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Alopecia

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ALOPECIA

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Contributors

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Meet the editor

Dr. Muhammad Ahmad is the only surgeon in the history of Pakistan to present a new classification system for hair loss, called "Ahmad's NPRT system." He also has a new classification on scalp hair (LGMA classification). He has published a new Ahmad's Cosmetic Surgery Scar Scale and is the first surgeon in the world to develop Ahmad's Hair Transplant Assessment Scale.

He is the only Pakistani plastic surgeon to receive the Merit Award by the Australian and New Zealand Burns Association in 2004 and also received the first prize at the annual meeting of the Asian Association of Hair Restoration Surgeons, Bangkok, Thailand, in 2017. He has more than 100 publications in national and international journals. He is also editor and reviewer of many international journals.

Contents

Preface

It gives me immense pleasure to be the editor of the book *Alopecia* published by the world-leading IntechOpen Publisher. The field of hair restoration is expanding rapidly and the current interest in alopecias is very intense and rising. The overwhelming desire of balding people to look younger has led to great efforts to understand the pathophysiological pathways of alopecias. The diversity in hair loss patterns has led to new classifications of baldness. The diagnosis of hereditary alopecia from non-hereditary alopecia is very important. Newer theories of hereditary hair loss are being postulated and investigated. These theories have opened new ways in the medical management of hair loss.

The current book has chapters emphasizing the different hereditary and non-hereditary alopecias. Furthermore, the administration of newer drugs may treat hair loss by a variety of mechanisms. All the clinical variants of alopecias are discussed in detail.

The book will help dermatologists, students, hair transplant surgeons, and physicians related to hair loss problems, giving them the opportunity to understand basic pathophysiological, clinical, and medical management options. The basic idea of the book is to diagnose alopecia correctly and outline a treatment plan.

Lastly, I thank my family who provided me with support during the various stages of manuscript preparation by providing the time meant for them.

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Chapter 1

Evaluation of Patients with Alopecia

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Additional information is available at the end of the chapter

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Abstract

This chapter outlines the clinical approaches for alopecic patients that are reliable in practice. We discuss three different categories of hair evaluation options: invasive methods (biopsy), semi-invasive methods (trichogram) and noninvasive methods. Besides describing the current status of diagnosis and quantification of alopecia, the chapter provides an objective assessment of these investigation tools: detailed medical history collection by structured interview and questionnaires, clinical examination of the scalp and other hair-bearing areas, laboratory investigations, assessment of hair loss distribution (patterned/diffuse/focal), dermoscopic evaluation, assessment of alopecia severity (by pull test, hair part width, counting hair test), common scales for hair loss staging, photography of alopecic areas, biopsy, trichogram, unit area trichogram, tug test, hair mount and microscopic evaluation, electron microscopy, hair card test, hair weight determination, hair densitometry, mechanical test of hair quality and computed hair analysis. Unfortunately, the disadvantages of most of these methods generate a lack of use in clinical practice, leading to few reliable evaluation methods for patients suffering from alopecia. We underline the necessity of easy, refined and precise evaluation tools for the assessment of alopecia patients.

Keywords: alopecia, hair loss, hair shedding, hair thinning, androgenetic alopecia, female pattern hair loss, telogen effluvium, anagen effluvium, alopecia areata, trichotillomania, tinea capitis, traction alopecia, evaluation methods, dermoscopy/trichoscopy, pull test, counting hair tests, trichogram, microscopic evaluation, hair weight determination, hair densitometry, computed hair analysis

1. Introduction

Hair loss and hair thinning represent frequent complaints of both female and male patients in clinical dermatology. Establishing a correct management can be challenging, beginning from

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the subjective description provided by the patient, the confirmation of a true hair disorder and its underlying pathogenesis.

Current literature describes a long list of evaluation methods for patients suffering of alopecia. The objective assessment of these methods enable us to outline the clinical approaches that are reliable in practice. There are three different categories of hair evaluation options: invasive methods (biopsy), semi-invasive methods (trichogram) and noninvasive methods (interview with detailed medical history collection and questionnaires, systemic evaluation through laboratory tests and other investigations, counting hair tests, hair pull test and tug test, hair weight determination, densitometry or HairCheck test, imaging tests such as global photographs, trichoscopy, phototrichogram, videodermoscopy, light microscopy, etc.) [1–3]. All these assessment methods have merits and demerits, but for some, the disadvantages generate a lack of use in clinical practice [4]. Also the patients seem to be more interested in performing only some of them, especially the non-invasive methods.

The diagnosis of androgenetic alopecia or female pattern hair loss is confirmed clinically and extra tests are not usually required. The dermatologist focuses on detailed medical history and physical examination, analyzing the scalp aspect, the hair loss pattern, the trichoscopic findings and sometimes the pull test results [5]. Basic hair evaluation methods also include scalp biopsy (each alopecia having a specific histopathological aspect), but this invasive method is only sometimes necessary for establishing a diagnosis [3, 6].

2. Detailed medical history

Collecting information is of main importance and can be done by carefully listening to the patients on the following subjects:

a. **date/age of onset** (congenital or acquired),

b. **type of onset** (sudden or insidious), correlated with other medical issues or personal events. Chronic or acute **stress,** for example, lowers the production of estrogens, leaving the genetically targeted hair follicles susceptible to testosterone action and inducing hair loss by premature onset of catagen [7].

c. **physiological changes**, such as childbirth (1–3 months after delivery hair follicles revert to telogen and begin to fall out as a new growth cycle of hair begins) [3, 8]. While breastfeeding prolactin can delay hair loss. Miscarriage or termination also lead to significant hair loss, especially after the first 3 months of pregnancy. In babies, alopecia is physiological up to 6 months due to a decrease of hormones after birth that will induce telogen effluvium. Finally, gradual thinning and finer textured hair are common with aging (involutional alopecia) and around menopause [9].

d. **illnesses and infections** prior to the onset of alopecia, or other possible **trigger factors** in the previous 2–5 months (high fever, chronic blood loss in women with prolonged heavy menses (anemia) or severe dietary protein deficiency, as they correlate with chronic

telogen effluvium (CTE) [7, 9]. Finding out if the patient follows a certain **diet** is also important to establish if the protein and calories intake is adequate. Anemia (lack of iron and low ferritin levels) may lead to diffuse hair thinning everywhere on the scalp, changes of hair texture (brittle hair) and strands breaking off in different lengths [9]. Sometimes an episode of telogen effluvium may uncover a latent AGA [8].

e. **past medical history** (chronic illnesses, surgeries, autoimmune, dermatologic and psychiatric disorders, etc.). The dermatologist should identify if other dermatological or endocrinological diseases are present, and if so, they must be properly investigated and treated. Atopy, autoimmune thyroid disease and vitiligo are commonly associated with alopecia areata [10]. Female pattern hair loss is common in patients with polycystic ovary syndrome (PCOS – with clinical signs: acne, increased facial hair as part of hirsutism, irregular periods, infertility, weight gain), congenital adrenal hyperplasia (an adrenal tumor causes a conversion of the hormones normally produced, increasing the testosterone levels), etc. [11, 12].

f. **medication** used by the patient, as some of them may induce hair loss: anticancer drugs, anticoagulants, anticonvulsants, antithyroid drugs, beta blockers, tricyclic antidepressants, and progestins with androgenic effects [7]. The contraceptive pills can act as treatment or cause of hair loss: in the first 3 months of use they determine either thicker hair regrowth or increased fall rate, especially after discontinuation [11]. Contraceptive pills with a high level of progestins may lead to hair loss, together with acne and hirsutism, and if so, they need to be switched to pills based on ethinoestradiol [9]. Anagen effluvium secondary to drugs is usually caused by cancer chemotherapy and immunotherapy drugs, having a more diffuse and rapid progression than telogen effluvium [13].

g. **rate of progression**, if it has any season pattern. If the patient has noticed hair regrowth since the onset of alopecia, this rules out cicatricial alopecia. The duration and extent of the hair loss process is important for the prognosis. Early, long-lasting and severe cases of alopecia areata have a less favorable prognosis, but no correlation has been found between the number of patches at onset and the subsequent severity of the disease [10]. Frontal fibrosing alopecia has an unpredictable evolution, and as well as central centrifugal cicatricial alopecia, if not treated, will burn out [6]. The prognosis in androgenetic alopecia and female pattern hair loss depends on the treatment: once discontinued the hair regrowth will be lost in 6–12 months and the hair pattern will return to the baseline state [14, 15]. In FPHL patients, complete hair loss on the central scalp is rare, in contrast to male-pattern baldness. Chronic effluvium telogen is usually a self-limiting condition, lasting more than 6 months, while anagen effluvium is entirely reversible, with hair regrowth after 6 months [7]. Scarring (cicatricial) hair loss tends to have a generally unfavorable prognosis.

h. **associated symptoms** such as: itching, tenderness, pain, burning sensation. It has been reported that 14% of the patients suffering of alopecia areata suffer a burning sensation and accentuated pruritus [10].

i. **type of treatment** (topic solutions, pills, laser, etc.) patients have used and the result from their point of view. Also focus on how the patient treats their hair on a daily/monthly

basis with: chemical agents (dying, coloring, etc.), thermic agents (heat from hair curler or straightener, etc.), hair cosmetics (dry shampoo, hair stimulating shampoo, balm, hair tonic, hairspray, gel, mousse or topical camouflaging fibers, etc.) and their frequency of use [11, 16].

j. the **family history of alopecia,** autoimmune, dermatologic or psychiatric disorders [17]. Androgenetic alopecia or alopecia areata may exist in the family, but cicatricial alopecias rarely occur in family members (except Central Centrifugal Cicatricial Alopecia). In a personal study, 57% of the patients with female pattern hair loss had a positive family history: the mother in 29% of the cases, the father in 21% of the situations [16].

k. **race of the patient** is also important. For example, central centrifugal cicatricial alopecia occurs in black women, resembling lichen planopilaris [6]. Dissecting cellulitis of the scalp is frequent mostly in black adolescents and adult males.

l. **different types of alopecia that may sometimes coexist**: alopecia areata with trichotillomania [18], both patchy and diffuse alopecia areata with androgenetic alopecia (AGA) [8, 13], female pattern hair loss (FPHL) with frontal fibrosing alopecia (FAA) [19].

2.1. Structured interview: standardized flowchart

A standardized chart represents an easy evaluation tool for the patient's course over time, so no data will be omitted or forgotten. It collects important information at each patient visit, providing a good overview and it is also a helpful reminder of all the items of interest, helping the dermatologist to pass through all necessary tests. The flow chart can contain the symptoms of the patients, the clinical sign, the dermoscopy findings, the pull and tug tests results, the extent of hair loss, laboratory results, biopsy results, treatments performed, etc. [6]. One can use a standard flow chart already published and applied in clinical practice or design a new chart that suits his own needs.

2.2. Questionnaires

Several questionnaires have been used so far for hair loss patients to assess the quality of life and associated lifestyle patterns. One study used the Hairdex, lifestyle indexes, Symptom check list 90R and output psychiatric rating scales, but the major complaint of the patients when completing the survey was that all together, the 4 questionnaires contained more than 120 questions, which was both time consuming and tiring [20]. In a personal research we developed and used for FPHL patients a new questionnaire, more concise (26 questions), time effective (30 min) and full of relevant information [20]. Our questionnaire collected data upon demographic items (race, age, level of education, urban/county environment), illness-specific data (patients complaint, status of scalp visibility/severity of disease, duration of hair loss, type of hair), risk factors (family history of alopecia, scalp diseases, possible cause of hair fall, cosmetic products and devices used on hair, covering of hair with products or items), psychosocial consequences (affected self-confidence, affected mood, how often they think of hair loss, what aspects of life are disturbed by alopecia), treatment (prescribed by doctor, therapy already performed, therapy the patient would like to perform, the main purpose for

the treatment in patients opinion), and last but not least the evaluation of the questionnaire (useful and easy to complete) [14].

Questionnaires of hair loss are available online (for printing or online completion) on the websites of different clinical practice offices and need to be completed before the first visit. The majority of the questionnaires refer to diffuse shedding or thinning, but some also have a hair loss in patches section. Ranging from 22 to 35 questions, they gather information about the background of hair loss, past medical history, diet and medication history, hair care practices.

Even though questionnaires seem to please the patients and to gather a large volume of information in a short period of time, some of the subjects do complain that it is a more distant kind of approach and that they lack the interaction with the doctor.

3. Clinical examination

For alopecic patients, the clinical examination begins as soon as they enter the doctor's office as you can notice right away if the scalp is visible across the room, if hair thinning is present [6]. For most of the cases you can assess from the first glimpse the **extent of alopecia**: localized or diffuse.

