted with SSEA-3 negative cells on Day 28. CTG-labeled cells were not detected in other healthy organs on Day 28 by fluorescent microscopy (data not shown). These results indicate that SSEA-3 positive cells have the inhibitory effect on arthritis and systemic administration of them is safety.

4. Conclusions

In our study, SSEA-3 positive cells were detected in RA synovial tissue even under pathological conditions such as RA. Although the synovial tissue we used was collected from various RA disease stages and surgical sites, SSEA-3 positive cells were detected with values of approximately 0.5–1% in all cultured SFMSCs. Collected SSEA-3 positive cells had higher gene expression level and differentiation ability in vitro compared with SSEA-3 negative cells

that were occupying most of mesenchymal stem cells. Wakao S., et al., reported that Muse and non-Muse cells had differentiation ability of osteocytes, chondrocytes, and adipocytes, while differentiation ability in non-Muse cells was lower [31]. We think that SSEA-3 positive cells in this study had a similar nature as Muse cells, considering also the results that SSEA-3 positive cells strongly expressed CD105 in FACS analysis. SSEA-3 positive cells can be systemically administered by intravenous administration like Muse cells and have possibility of differentiation into osteoblasts, adipocytes, and chondrocyte. These suggest the possibility of repairing degenerative cartilage and destroyed joints in RA. In the CAIA mice experiment, SSEA-3 positive cells that were systemically administered had an inhibitory effect on arthritis. In the transplanted group consisting of mice transplanted with SSEA-3 positive cells, arthritis score quickly decreased after the onset of arthritis compared with SSEA-3 negative cells group. There were some previous studies on immunosuppressive effect of BMMSCs [38–40] and SFMSCs [24] as mentioned earlier. Especially, SFMSCs extracted from healthy subjects are able to inhibit T-cell proliferation [25]. However, immunomodulatory function of SFMSCs and SSEA-3 positive cells may be disturbed and cause an inefficacy of SFMSCs and SSEA-3 positive cells in inflammatory environment like uncontrolled RA [41]. In RA synovial tissue, fibroblast-like synoviocytes (FLS) are key players in the perpetuation of joint inflammation and destruction. The link between FLS and SFMSCs plays an important role in controlling the inflammation and immune hemostasis in RA. In our mice study, autologous SSEA-3 positive cells proliferated in vitro might have altered the balance of immune regulation with FLS.

Our study suggests the possibility of inhibiting arthritis and joint destruction by SSEA-3 positive cells derived from synovial tissue in RA. Further study of SSEA-3 positive cells for clinical application in humans will lead to future development as a new treatment in RA.

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