Pay attention to the patient's **hair style**, as it might try to hide the alopecic areas, but also gives you hints about how the patient treats the hair, underlying possible **risk factors** [5, 17]. Ask the patients if they use back combing, hair curling wand or hair straightener, decolorizer, hair dye, or mechanic aggression. Overuse of heat and chemical treatments leads to hair shaft damage and hair loss, also to an increased tendency to develop traction alopecia [21]. Patients with traction alopecia usually wear their hair in positions which induce prolonged and repetitive tension on the hair (for example pony tails or braids) [6]. The presence of retained hairs along the frontal and/or temporal rim, is called the "fringe sign" and is a useful clinical marker present in both early and late traction alopecia [22].

The evaluation of an alopecic patient should include the **examination of other hair-bearing sites** besides the scalp in order to determine if hair loss is present in: eyebrows, eyelashes, limbs, axillae or pubic area [23]. The examination of the **skin, nails, oral or genital mucous membranes** is also needed (for evidence of associated dermatoses, such as lichen planus) [3, 11, 24].

After a thorough clinical examination in order to evaluate impaired vision, defective hearing, dysmorphic features, clues to autoimmune or metabolic diseases, or ectodermal anomalies, the dermatologist should rule out if the patient has a condition defined by sparse hair (autosomal recessive hypotrichosis—wooly, easily broken hair) or loss of hair (alopecia). Then decide what type of alopecia it is: localized or diffuse, scarring or non-scarring [25].

In order to establish a diagnosis, the doctor will inspect the scalp for inflammation, scaling, erythema, follicular openings, then examine the pattern distribution and determine the hair density, finally study the caliber, fragility, length and shape of hair shaft [2, 23].

3.1. Scalp assessment

For clinical examination, the patient must remove hair pieces, pins, clips, braids, hair extensions and preferably sit on a chair than on the examination table [6]. **Good lighting** is essential, when pictures are taken [6, 11]. The dermatologist must examine the hair and scalp from above, then proceed with a magnifying light or dermatoscope for trichoscopic assessment. A thorough examination requires a serial parting of the hair starting at the center of the scalp, with fingertips, disposable combs or the wooden end of a Q-tip [6].

If during the clinical exam, diminished or absent **follicular ostia** is noticed, a diagnosis of cicatricial alopecia is most likely positive and the detailed history of the patient should be guided in this direction [6]. In patients with darkly pigmented skin, in which it is difficult cu appreciate the follicular ostia, it is recommended to perform the skin biopsy.

The area with hair loss must be examined closely to determine whether it is **bare** as "as baby's bottom" (characteristic of alopecia areata) **or it maintains residual hairs** or fine textured and miniaturized hairs, as in AGA [13, 22].

The **color of the affected scalp** is also important (pink, peach-colored or skin colored, with hypo- or hyperpigmentation, with telangiectasias, with erythema present perifollicular or in patches, etc.).

The scalp must be examined for **signs of atrophy or scaling** (perifollicular or in patches), for **edema, papules, pustules, crusts, follicular hyperkeratosis, ulceration and scarring** [22, 24].

Atrophy, loss of follicular openings and tufted hair are usually present in scarring hair loss (in non-scarring hair loss they are absent), while erythema, scaling or pustules may be present in both types of alopecia. An almost normal scalp skin with a pale, shiny or mildly scarred aspect is present in frontal fibrosing alopecia, a form of lichen planopilaris [19, 21].

Most of the time, patients leave their hair unwashed and uncombed for the doctors' appointment to show how oily it is or how much hair falls down when combing [5, 11]. In a personal study we have reported that 68% of the patients suffering of female pattern hair loss presented also hyperseborrhea and 7% had seborrheic dermatitis with annoying pruritus [14].

Hyperseborrhea is the hyperactivity of sebaceous glands leading to an excessive production of sebum with immediate symptoms such as itchiness and pain. Hormonal imbalances, metabolic disorders (nutrition and elimination), digestive problems (hepatic and intestinal dysfunctions), nervous factors and stress favor its appearance. Hyperseborrhea may lead to: hair loss, accelerated skin whitening, oily hair, hair blemishes and deterioration, bad-smelling skin, folliculitis, acne, pityriasis steatoides, seborrheic dermatitis [11, 13].

Seborrheic dermatitis is not infectious, but it involves the proliferation of yeast normally present on one's skin (malassezia yeast being lipophilic, the sebum becomes a source of food). The sebum usually represents a scalp protection barrier with antibacterial and antidehydration property, that moisturizes, protects and waterproofs the hair shaft. Seborrheic dermatitis may non-specifically cause diffuse hair loss as hair follicles find an unsuitable development environment in inflamed skin which will generate a shorter anagen [24].

3.2. Pattern or distribution of hair loss assessment

By analyzing the extent, pattern or distribution of hair loss (focal patchy, patchy all over, diffuse, central, intact frontal hairline, loss of hair in the eyebrows, eyelashes, limbs, axillae, pubic area, etc.) the dermatologist can rule out some diseases in the differential diagnosis process.

3.2.1. Diseases with patterned hair loss

There are some hair loss conditions which seem to affect a certain part of the scalp. For example, **androgenetic alopecia (AGA)** has an early age of onset with an "M shaped" hair thinning over the frontal area and parietal scalp, but conserving a greater density in the occipital scalp [11, 13, 14, 20] (**Figure 1**).

Figure 1. Male pattern suffering of androgenetic alopecia (AGA), grading on Hamilton-Norwood scale: (a) grade II lateral view; (b) grade II view from above scalp; (c) grade VI lateral view; and (d) grade VI view from above.

In the **female pattern hair loss (FPHL)** there is a "Christmas tree" progression of hair loss (central thinning) or it can happen in a diffuse manner, but with the retention of the frontal hairline [3, 16]. Advance thinning occurs in the frontal hairline and above the ears, when markedly elevated circulating androgens are present [5, 26]. The dermatologist must check if clinical signs and symptoms of androgen are present (menstrual irregularities, infertility, hirsutism, severe cystic acne, virilization, galactorrhea) [25]. Common complaints in females might be that the hair does not grow in length any more or that the pony tail is smaller in girth [8]. The clinical diagnosis of AGA and FPHL is supported by the pattern of hair thinning, the presence of miniaturized hairs (increased spacing between hairs, shorter and fine hairs of various lengths and diameters) and usually lack of increased shedding [8, 9, 11] (**Figure 2**).

AGA and FPHL are mostly seen as hair thinning conditions above the ears, sometimes extending posteriorly to the occipital hairline [27]. Another affected area is at the nape of the patients (**back of the scalp**) and possible causes are: the 'ophiasis' form of alopecia areata, Frontal fibrosing alopecia with hair loss starting as a band-like distribution around the frontal hairline but also at the sides, just behind the ears, Monilethrix (abnormality in how the hair shaft is produced, leading to hair breakage), overuse of heat and chemical treatments leading to hair shaft damage and hair loss at the nape [20, 21].

Figure 2. Female pattern hair loss, grading on Ludwig scale: (a) grade I, (b) grade II, and (c) grade III.

The "moth-eaten" pattern of hair loss is the most common type and it is considered to be a pathognomonic manifestation of **secondary syphilis** [28]. Still, in some cases, alopecia can be the only presenting feature of syphilis, without any other clinical feature such as roseola syphilitica, mucous patches, condylomata lata, and ophthalmologic or auditory findings. Differential diagnosis is hard when the hair loss is not just moth-eaten, it is diffuse or both. Syphilitic alopecia can mimic alopecia areata both clinically and histopathologically, with lack of "exclamation point" hairs, characteristic of alopecia areata. Syphilitic alopecia can resemble a noninflammatory Tinea capitis, but laboratory testing of scrapings will indicate the presence of fungus. Trichotillomania can also be suspected, but it would be confirmed by history and findings of a biopsy [28].

3.2.2. Diseases with diffuse hair loss

Chronic telogen effluvium (CTE) affects women in the fourth to sixth decade, with a sudden onset (frequently related to physiologic and emotional stress) of marked shedding of telogen hairs (which sometimes is prolonged) from the entire scalp, yet scalp hair density appears normal or minimally decreased. The dermatologist will not see miniaturized follicles at examination [9]. **Anagen effluvium** occurs days to weeks after chemotherapy in 65% of the cases and the hair loss is reversible [11, 13] (**Figure 3**).

Figure 3. Female patient suffering of telogen effluvium: (a) clinical view and (b) patient brought a small plastic bag full of the hair collected during the standardized wash test.

DUPA or diffuse unpatterned alopecia in males is characterized by hair thinning on the top (without progression to complete baldness) and diffuse miniaturization throughout the occipital area also, being a contraindication for hair transplant [23].

Alopecia areata can affect any hair-bearing area, being described as alopecia totalis (scalp hair loss), or universal (loss of all hairs from body and scalp, including eyebrows, eyelashes, etc.) [10].

Induced metabolic disorders lead to diffuse hair loss [11].

The presence of alopecia in infants and children may be due to congenital or acquired causes. Congenital diffuse hair loss could consist of congenital hypotrichosis/atrichia or hair shaft anomalies, such as Nethersons (bamboo hair, atrophy), Menkes (beaded hair) or Monilethrix (wiry hair, pale skin, pili torti). Acquired **diffuse hair loss in children** occurs in history of prolonged illness (telogen effluvium) or exposure to radio/chemotherapy (anagen effluvium). Medical conditions such as thyroid disorders or hyperpituitarism can also cause diffuse hair loss [29, 30].

3.2.3. Diseases with focal hair loss

Alopecia areata most often affects the scalp and is described as acute circumscribed hair loss (80% of the patients having just a single patch). The clinical diagnosis is made by the aspect of hairless patches with a normal skin and preserved follicular ostia [10] (**Figure 4**).

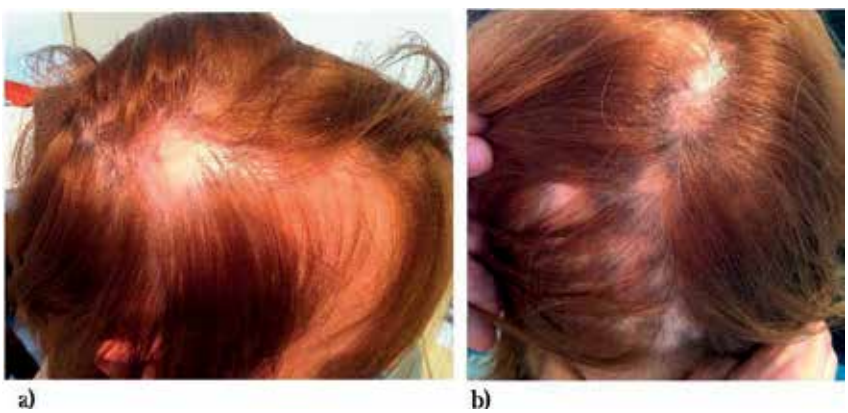

Figure 4. Female patient suffering of alopecia areata (AA), having: (a) a single AA patch and (b) multiple, confluent AA patches.

Tinea capitis represents a dermatophyte infection of the hair shaft and follicles, leading to fragile and easily broken hair [13, 24].

Trichotillomania is a type of alopecia with irregular patches, determined by the patient who twirls or pulls his hair compulsively. Patches occur especially frontoparietal, progressing backward and may include eyelashes and eyebrows, being described as incomplete thinning with stubble [8, 23].

Traction alopecia refers to frontal and temporal loss of hair. Not only some hair styles cause traction alopecia, but also facelifts, due to the tightening and trimming of the skin. The dermatologist must distinguish traction hair loss from hair loss recession due to frontal fibrosing alopecia or alopecia areata [3, 24].

Primary cicatricial alopecias have many subtypes mostly presenting patchy hair loss and some of them cellulitis or folliculitis. The clinical features of **lichen planopilaris** are itchy, multifocal or central patches on scalp and nonscalp areas with follicular hyperkeratosis and perifollicular erythema. **Chronic cutaneous lupus erythematosus** presents single or multifocal patches with intense activity in the patch center, ulceration, follicular plugging, atrophy and depigmentation. **Brocq pseudopelade** consists of small or large irregular patches on the scalp with no detectable symptoms or inflammation, with end-stage burnout. Clinically **folliculitis decalvans** is described as a single patch of complete alopecia on the hair-bearing periphery of the scalp (it expands circumferentially, slowly over the years) and pustules, honey-colored crusting and tufting. **Dissecting cellulitis of the scalp** presents multiple fluctuant nodules across the scalp, interconnected by sinus tracts and sometimes patients also suffer from acne conglobate [6, 29].

The congenital focal **hair loss in infants and children** may be due to traumatic events (birth trauma) or nevoid conditions, such as velvety smooth (nevus sebaceous), warty (epidermal nevus) or absent overlying skin (aplasia cutis). Acquired focal hair loss in children occurs in trichotillomania (with irregularly broken hair due to mechanic repetitive traction performed by the child itself), tinea capitis or ringworm (fungal infection which leads to patchy bald spots with red, flaky scaling, easy pluckability of hairs and cervical lymphadenopathy) or alopecia areata (smooth, round, totally bald areas with the detection of exclamation point hair in trichoscopy and nail pits). Even traction alopecia (from tight ponytails) can result in focal hair loss [30, 31].

3.3. Dermoscopy

Trichoscopy represents the dermoscopy imaging of the scalp and hair. Pigmented patterns must be assessed by dermatoscope with an interface solution, while interfollicular patterns are visualized only with a polarizing light source (or filter) or a videodermoscope, as direct contact can produce bleaching [32–34].

Trichoscopic evaluation of the scalp is based on the observation of follicular patterns (white dots—destroyed follicles that are replaced by fibrous tracts, yellow dots—follicular infundibulum with degenerating keratinocytes and sebum, black dots—stubs of cadaverized hair, fractured before emerging from the scalp), interfollicular patterns (pigment pattern and vascular pattern with interfollicular simple red loops or twisted loops or arborizing red lines) and hair shaft characteristics (specific features in various genetic and inflammatory disorders) [35–37].

The assessment of the scalp must begin with the observation of the follicles presence, suggesting a noncicatricial disease. Afterwards, the examiner must identify if yellow dots are present and if so, focus on the hair diameter.

AGA trichoscopic characteristics are: hair shaft diameter variation of more than 20% hair shaft (miniaturization), peripilar halo in early stages, predominance of follicles bearing single hair, hypertrophy of sebaceous glands [11]. In severe AGA cases trichoscopy shows the presence of empty follicular ostia, brown dots and honeycomb-like pigmented network in bald, sun-exposed areas [38]. The latest reports indicate that although miniaturization of hairs (progressive thinning of hairs) is a specific feature of AGA and FPHL, it is also found in alopecia areata and traction alopecia cases [39].

For FPHL, a study suggests that 2 major criteria (more than 4 yellow dots in a field of view in frontal area/a lower mean hair thickness in frontal area/more than 10% thin hairs <0.03 mm in frontal area) or one major and two minor criteria (ratio of single-hair units, frontal area to occiput >2:1/ ratio of vellus hairs frontal area to occiput>1.5:1/ratio of hair follicles with perifollicular discoloration frontal area to occiput>3:1) are necessary for a trichoscopic diagnosis with a 98% specificity [40]. Previously, FPHL has been diagnosed on two dermoscopic criteria: more than 20% variability in hair shaft diameter and more than 7 short regrowing hairs in the frontal scalp [11, 41] (**Figure 5**).

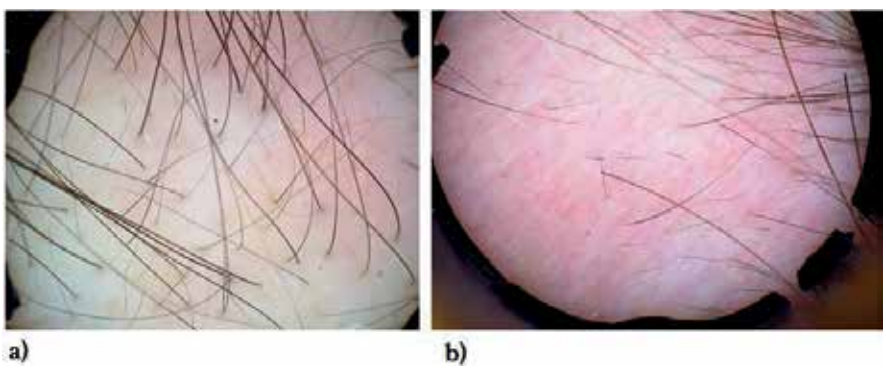

Figure 5. Trichoscopy of (a) AGA patient (hair miniaturization with diameter diversity >20%, thin hair, predominance of follicles bearing single hair, peripilar halo, yellow dots, vellus regrowing hair) b) AA patient (exclamation marks, cadaverized hairs-black dots, yellow dots) (DermLite DL100).

The dermatologist must identify if black dots are present, which is suggestive for AA, trichotillomania or tinea capitis (black dots tinea).

In trichoscopy, AA presents: yellow dots with short vellus hairs or empty follicles, dystrophic and tapered hairs and black dots representing cadaverized broken hairs. The trichogram may show dystrophic fractured and telogen roots [36] (**Figure 6**).

Trichotillomania is usually recognized by: curled hairs with hair shafts of variable length, longitudinal splitting of hair shafts and coiled fractured hair shafts. New trichoscopy findings that seem to be specific features of trichotillomania are: flame hairs, the V-sign, hook hairs, hair powder and tulip hairs [42].

Telogen effluvium is a diagnosis of exclusion as it is described by decreased hair density and presence of empty follicles. It is differentiated from AGA due to the absence of a certain site predilection, hair shaft diameter variation or peripilar halo [32].

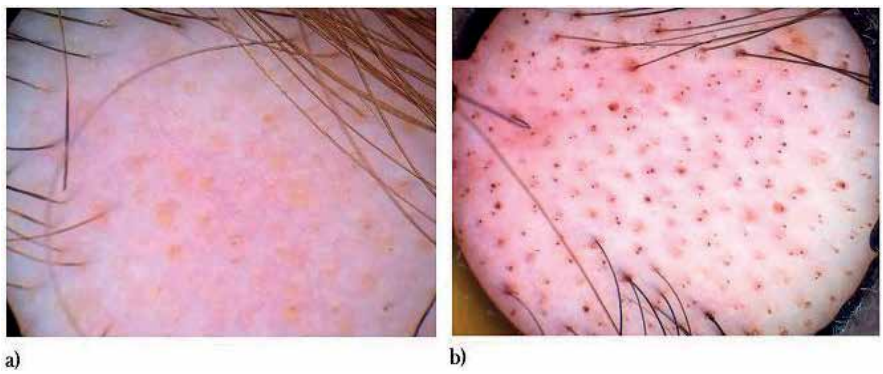

Figure 6. Trichoscopic aspect of (a) chronic AA patient (keratotic plugs in the follicular openings) and (b) hair dye on the scalp surface in an AA female patient (dye is within the follicular openings, simulating interfollicular hyperpigmentation and dots) (DermLite DL100).

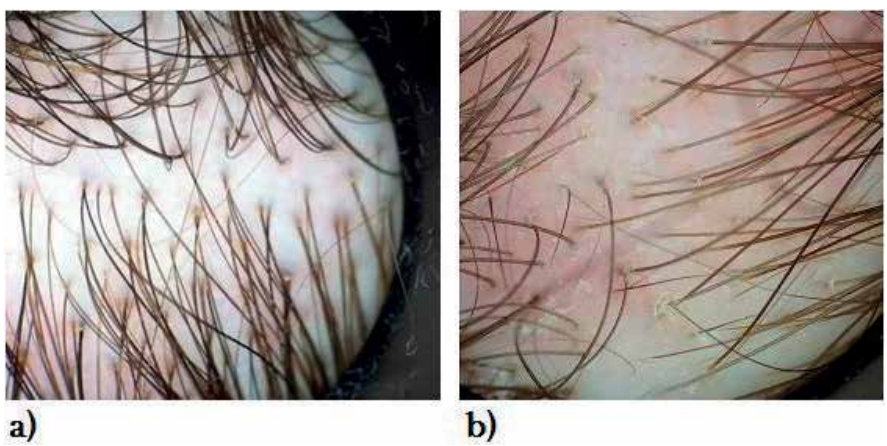

Figure 7. Trichoscopy of: (a) normal scalp, patient without alopecia and (b) patient with lichen planopilaris (peripilar scale, reduced hair density, erythema) (DermLite DL100).

If the trichoscopic assessment of the scalp does not reveal the presence of the follicles, but milky-red or ivory-white areas lacking follicular openings, the dermatologist must think of forms of cicatricial alopecia.

Dissecting cellulitis characteristic findings are: "3D" yellow dots imposed over dystrophic hairs, large, yellow amorphous areas and pinpoint white dots with a whitish halo [7]. Early stages of the disease may mimic AA findings, while the exclamation mark hairs and white dots are markers of disease chronicity [43, 44].

Trichoscopy features in Tinea capitis are: comma shaped stubs/hairs, black dots tinea (stub of broken hair shafts with scaling), blotchy pigmentation, erythema, scaling, pustules and follicular scale-crust formation. It has fluorescence in UV examination [32].

Lichen planopilaris (LPP) can be identified by: peripilar casts, target pattern "blue-gray dots", spared intervening follicles, white dots. Trichogram examination may show anagen roots [45] (**Figure 7**).

The most characteristic trichoscopy features in Discoid lupus erythematosus (DLE) of the scalp are thick arborizing vessels and large yellow dots, atrophy, complete follicular paucity/plugs, hyperkeratotic scales and dark brown pigmentation. A good prognostic factor of hair regrowth is represented by the presence of Red dots, difficult to be observed in brown skin color patients [46] (**Figure 8**).

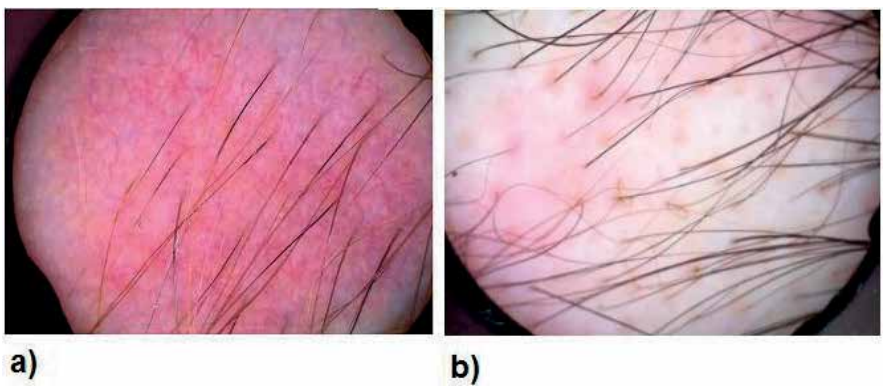

Figure 8. Trichoscopic aspect of (a) discoid lupus erythematosus (arborizing vessels with red lines and dots, large yellow dots) and (b) pigtail hair in an AGA patient (DermLite DL100).

Hair shaft disorders have a particular aspect: beaded shaft in monilethrix, brush fractures in trichorrhexis nodosa, shaft nodes in trichorrhexis invaginata, twisted shafts in pili torti [35, 47, 48].

Advantages of trichoscopy: it represents a handy and reliable tool for establishing a diagnosis, it allows a rapid examination of different areas of the scalp, easy capturing of photographs for documentation and comparison with pre-treatment images [33, 36, 38]. The disadvantages are that the examiner needs to acquire the skill and expertise necessary for a correct interpretation, to take into account the race (trichoscopy of vascular patterns is difficult in darker populations) and the shampooing habits of the patient (yellow dots may not be present in freshly washed scalp) [32, 49].

3.4. Videodermoscopy

Videodermoscopy represents a noninvasive tool for the observation of the scalp skin and hair, being a useful tool in the evaluation of hair loss, both for differential diagnosis and for prognostic evaluation [34, 50].

The videodermoscopy devices have the ability to capture digital images in a high resolution (magnification available up to 1000×) and to store them for later comparison. It is an adjuvant of trichoscopy and a prior step to performing a biopsy, also helping the dermatologist decide which is the right place to take the skin sample from [50].

4. Assessment of severity

4.1. The pull test

The dermatologist should briefly describe the method to the patient and tell him that the hair will be gently pulled several times. The technique will be repeated in different parts of the scalp: at a margin of a hair loss patch (in alopecia areata or trichotillomania) to see if it is active and in an unaffected scalp area to see if there is pendant activity, if the new grown hairs are stable [3, 5]. In diffuse telogen effluvium, androgenetic alopecia and female pattern hair loss, the two sites that are examined are: frontal (2 cm behind the forehead and lateral) and occipital (2 cm lateral from the occipital protuberance) [51].

Using the thumb and forefinger, the dermatologist will grasp a small section of hair (about 60 strands) close to the scalp and gently but firmly slide the fingers away from the scalp at a 90° angle along the entire length of the hair swatch (**Figure 9**). The number of extracted hairs is

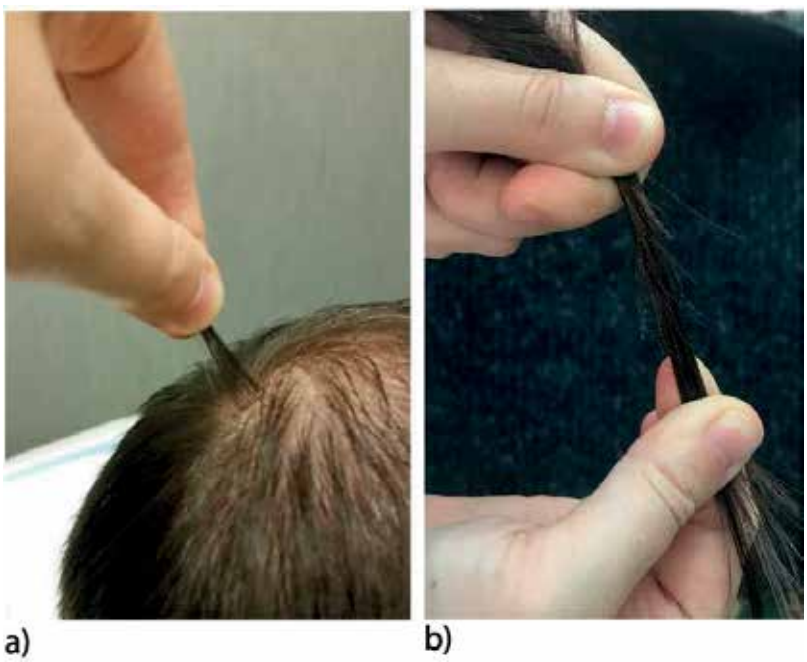

Figure 9. (a) The pull test and (b) the tug test.

counted [11]. If 6 more than 10% of the hair strands fall at the test, it means that the patient has an active hair loss, with one of the following differential diagnosis: telogen effluvium, anagen effluvium, loose anagen syndrome (common in young children), early androgenetic alopecia or female pattern hair loss or advanced alopecia areata. Also, it is important to establish what type of bulb do the extracted hairs present: anagen bulbs or telogen bulbs. Anagen bulb hairs

are not usually pulled easily out of the scalp, but they may be extracted in a pull test in the following situations: active primary cicatricial (scaring) alopecia, loose anagen syndrome or anagen arrest during chemotherapy [6]. An active phase of CTE would show a positive test with 6–8 telogen roots in light microscopy, while a loose anagen syndrome would present a highly positive pull test with anagen hair roots mostly lacking the hair sheath (anagen dysplastic) [37]. In Alopecia areata, after a positive pull test, a light microscopy assessment would show dystrophic anagen and telogen stage. In active AGA, the examiner can find a positive test in vertex and negative in the occipital area. In the active phase of anagen or telogen effluvium an increased number of anagen or anagen dysplastic hairs or normal telogen hairs would fall. Trichotillomania patients have a negative pull test.

The pull test has several disadvantages, being a rough and no standardized method, due to the different pulling strength of each investigator, not uniformly distributed over the hair bundle and variations of density or thickness of hair shafts. Mention should be made that on the day of shampooing, the pull test can be negative, as the telogen hairs have been rinsed away [37]. The pull test is currently performed in clinical practice, but it is useful only if the same examiner performs it in acute phase of hair loss in the more severe conditions for diagnosis and therapeutic follow-up [37].

4.2. Hair part width assessment

Assessment of hair density is important in patients with active hair shading or thinned hair. Hair part width is a test used for the assessment of hair density in different areas of the scalp: frontal, vertex, occipital, etc. In a scalp area with normal density, the hair parting with a comb discovers a very fine and thin line, while an alopecia area has an irregular line with small clear areas on both sides of the part line (**Figure 10**). The dermatologist must take into consideration the fact that usually, the hair density on the vertex is lower and that thinning increases with age [51].

Figure 10. Hair part width assessment in female patients: (a) healthy subject, without alopecia, (b) telogen effluvium subject, and (c) female pattern hair loss grade II on Ludwig scale.

4.3. Counting hair tests

Literature reports that shedding over 100 hairs per day is abnormal, but for the patients with advanced stages (who already lost more than 50% of the hair), around 50% hairs/day

represents increased shedding. Usually when patients loose around 80–100 hairs/day they address the doctor's office. The number of 100 hairs per day (in telogen phase, representing 10% of hairs on the scalp, which could contain up to 100,000 hairs) is taken as an approximate value, it was not validated as a standard reference and it cannot be globally applied to all the patients suffering of alopecia [5, 11, 52].

The number of hairs shed can be misleading in women who do not comb their hair after shampooing and leave it to dry naturally, because when their hands 'comb' through the hair many loose hairs will appear on the fingers [5].

More refined ways of assessing hair loss were needed and dermatologists have developed the following counting hair tests:

Daily hair count is recommended to be performed by the patients who observe intense hair shedding on the brush, comb, pillow, floor, in the washing tub. The patient would collect all the hairs shed during a day and place them in a plastic bag, then handle it to the doctor [4, 5]. The test could provide inaccurate results because numerous hairs usually escape detection and it is disliked by the patients who complain that it is stressful and difficult to perform [2, 4, 5].

The 60 second hair count test consists of the following steps: using the same comb/brush for combing the hair for 60 seconds (over a sheet of contrasting color for easier collection of hairs) from the back top moving to the front of the scalp, repeating the procedure before three consecutive shampooings, counting of hairs from comb/brush plus sheet and recording the value. This procedure must be repeated monthly, for 6 months. The researchers applied this test to 60 men, not previously diagnosed with alopecia and concluded that the similarity between investigator and subject hair counts indicates a reliably hair count technique. The low intra patient variability proved dependable results over a longer period of time. The test seems a simple, practical and objective tool for monitoring conditions associated with hair shedding, but needs to be applied to FPHL patients as well [52].

For the **standardized wash test** patients have to restrain from shampooing for 5 days, then wash the hair in a basin with a gauze (covered drain) that allows the collection of the hairs (**Figure 3**). This non-invasive method involves the counting of hairs, but the results cannot be properly interpreted because a hair loss considered normal can range between 30–70 and 200–250 hairs on shampooing days [4, 37, 43]. The wash test is usually disliked by the patients because it involves a bad self-hygiene and unpleasant hair aspect that could interfere with daily activities [5, 11].

The daily hair count and standardized wash test were not practical methods for monitoring hair shedding, so improved tests were developed [52].

The modified wash-test was developed by Rebora et al. and involves the same technique: washing the patient's hair 5 days after the last shampoo in a sink with covered drain. The collected hair is analyzed by the doctor and separated upon length: under 3 cm (belonging to short telogen vellus hairs), intermediate (between 3 and 5 cm) and long (above 5 cm). The test provides the total number of telogen hairs and the percentage of telogen vellus hairs and makes the difference between FPHL (with increased rate of vellus hairs, around 59%) and telogen effluvium (less than 4%). The test is difficult to apply to patients with very short hair and curly hair [53].

Both washing tests have the following disadvantages: unstandardized method with difficulties because of the shampoo, water, duration, strength of the massage and significantly time-consuming.

4.4. Common scales for staging of hair loss

Staging of hair loss patients is important in assessing the disease severity, to record the progression of alopecia or determine the response to therapy. Various classification systems have been suggested from simple versions based on the recession of the hairline to advanced multifactorial ones, based on morphological and dynamic parameters that affect both the scalp and the hair [54]. Hamilton (1951)-Norwood (1975) classification for males and Ludwig (1997) scale for women are the most commonly used [12, 27, 55] (**Figures 1** and **2**).

An easy to memorize and novel classification for alopecia in both genders is the Basic and Specific classification (2007), which includes the shape of the anterior hairline (basic classification with four types: L, M, C, U) and the hair density on distinct areas such as frontal and vertex (specific classification with two types F and V) [54].

The photographic assessment of hair loss severity developed by Sinclaire (2004) (scalp with hair parted in the center), was especially useful in chronic telogen effluvium [54] (**Figure 3**). Recently, Martínez-Velasco et al., issued the hair shedding visual scale, considered a fast and effective method of evaluating hair-shedding amounts in an office setting [41].

5. Photographs of the affected areas

Besides being present in the Sinclair scale (as a classification of the degree of hair loss severity), photographs are used for their general purpose, documentation of alopecia extent or evaluation of treatment efficacy (**Figure 10**).

Photographs should include both close-up views and global views to identify nearby landmarks. Four standard views (vertex, midline, frontal, temporal) should be captured with the same camera, magnification and lighting conditions [56]. The Canfield technique is the most commonly used for alopecic patients and needs the following requirements to be fulfilled: little extraneous information such as distracting clothes or backgrounds, a certain stereotactic position of the device and of the patient's chin and forehead, the same hair preparation each time (the doctor or a technician should perform the same hair parting each time, comb the hair), and the hair should be clean and dry. Oily or wet scalps have increased reflection, revealing more skin than hair, giving the false impression of less hair. In order to record the patient's cosmetic state at each follow-up visit it is recommended that the patient maintains the same hairstyle and hair color in order to decrease the variability of the technique and assure an easy assessment [5, 11]. The serial photographic documentation of hair loss or hair regrowth represents an objective and useful assessment method only if the doctor uses a regimented approach at each photographic session [56]. Up to the present, clinical researchers studying androgenetic alopecia used controlled photography to determine the efficacy of therapy, but this technique should be applied to various types of alopecia [5, 56].

In spite of its advantages, the imaging tests do encounter some problems in female patients, who usually try to hide the hair loss condition by changing the hairstyle (cutting the hair shorter to let the impression of more volume) and the color (dying the hair into brighter shaded in order to give the impression of increased density and hide the alopecic areas) [5, 11]. Global photographs can be combined with other evaluation methods, such as: trichogram, phototrichogram, TrichoScan or hair weighting [26, 37, 50].

Recently, photography of alopecic scalps has overpassed its medical use and has become a form of art meant to raise awareness about alopecia areata, in a project by Sigriour Frimannsdottir called Baldwin (meaning strength).

6. Assessment of hair characteristics with different tests

6.1. Trichogram

The trichogram is used for hair root and hair cycle investigation. It is semi-invasive: 60–80 hairs are plucked with a rubber-armed forceps in a quick pull perpendicular to the scalp, in the direction of hair growth. Two areas of interest are investigated in AGA, diffuse effluvium and loose anagen hair: the occipital area (2 cm lateral from protuberans occipitalis) and 2 cm behind the frontal line and 2 cm from the midline respectively. In AA the investigation focuses on the border of the alopecia patch and on the contralateral, unaffected side. After hair collection, the bulbs are placed on a glass slide, in an embedded medium for later examination. Hair roots are investigated under magnification lens or a low-power microscope and the number of hairs in each stage of the hair cycle is provided as percentage of the total number of plucked hairs [57]. Besides being laborious and time-consuming, the technique has another disadvantage: the superfluous remaining hair dyes in furrows mimic hair leading to false results [37].

6.2. Unit area trichogram

The unit area trichogram represents a semi-invasive quantitative method used until now in clinical trials to observe hair growth cycle and monitor different hair growth therapies [57]. It focuses on the main growth parameters: hair follicle density, proportion of anagen fibers, hair shaft diameter. The investigated scalp area is degreased with an acetone:isopropanol (60:40) mixture to remove surface lipids and the hair is plucked from an area of more than 30 mm^2 with a single smooth action in the direction of hair growth to minimize root trauma. The hairs are mounted on a double-sided tape and ordered by length. By microscopic analysis the investigator will establish the hair growth phase and measure the hair length, the major and minor axis of hair to determine the hair shaft diameter. The disadvantages of the method are: patient discomfort, extended hair regrowth time until obtaining any test results, time consuming, unsuitable for a large number of patients [37, 57].

6.3. The tug test

The tug test is performed when hair fragility and hair shaft abnormalities are suspected [5]. With the fingers of one hand, a section of hair is grasped, holding it near the root while the distal ends or hair tips are plucked (as plucking feathers) [6] (**Figure 9**). The dermatologist tugs to see if the strands break in the middle into small bits. If positive, the test gives information about hair brittleness or fragility [3, 11].

The hair feathering test is usually performed in patients who complain that their hair does not grow or breaks off. The distal 2–3 cm of hairs are rubbed between the thumb and index finger, then a brisk pull is made on the ends of the hairs. A positive test would reveal short broken hair fragments on the examiner's fingers [37]. The next step is the light microscopic inspection of the hair shaft to discover the underlying defect.

6.4. Hair mount

The short segments of hair shaft from the tug test are placed on a clean microscope slide. The examiner must place them parallel to each other. One or two drops of mounting medium such as Permount or similar, are used on slide and hair, then a cover slip is placed, avoiding the presence of air bubbles. The slide is then examined under a light microscope [6].

6.5. Microscopic evaluation

The following equipment is necessary when assessing the hair: a stereomicroscope with a magnification up to 100× (for the initial examination of mounted and unmounted hairs), a high-quality transmitted light microscope (examine and identify the microscopical characteristics of hairs in a range of 40–400×) or a high-quality transmitted light comparison microscope (for comparing the microscopical characteristics of hairs). For comparison microscopy it is desirable to have a second hair examiner verify the microscopical hair association that may have probative value. Usually hair comparisons are conducted among hairs from the same somatic region, with hairs of similar length, each with a root present or in a similar growth phase as the questioned hair [58].

A polarized light microscope is helpful in order to examine certain features and to determine the cross-sectional shapes of the hairs.

The hair examiner should be familiar with the instruction manual, maintenance requirements, performance and calibration checks and color balance. The hair sample may present adhering material (if considered significant it should be removed and preserved for later analysis) which can be removed by washing or cleaning the hair, then allowing it to dry prior to mounting [58].

Macroscopical and stereomicroscopical examinations enable the description of hair characteristics (color, length, shape, texture, etc.) and the identification of hairs suitable for microscopic comparison or the ones which have roots suitable for DNA analysis [11, 59]. Microscopical examination is helpful to complete the hair wash tests for hair cycle phase differentiation, shaft abnormalities and morphologic appearance of the distal tip [37] (**Figure 11**).

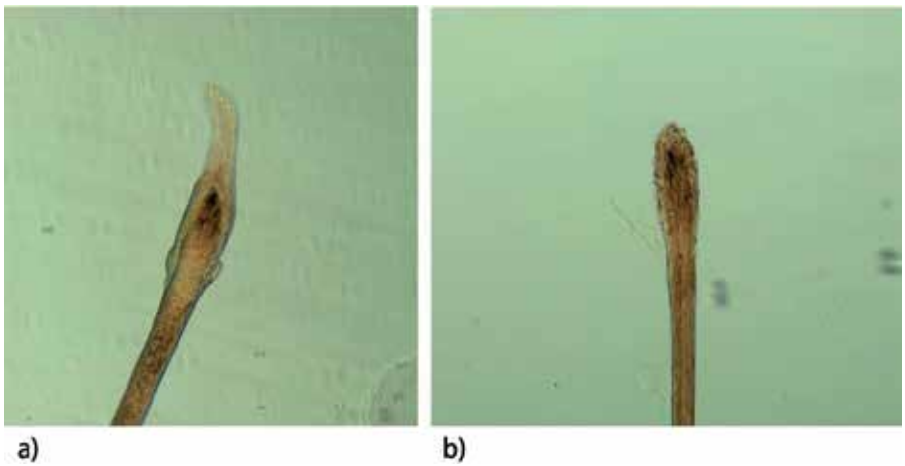

Figure 11. Embedded hair roots under 10× magnification (optical digital microscopy Leica DMD108): (a) catagen hair in a female with telogen effluvium and (b) telogen hair in an AGA patient.

Macroscopical examinations are useful for observing hair characteristics such as color (white, blonde, red, brown, black), hair shaft form (straight, arched, wavy, curly, twisted, tightly coiled, crimped), hair length (in cm) or hair shaft overall thickness (fine, medium, coarse).

Microscopic examination enables the observer to describe the color, natural pigmentation (pigment size, pigment aggregation, aggregate size, pigment density, pigment distribution) or the color treatments performed, such as dyes (permanent, semipermanent, temporary), bleaches or lighters. Hair characteristics assessment performed by microscope include the shaft description: diameter (in μm), cross-sectional shape (round, oval, triangular, flattened), shaft configurations (buckling, convoluting, shouldering, undulating, splitting, regular), medulla/cuticle/cortex configurations, description of proximal ends with root present (telogen/catagen/anagen/sheathed/follicular tag/ postmortem banding/putrid) or root absent (severed, decomposed, crushed), and description of distal ends (tapered or uncut tips, rounded or abraded, square cut, angular cut, frayed, split, crushed, broken or singed).

Acquired characteristics are also available for analysis and include: artifacts (nits or lice, old, fungal tunnels, insect bite marks, debris or blood), artificial treatments other than color (hair sprays, gels, permanents, cosmetics), environmental/chemical/mechanical damage markers and hair abnormalities (pili annulati, trichoschisis, monilethrix, trichorrhexis nodosa, trichorrhexis ingavinati, pili torti, trichonodosis, trichoptilosis) [58, 59].

In pili annulati the hair is stripped, with a pattern of light and dark banding. Trichoschisis represents a sudden shaft break across the diameter of the fiber with the localized absence of the cuticle at the site of fracture. In monilethrix, individual strains of hair have a "beaded appearance", like the beads of a necklace, presenting a periodic narrowing of the hair shaft [47]. The abnormality in trichorrhexis nodosa is the formation of nodes along the shaft as a response to physical or chemical trauma [37]. Trichorrhexis invaginata (bamboo hair) is due to abnormal keratinization leading to weak hair shaft cortex at specific points along its length. In pili torti, hair has a flattened shaft with clusters of narrow twists at irregular intervals.

Trichonodosis is characterized by knotted hair on the distal portion of the shaft, sometimes due to mechanical factors such as scratching or combing. In trichoptilosis, hairshafts become dry and exhibit splitting or fraying of the hair due to excessive exposure to chemical, thermal or hairdressing procedures [48]. If the hair shaft is apparently normal in microscopy, other tests can be performed, such as: KOH mount or root exam [59].

Besides the presence of the above mentioned genetic hair shaft abnormalities, the individual hairs in an affected area may be notably curly or kinky due to dermal fibrosis and subsequent follicular torsion [6].

Electron microscopy is distinguished by its high spatial resolution in the nanometer range, compared to optical microscopy. Even though it reveals higher details, the pretreatment required of the hair is more extensive and usually leads to artifacts. Another type of microscopy used for hair is atomic force microscopy (AFM), which uses the principles of scanning tunneling microscope and the stylus profilometer in order to provide 3D images with high resolution at the nanometer scale, together with qualitative and quantitative measurements of the sample [37]. AFM is limited to the measurement of the topographic morphology perpendicular to the sample plane (re-entrant surfaces and surface information cannot be detected), so it is not used in clinical practice.

6.6. The contrasting felt examination (hair card test)

The purpose of this test is to evaluate the number of new hair strands that are growing and to examine the health of the hair shafts. Good lighting is essential. The dermatologist creates a part in the hair and uses a small rectangular index card with black felt glue on one side and white felt on the opposite side [5, 11]. The hair card is placed as close to the skin surface as possible and is used on the scalp, brows, eyelashes, etc. Hairs shaft and tips will be held by the doctor against the contrasting black or white background, depending on the color of the hair, for maximal contrast (if dark hair is examined, use the white side) (**Figure 12**). New hair strands, fine short miniature or broken hairs will project up along the edges of the felt card, so that they can be counted and examined [5]. If the distal ends are tapered or pointed (like the ends of the eyelashes) they indicate new hairgrown [6]. If the distal ends are blunt or straight, they may have been broken or cut. If some of the new short hair is thinner in diameter than the rest, it indicates miniaturization [6]. The test is useful in the recognition of very thin strands in telogen effluvium, short vellus hairs (with miniaturization) in AGA and FPHL patients or short strands with broken tips in hair shaft abnormality disorders.

The hair card has a ruler portion which can be used for measuring: the length of new growth, the dimension of hair loss area or the temporal recession [6] (**Figure 12**). Using the ruler side of the hair card, one can measure the distance from the lateral end of the brow to the apex of the temporal recession (in males the normal distance would be 6–6.5 cm) [6].

6.7. Hair weight determination

Hair weight determination involves the selection of a scalp area (usually an area of 1.34 cm^2 in the frontoparietal region), where the hair is clipped under magnification to a length of 1 mm at

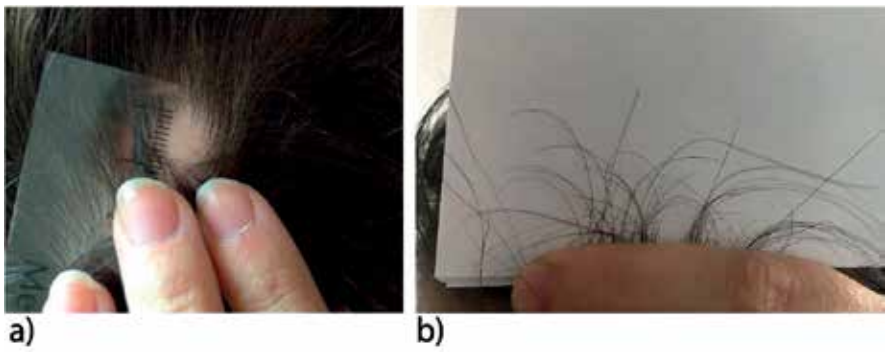

Figure 12. (a) Measurement of AA single patch dimensions using a ruler and (b) the hair card test performed on a telogen effluvium female patient.

baseline, the scalp is permanently marked by tattoos, the hair is allowed to grow for a period of time (from 4 to 24 months, depending on the treatment performed), then clipped again, collected carefully and weighed by an experienced technician [5, 11]. The method is a quantitative one, but unfortunately it is not standardized and precise. It has been used in clinical trials, but was not applied in clinical practice due to its demerits: time consuming, no immediate results are available, no specific scalp areas to be assessed, improper capturing of the hairs, frequent mistakes during the clipping process, incomplete trimming of hairs from that area, unspecified time for regrowth [4, 49]. Also, the method has been rejected by the patients, especially female patients who do not agree to have the hairs clipped/cut from visible scalp areas, such as the frontal area [5].

6.8. Hair densitometry

This determination was classically performed since 1993 with a densitometer: a handheld magnification lens device with an opening of 10 mm^2, used to check for miniaturization of the hair shaft, to describe the follicular unit composition and to assess the hair density [50]. First, the doctor clips the hair, about 1 mm short, and the instrument is then placed on the scalp. Then the total number of hairs in the field are count, the number of hairs per follicular unit is assessed, as well as the diameter of the hair, looking in particular for abnormal levels of miniaturization (decreased hair shaft diameter) [5].

In practice, hair densitometry is used to evaluate a patient's candidacy for hair transplantation, as it assesses a person's donor hair supply and anticipates the esthetic outcome of the hair restoration procedure [50]. Otherwise, the method is extremely laborious and tiring, less accurate than computed hair analysis.

A modern version for determining hair densitometry is the HairCheck device (Divi International Co., Miami), a cross-section trichometer. This quantitative method enables a precise evaluation, since it indirectly measures the density (n/cm^2) and diameter (μm) of hairs by directly determining the cross-sectional area of all the hairs in a premeasured area of scalp skin [50]. The measurements are performed without cutting the hair, by using an inked four legged device and selecting an area of 4 cm^2. The hair from that area is gathered with a pinhead tool, then

clipped between fingertips and introduced as a bundle in the J type hook of the measuring device [4, 60]. The HairCheck tool produces a compression of the hair bundle which is engineered to deliver the same predetermined load and the force without damaging the captured hair [60]. On a LED display appears the trichometric index, which represents the height of the compact bundle in its capture chamber, with a value between 75 and 100, expressed as square millimeters of hair per square centimeter of skin × 100 ($mm^2/cm^2 \times 100$). Several tests done by Cohen have proved its accuracy and concluded that there was a direct correlation between the bundle's cross-sectional area and the number of surgical silk fibers/filaments, the diameter of the filaments and their dry weight [60, 61]. The HairCheck method is used to quantify by comparison the amount of hair lost or gained and it is extremely helpful as it offers regrowth information by direct assessment of the hair mass (on density and diameter changes) [4]. In one personal study on FPHL, we have successfully used the HairCheck assessment on monitoring the patients' evolution under treatment [5, 11] (**Figure 13**).

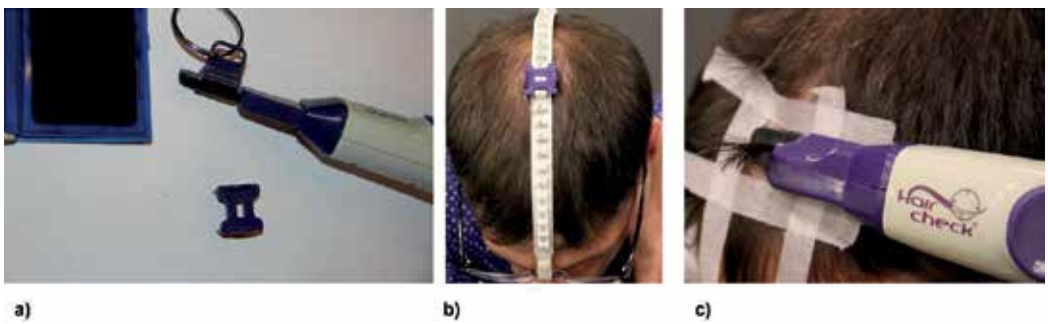

Figure 13. Hair density measurement also known as hair mass index (HMI) with the HairCheck device: (a) pinhead tool (area for measurement), a template with inkpad to demarcate a pre-measured site, calibration tool; (b) a locating strip attached to glasses; and (c) the determination of hair mass by compressing the hair bundle in the J hook of the device.

The HairCheck device offers also the possibility to determine the hair breakage index (HBI) or percentage of broken hairs, which is performed with a proximal and a distal HMI measurement on the same isolated bundle (**Figure 14**).

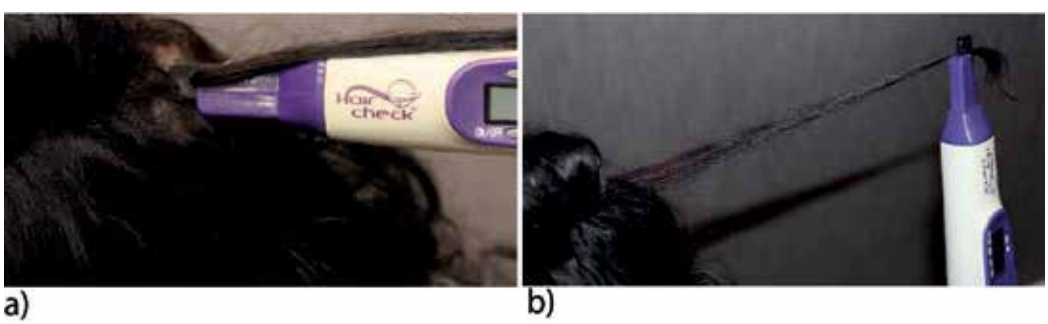

Figure 14. Hair breakeage index (HBI) measured with the HairCheck device (a) at proximal part of the hair bundle and (b) at the distal part of the hair bundle (tip of hair stand).

6.9. Computed hair analysis

Conventional approach to evaluate an alopecic patient implies visual evaluation, which may hinder an objective assessment. For this reason, several researchers tried to develop a quantitative method using a computer-aided imaging system. A study has used a series of digital image processing techniques to measure the width of central balding area of FPHL: the balding area was identified by the computer, which measured the ellipse of balding [26]. The values obtained were significantly correlated with the Savin clinical scale.

A reliable computer-aided imaging system besides staging the severity of the alopecia, could also monitor hair loss and treatment responses.

TrichoScan is a new device, based on epiluminescence microscopy, combined with automatic digital image analysis for the measurement of human hair, focusing of the following 4 parameters: hair density (number per cm^2), hair diameter (μm), hair growth rate (mm per day), and anagen/telogen ratio [54]. The investigator chooses and clips an area of hair loss between normal hair and the balding region. Images are obtained by pressing onto the scalp a digital camera with rigid "contact lens" (so they are taken at the same distance from the scalp) fitted with a close-up microscopy attachment [37].

TrichoScan represents an automated image analysis tool that can determine the surface, miniaturized hair density, terminal hair density, percentage of terminal and miniaturized hair, anagen and telogen percentage. The device is precise and has an intraclass correlation of approximately 97% for different TrichoScan operators. The advantages of the TrichoScan examination make it a useful tool to assess placebo versus treatment, to compare different capacities of hair growth promoting substances, to study AGA and diffuse hair loss, to evaluate the effects of drugs and laser treatment on hypertrichosis and hirsutism [62]. One disadvantage consists of the fact that gray or fair colors have limited contrast with light scalp skin and need to be dyed for 15 min with a solution provided with TrichoScan, that needs to be mixed 1:1 with development cream.

Literature reports underline that computer-aided imaging systems are valuable methods of quantifying hair loss, than can assist the physicians in evaluating the balding area more precisely for clinical staging.

6.10. Optical coherence tomography

Optical coherence tomography is used for measuring hair shaft longitudinal and transverse diameters, cross-section-surface area and hair shape, similar to histology, but in vivo. This procedure uses low-coherence interferometry to produce a two-dimensional image of optical scattering from internal tissue microstructures analogous to ultrasonic pulse-echo image, which works with ultrasound. The results consist of a running time of a near-infrared signal to a studied specimen and back, that will be compared to a known reference signal [37].

6.11. Hair analysis methods

Hair analysis methods are used for the evaluation of genetic disorders, the investigation of physicochemicals properties of hair in disease, the study of exposure to certain substances, etc. Plucked hairs from the temporal area are preferred. The hair should be uncontaminated: a non-exposed part of a growing anagen hair fiber or close to the infundibulum of the hair follicle. The sample of hair is cleaned and put through a number of spectrographic processes capable of identifying as many as 40 trace elements [37].

6.12. Mechanical tests of hair quality (elasticity, strength, fragility)

The evaluation tools for the physical properties of hair focus on the integrity of the internal structure of the fiber and its alteration due to environment, cosmetics or treatments applied. The measurements of mechanical properties are easy to perform and the most common is the tensile property test, which focuses on the stress/strain curve of single hair fibers and is measured with an extensometer. The hair is fixed between two ferrules in a sample cassette of the instrument and a constant speed of extension is exerted until the hair fiber breaks [37].

7. Scalp biopsy

A scalp biopsy is recommended to be taken as soon as the dermatologist suspects cicatricial alopecia [5].

7.1. Scalp biopsy technique and requirements

There are some requirements for the patient, such as refraining from topical steroids usage (Clobetasol, Betamethasone, Fluocinonide, Clobex, Luxiq, etc.) for 1 week prior to the biopsy. On the day of the biopsy the hair should be washed and without any hairspray, gel, mousse or topical camouflaging fibers and agents (Toppik, DermMatch, Couvre, etc.) [21]. Certain lifestyle choices (smoking, excessive drinking) increase the risk of side effects such as bleeding and slow healing [11]. The best position for scalp biopsy harvesting is with the patient sitting down on a chair, leaning over the examination table, bracing their head with their hands, also known as "the Thinker position" [5]. For a biopsy from the occipital scalp, the patient may lie on the examination table facedown or sit on a chair and rest his head down on the evaluation table, "taking a nap on the school desk position" (**Figure 15**).

The doctor should select an active hair-bearing area with an advanced thinning and a preferred positive anagen pull test (more than six hair fall), clean the area and disinfect it with alcohol, iodine or similar solution, sometimes trim a few hairs from the area [9, 21]. Some dermatologists select an area with active inflammatory disease (early thinning with visible erythema and mild scaling), an incipient one, not with end-stage changes of scarring [63]. This type of area is preferable in cicatricial alopecia, in which it is highly recommended to take the biopsy

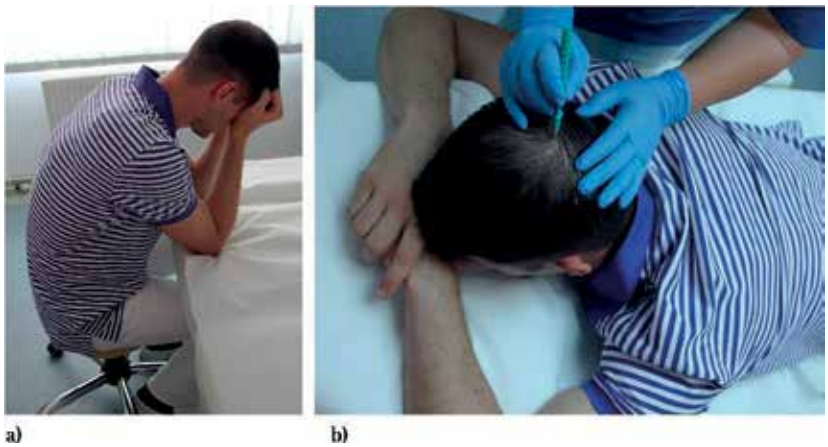

Figure 15. Positioning of the patient for scalp biopsies and injections: (a) sitting on a stool, leaning over the exam table, bracing their forehead "the Thinker position" and (b) patient lying on the examination table facedown.

at the periphery where compounding is not present. Taking biopsy specimens of tuft of hairs (polytrichia or compounding follicles) is not helpful as these nonspecific structures are end-stage features of many cicatricial alopecias, including lichen planopilaris, central centrifugal cicatricial alopecia, also lupus [63].

Local anesthesia is usually performed with 1% lidocaine with epinephrine and the wait time should be 10 min for a maximum vasoconstriction. Hemostasis can be performed with the help of an assistant using gauze squares and Q-tip [5]. Scalp samples can be obtained either by classic surgical collection in an elliptical incision of the skin or by punch biopsy [9]. The most common method is by using a 4 mm-punch biopsy (12.6 mm^2) for vertical sectioning or horizontal sectioning [55]. The punch tool is placed on top of the scalp, pressure is applied until the doctor samples down to subcutis and with the help of the needle the excised skin is removed [11]. Some dermatologists consider that both vertical and transverse sections of scalp biopsy specimens are needed. Transverse sections seem to provide a better assessment of the histological features than vertical sections in specimens provided by alopecia areata patients [64]. On the other hand, some dermatopathologists strongly prefer vertical sections, especially in cicatricial alopecias, as they allow the assessment of alopecias associated with interface changes, lichenoid infiltrates and subcutaneous pathology [63]. The vertical sectioning biopsy technique represents a qualitative approach, including just 10% of the hair follicles, and being susceptible to sampling errors. On the other hand, the transverse section is a quantitative approach providing all the hair follicles present in the biopsy, offering data of follicular cycling, as well as morphometric evaluation of the hair follicles throughout their entire length. The transverse biopsy enables the detection of even focal pathology and is preferred in most of the cases of nonscarring alopecia, because of the larger number of follicular structures that can be studied [55].

The dermatologist can close the defect with suture with classic stitches (which should be removed in 10–14 days) or dissolving stitches (which dissolve fully in 6–8 weeks) [11]. In case of punch biopsy, a single stich is common or some dermatologists leave the wound open [9]. The

next day after the biopsy the washing and shampoo is allowed, usual product used on scalp can be used the day after, while dying and coloring of hair is permitted 1 week after the biopsy [63].

7.2. Scalp biopsy results

Besides sending the biopsy specimen to the dermatopathologist, the dermatologist must provide the patient characteristics (age, race), duration of condition and clinical pattern, and sometimes a photograph is helpful, if available. There are differences in normal hair densities depending on race, also certain racial groups have higher predilections for some diseases [63, 65].

If the biopsy provides a diagnostic, it can offer information about a systemic disease (discoid lupus erythematosus, sarcoidosis, lichen planus follicularis, necrobiosis lipoidica diabeticorum, etc.), an infection (fungal, bacterial, protozoan) or even a neoplasm (basal cell carcinoma in the morpheaform, squamous cell carcinoma, metastatic carcinoma in the alopecia neoplastica form, lymphoma or adnexal tumors, etc.). If the biopsy is not diagnostic, the dermatologist should suspect a hereditary disorder or trauma/injury (mechanical trauma, burn, caustic agent, exposure to radiation, etc.) [64].

In **AGA**, the biopsy sample contains increased number of miniaturized hairs, abundant enlarged sebaceous glands and minimal inflammation. A ratio of terminal (T) to vellus (v) ratio 3:1 or less is considered to be diagnostic [63, 64, 65].

Patchy and diffuse alopecia areata present peribulbar lymphocytic infiltrate around anagen hair bulbs, rich in helper T cells, evidence of an autoimmune process. Alopecia areata should be suspected when high percentages of telogen hairs are present, even if the peribulbar infiltrate is not present. Both types of AA may sometimes coexist with Androgenetic alopecia [5, 9, 64].

Histologically in **LPP** the pathologist can find two patterns: hair follicles and the perifollicular dermis mainly involved in the pathologic process (with no involvement of the interfollicular structures) or the pathologic changes extended to the interfollicular epidermis and papillary dermis [55]. Direct immunofluorescence shows the presence of colloid bodies in the peri-infundibular area staining with IgM, while the immunohistochemistry staining shows a significant alteration in the basement membrane structure, which differentiates it from active DLE lesions [66].

The distinctive clinical features of **DLE** of the scalp are the presence of erythema, scaling, telangiectasia, mottled hyperpigmentation within the areas of scarring alopecia and the presence of hyperkeratotic papules in the central part of the bald area in DLE, while in LPP it is present at the margin of the alopecia patch [67]. When routine histological findings are equivocal, direct immunofluorescence (DIF) helps, but light microscopy should be performed before DIF. The suggestive findings are: multiple immunoreactants deposits around hair follicles (not seen in other scarring alopecias) typically IgG and IgM, in a special pattern (bright in intensity, continuous, perifollicular, and granular) [67].

7.3. Scalp biopsy complications

Patients should be aware that a small scar will be present permanently in the area where the biopsy was taken, resulting in a new area with no hair regrowth [9, 19].

As with any medical procedure, there are some possible preoperative, intraoperative and postoperative risks [68]. Preoperative risks can be related to the used anesthetic. Performing of a patch test is recommended if true sensitivity to the anesthesia is suspected, in order to avoid an anaphylaxis, situation which rarely occurs. History of syncopal attacks are important, as a vasovagal attack can be present in this clinical setting [68]. Intraoperative risks involve bleeding, difficulty in closing the skin defect, pain and discomfort caused by insufficient anesthesia [68].

Postoperative risks such as pain, discomfort, bleeding, swelling, tenderness develops, and some of the symptoms can be relieved by medication, prescribed by the dermatologist. Besides scarring and hyperpigmentation, postoperative infection develops in 22% of the cases, but it can be easily treated with antibiotic. Another possible complication consists of temporary numbness or weakness, due to nearby nerve structures damage, which usually represents a nonpermanent situation [68].

8. Laboratory investigations

There are no laboratory tests indicated in AGA male patients who are using topical minoxidil or finasteride [3].

Extensive hormonal testing is not required in female patients, unless symptoms and signs of androgen excess are present (hirsutism, acne, virilization, etc.). The female patients who require endocrine evaluation are identified with careful inquiry regarding: menstrual irregularity, history of infertility, galactorrhea, etc. If positive, laboratory measurement of the following hormones is necessary, in order for the dermatologist to have a clinical evidence of the androgen excess: serum total testosterone, free testosterone, dehydroepiandrosterone sulfate (DHEA-s) and prolactin levels [3, 34]. All the above plus the follicle stimulation hormone (FSH) and luteinizing hormone (LH) are recommended to be performed in FPHL. Habif recommends that testosterone-estradiol–binding globulin (TeBG) should be also tested, in order to obtain the level for the total testosterone/TeBG ratio [17, 26]. If elevated, this androgenic index may indicate a pituitary disease (e.g., pituitary prolactinoma 8).

Tosti considers that 30% of all hair loss is caused by polycystic ovary syndrome PCOS and for diagnostic a pelvic ultrasonography is required [3].

Every patient with hair loss should have the following baseline studies: complete blood count (CBC) and iron study including serum iron and ferritin to rule out iron deficiency [12]. Also, thyroid function tests (free T3, free T4 and TSH) and serum thyroid autoantibody (anti-TG and anti-TPO levels) need to be done to rule out a possible thyroid disease, especially in telogen effluvium [3, 53, 60]. Estimation of blood cadmium (Cd) levels may be important in cases of chronic telogen effluvium as its toxicity can be an underlying hidden cause [69].

Other common causes of hair loss investigated by measurement of different serum levels: serum thyrotropin and vitamin D 25OH (deficient serum levels of the vitamin are present in AA patients and inversely correlate with disease severity) [70]. In AA it is also recommended to test: erythrocyte sedimentation rate (ESR) and rheumatoid factor (RF).

In order not to miss other possible factors, antinuclear antibodies (ANA) test is performed for the diagnostic of systemic lupus erythematosus. Reagin plasma response (RPR or VDRL) test is necessary to rule out syphilis.

In tinea capitis, the following tests are recommended: culture swab, potassium hydroxide (KOH) examination and fluorescence examination with Wood's lamp. In dissecting cellulitis of the scalp discharge is common and should be cultured [12, 60].

If all the laboratory results are normal, the dermatologist can consider:

- Other nutritional deficiency (malnutrition, sprue, zinc deficiency)
- Trauma (Trichotillomania, traction alopecia)
- Hereditary syndromes [8, 57]

Laboratory investigations are not only used as diagnosis tools, but are also necessary in the treatment initialization or evaluation. Systemic medication that inhibits androgen production or its effects (spironolactone, cyproterone acetate, flutamide) represents a second-line treatment in FPHL. For safety purposes, women taking spironolactone should have potassium levels checked prior to therapy [3, 12]. Women taking antiandrogens or oral contraceptives at 3–4 months after the onset of therapy must have the levels of free testosterone and dehydroepiandrosterone (DHEA)-sulfate measured [13, 14].

9. Conclusion

Although dermatologists have a large number of evaluation methods for hair loss and hair regrowth, most of them are not standardized and their applicability is limited in clinical setting. The majority of these evaluation methods are rejected by the patients because they are too invasive, time consuming and difficult to perform or involve a bad hygiene and hair aspect that interferes with daily activities. Taking into consideration all these problems that occur in clinical practice we underline the necessity of more refined and precise evaluation tools for assessing hair loss patients.

Conflict of interest

The authors have no conflict of interest to declare.

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Chapter 2

Perspectives of Alopecia behind the Regulation of Foxn1 Gene Exposes the Human Nude Phenotype

Shahnaz Begum, Md Jamil Hossain, Li Juan Gu and Chang Keun Sung

Additional information is available at the end of the chapter

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Abstract

The hair follicle (HF) is remarkable for its dynamic structure and one of the most prominent mini organs of the skin. The most visible end product formed by the hair follicle known as "hair shaft"- a tissue with a highly keratinized protein. Therefore, alopecia or baldness issue largely depends on the equilibrium between keratinocyte growth and differentiation of the HF. However, molecular nature in mice, rats, and humans, loss of transcription factor Foxn1 the keratinization processes is significantly impaired. Hence, this nude gene lack of function makes very similar hair pattern baldness in both human and nude mice (Foxn1nu/Foxn1nu). Thus highlighting the usefulness of mouse mutants and mouse genomics as a research tool for better molecular controls of human hair biology. To enhance research efforts of Foxn1 target gene regulation and the pharmacological manipulation of the nude phenotype are important open questions for promising research strategies related with Foxn1 biology. Taken together these issues may open the discovery of the investigative dermatology that promises the controls of epithelial differentiation in mammalian skin.

Keywords: hair follicle, nude mouse, alopecia, Foxn1, T-cell immunodeficiency, keratinocytes

1. Introduction

The hair follicle (HF) is the most prominent mini organ of the skin and it undergoes repeated cycles of regression and regeneration throughout the lifetime of a mammal. Each phase of the hair cycle is distinctively characterized. During the anagen phase hair is actively growing

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with a progression of tissue proliferation, a short resting phase with a massive apoptosis of hair follicle (catagen) and the relatively quiescence of telogen follicle, thus maintaining hairy phenotype in mice, rats, and humans [1–3].

The natural hair cycle in human poses a unique paradox involves many signaling molecules, transcription factors, and structural components are differentially and sequentially expressed and generate this organ. The differential expression of receptor and enzyme also provides basics for the variable responsive into the active hair follicle. On the other hand, genetically acquired disorders (nude gene) also inhibit to generate well differentiated hair follicles related with abnormal keratinization of hair fiber; taken together all these phenomena may cause baldness in human (alopecia, hair loss/balding); is a serious psychological distress in human society [4–7]. All these regulatory phenomena of hair cycle are highly conserved between rodents and humans. Although, some clinical differences might be present at the molecular level between the human and animal models; but it may not fully represent the morphological presentation of disease in humans. Consequently, especially when it comes up with the pathogenesis of human hair growth and disorder, it is essentially required to carry out research on human hair follicles in to animal models studies in the parallel lines.

Mammalian models, especially the development of genetically engineered mice which is role model to study for hair biology and hair disorder research those includes (1) failure in hair follicle formation and consequently abnormally low number of hair follicle in epidermis, (2) disorder of hair morphogenesis causing to fail the hair shaft to penetrate the epidermis, (3) hair follicle structure disorder leading to hair shaft defects and alopecia and also (4) immunological abnormalities resulting in alopecia [8]. Moreover, rodents those are genetic manipulation has been conducted to produce knockout (gene inactivation) mice for specific gene of interest the regulatory events of hair follicle development and hair growth, directly relevant to the hair follicle biology in humans [9].

During embryogenesis the development of the hair follicle appendage formation involves a complex sequence of signals interacting with the ecoderm and mesenchyme to form a mature hair follicle [10]. Once the hair follicle is generated, it displays dynamic cell kinetics: anagen (growth phase), catagen (regression phase), and telogen (resting phase), throughout postnatal file. Among the skin appendages, the hair follicle has the most complicated structure, composed of several distinct cell types that produce highly specialized protein. The anagen hair shaft has a common structural organization, in which a multicellular cortex is encased in a cuticular layer of flattened cell, often with a medulla layer centrally placed in the cortex. The hair is surrounded and supported by the inner root sheath (IRS), companion layer, and outer root sheath (ORS). The IRS consists of three distinct layers: IRS cuticle, Huxley layer, and Henle layer. The matrix cells in the hair bulb, which originally derive from the stem cells located in the bulge region, actively proliferate and differentiate into these cell layers except for ORS [11]. Like the epidermis, the hair follicle is also a highly keratinized tissue forms a rigid structure and also the end-product of the hair follicle. Recently, more of the genes that control the expression of hair keratins have been defined, including Foxn1 (FOXN1 in humans), which was first to be found using linkage analysis and an autosomal recessive mouse mutant, “nude“. This gene encodes a member of the Forked/Winged-helix domain family designated as Whn (Winged-helix-nude)

or Hfh11 (hepatocyte nuclear factor 3/forkhead homolog 11) but was later renamed Foxn1 (Forkhead box n1) [12–15]. In the anagen hair follicle, Foxn1 is strongly expressed in the upper matrix, precortex and cortex of the hair fiber [12, 16] suggesting that Foxn1 might activate genes essential for hair fiber differentiation. Several lines of evidence support this suggestion, specially about the involvement of Foxn1 in the expression of hair keratin genes.

It is known that nude mice have disrupted postnatal hair growth and show T-cell immunodeficiency due to thymic aplasia [17]. Hairs of nude mice are very thin and easily forms coils within the hair follicle, indicating a defect in hair keratinization. Also, nude mice possess homozygous mutant Foxn1 alleles, which results in the trunked protein lacking both the DNA- binding and transcriptional activation domains as pointed as the need for DNA-binding of Whn to fulfill its function [12]. Finally, in 1999, Frank et al. [18] reported the crucial finding that the nude phenotype is by no means just a peculiarity of the nonhuman animal kingdom, but also occurs in humans equivalent of the "nude" murine phenotype was first described in two sisters in 1996 and also described by Lin et al. [19] in consanguineous Chinese family affected by PHNED and identified a homozygous nonsense mutation. This made the nude gene the second gene to be defined after hairless [20] whose lack of function generates a very similar hair phenotype (alopecia) in both human and mouse, and it underscored the usefulness of mouse mutants and mouse genomics as a research tool for better understanding the molecular controls of human hair biology [21].

2. Morphological characteristics expressed by Foxn1 gene in the skin

2.1. Imperfect haircoat leading to immunodeficiency

The role of Foxn1 gene expression unambiguously expressed in the skin of nude mice with a lack of haircoat compared with the wildtype and heterozygous animals. As this phenomenon can also be selectively expressed in the thymus [22] and the epidermis shows the sign of abnormal differentiation and the nails of nude animals are severely malformed [16, 23]. In 1966, S.P. Flanagan from the Institute of Animal Genetics in Edinburgh, UK, nude mouse Foxn1nu/Foxn1nu (hereafter called nu/nu) established a classical mouse model with a lack of fur coat and an increased rate of postnatal mortality [17]. On that time, Flanagan failed to notice that nude mice suffer from agenesis of thymus and as a results leads to severe immunodeficiency [24]. It caused by lack of mature T cells thus makes nude mice also an ideal model system for immunological research and experimental [25] oncology. Later advanced studies have demonstrated that both defects are the pleiotropic effects of the same gene [22, 26]. Thus, restoration of a thymus gland may not govern the hair growth of nude mice. Hereafter, it becomes an attractive mouse model for immunodeficiency caused by congenital athymia and also for great research tool for congenital alopecia.

According to Flanagan's observation, at birth nude mouse found no histological abnormalities. The impaired differentiation of nude follicles exhibits structural imperfections (**Figure 1**) of the cortex, hair cuticle and inner root sheath (IRS) [27]. The most remarkable phenotypic characteristic

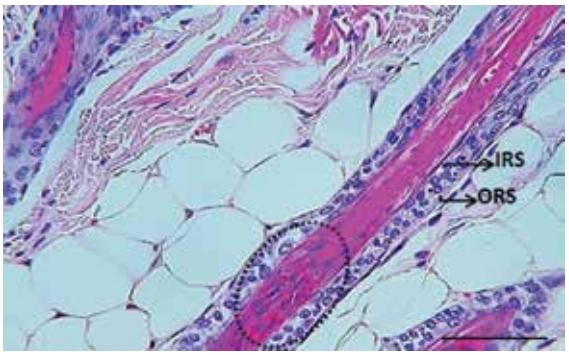

Figure 1. Cortex formation is severely injured in nude follicle and exhibits structural imperfections of hair cuticle and inner root sheath. Scale bar: 100 μm.

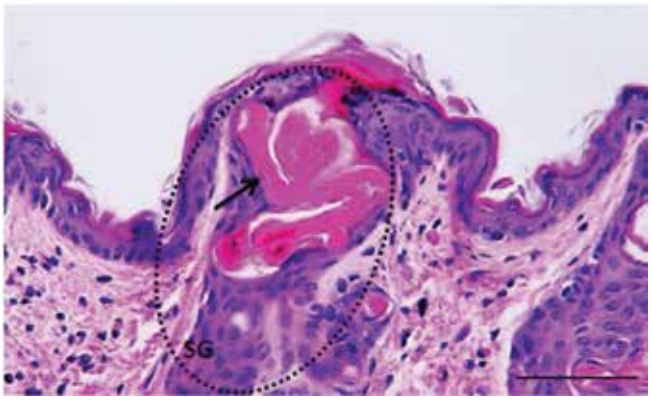

Figure 2. The follicular infundibulum, hair shaft twist and coil (arrow) at the level of sebaceous gland that does not penetrate the epidermis. Scale bars: 50 μm.

of nude mice skin is the disintegrated IRS leads to bend and coiling inside the sebaceous gland thus fails to achieve to penetrate the epidermis on postnatal day 8 [28, 29]. Subsequently, the follicular infundibulum, lined by a hyperplastic epithelium, becomes dilated (**Figure 2**) by keratinaceous debris and a small and curly hair shaft that does not penetrate the epidermis [30].

Finally, inadequate sparse hairs are visible at the head, neck, and the front extremities of nu/nu mice by approximately postnatal day 10 [17, 30–32], and later on spread it over the trunk area. Hair shafts those are able to enter the epidermis are often rigorously twisted or locally thickened and often breakdown before achieving a substantial length [30]. However, these are the molecular consequences of the nude phenotype cause by the mutations in the Whn gene [12, 13, 18, 33–35].

2.2. Nude mouse hair follicle undergoes normally cycling and usual hair bulb number

In general, *Foxn1*^{nu} nude mice exhibit the same number of hair bulbs as normally mouse at day 42 postnatal [32], although marked defects observed within the nude mouse hair shaft, as which is mainly responsible for synthesizing the active hair shaft [36].

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The *Foxn1*^{nu} nude mouse hair follicle, it passes through a regular cycle of hair growth (anagen), regression (catagen), and a resting period (telogen) and it does not directly interrupt with well-differentiated the three phase (**Figure 3**) of the hair growth cycle [17]. Flanagan observed that during the third week postnatal hair follicles undergoes rapid organ involution, corresponding to the catagen stage of normal skin. During catagen phase the follicles in nude mice also shorten and build a club hair (**Figure 4**) that stopped growing any more as also observed in normally haired mouse skin [17].

Therefore, hair follicles in nude mice go through a normal cyclic transformation, as was already mentioned by Flanagan in 1966 and later on validated by some other researcher [25, 37, 38]. According to the typical morphological criteria during hair follicle formation events [39], no profound weakness were formed by the nu/nu mice, comparing to normally haired littermates [40]. Only the peculiarities found in the sebaceous glands as also been assumed by Flanagan [17], but sebaceous glands are simply a little displaced by the bending of developing

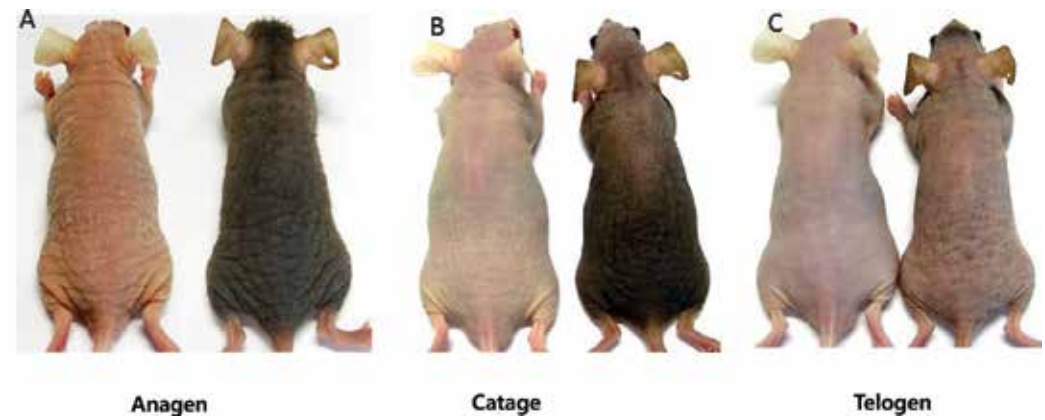

Figure 3. The Foxn1nu nude mouse (albino and pigmented) hair follicle passes through a regular cycle of hair growth A (anagen), regression B (catagen), and a resting period C (telogen). Inadequate sparse hairs (A) are visible at the head, neck, and the front extremities of nu/nu mice by approximately postnatal day 10.

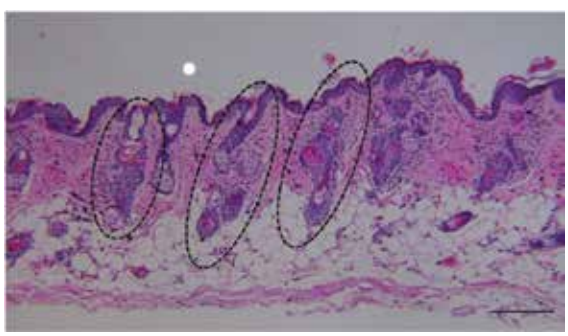

Figure 4. Catagen phase of HF in nude mice become shorten and fragmented hair shafts with keratinized debris that are heavily twisted. Scale bar: 100 μm.

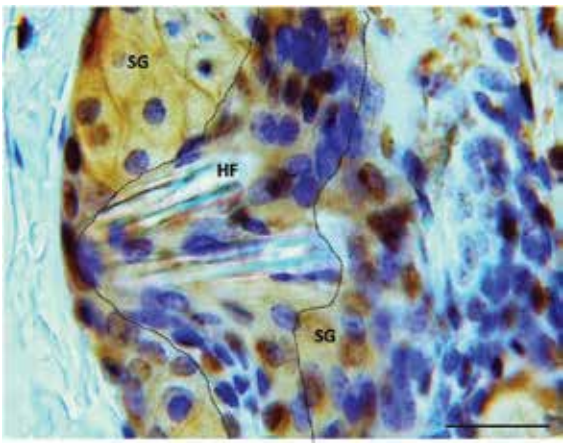

Figure 5. Sebaceous glands are simply a little displaced by the bending of developing hair shaft. BrdU labeling. Scale bars: 50 μm.

hair shaft and the dilated (become wider or larger) infundibulum is formed as like as funnel like structure (**Figure 5**), but without any morphological and structural abnormalities in the sebocytes themselves [30].

2.3. Expression of Foxn1 gene in epidermis

Foxn1 gene expression expressed in typical regions of developing skin, first in the nasal region as early as day 13 of gestation, subsequently *Foxn1* mRNA, the FOXN1 protein, and keratinocytes can be detected in the developing suprabasal epidermis but not found in the hair bulb as the first stage of hair follicle development on day 16 as it confirmed by using a reporter marker Beta-Galactosidase [16]. In addition, an increased proliferation and an impaired keratinocyte differentiation have been found in interfollicular epidermis, and the hair follicle of the nude mice, which overexpress Foxn1 in terminally differentiating cells [41]. Within the mature hair follicle and all anagen stage hair follicles, Foxn1 is preferentially transcribed in the supramatrical region, in the hair shaft, and in the inner and outer root sheath [16].

For the formation of an active hair follicle, interaction between the epithelial and dermal cell plays a vital role. The dermal papilla cell delivers signaling activators to induce follicle development [42–44]. Now the link between a mutated nude gene and its changes in molecular level in keratinocytes was presented by [45] as follows: While the reconstitution grafting assays was conducted with the nude keratinocytes recombined with wildtype follicular papilla cells, the resultant hair follicles trait expressed the nude phenotype [45]. Hence, functional defect of nude keratinocytes cannot be restored by wild-type dermal cells. Consistently, primary keratinocyte cultures derived resulting Whn gene is expressed either from the epidermis or developing hair follicles [45].

Taking these studies together, whn activity appears specific to epithelial cells, but the location and timing of whn expression in the skin is not clear at all. The epithelial cells of the epidermis

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and hair follicles are similarly divided into proliferative and postmitotic compartments. During epidermal or follicular self-renewal, postmitotic cells originate from the proliferative populations, and the loss of the ability to multiply is accompanied by the initiation of terminal differentiation. While the precise characteristics of differentiating cells may vary depending on the location, the differentiation programs of the epidermis and hair follicles share certain features, including keratin accumulation and the eventual death of the cell [16]. The whn expression is present in many tissues during development and is also confined to the epithelial cells at different stages of maturation. In the epidermis and hair follicles, whn expression is associated primarily with the early stages of terminal differentiation but is also induced in a small subset of multiplying cells. Given this expression pattern as well as the effects of nude mutations, it is likely that whn influences the conversion of proliferative epithelial cells to postmitotic, differentiating cell resulting epithelial cell proliferation is enhanced and their terminal differentiation is disrupted in the absence of FOXN1, leading to epidermal thickening persistent anagen [16, 41].

2.4. The structural defects of nude hair shaft but hair bulb region entirely unaltered

According to hypothesis from Flanagan 1966 [17] and also supported by Mecklenburg et al. [30] defect of keratinization as the cause for hair shaft abnormalities, it could be found in nude mice exhibited with multiple fractures and locally twisted or thickened (**Figure 6**).

Indeed, in nude hair fibers, contains a reduced sulfur concentration was confirmed by elemental X-ray microanalysis as also been supported by the concept of impaired keratinization as observed for the hair shaft abnormalities [46]. Hair follicle ultrastructural analyses exposed that the cuticle of the IRS and the cuticle of the hair shaft are filled up by abnormal globular aggregates, that the hair cortex is fragmented into irregular cornified material, and that the hair medulla is partially lacking [32]. Whereas the Henle and Huxley layers of the IRS are normally keratinized in both nu/nu and wildtype mice, both the IRS cuticle and the cuticle of the hair shaft are fragmented into globular amorphous structures [29, 32]. A similar fragmentation is observed within the hair shaft cortex [32].

Figure 6. Short and sparse hair bending and exhibited with multiple fractures and locally twisted or thickened. Digital images were acquired using Kong, Bom-Viewer Plus software at 80×, 300× magnifications.

This possibly cause of this results is a complete lack of hair follicle keratin gene in nu/nu mice, particularly mHa3 a mouse ortholog of human acidic hair Keratin gene, which is normally expresses in the IRS and the hair shaft cortex of [47, 48]. Whereas previously thought that the hair shaft medulla is normal in nu/nu mice [30, 32], this observation has recently been challenged based on new experimental evidence [49]. According to Johns et al., the medulla of nu/nu hair shafts is less septulated than in heterozygous or wildtype animals, possibly due to a reduced expression of the adhesion molecule Desmocollin-2 in keratinocytes of the hair shaft medulla [49]. Taken together, these investigations support the concept of an impaired keratinization, both within the hair shaft and its cuticle and within the IRS, although the precise underlying molecular defects are still ill defined.

In spite of the great morphological changes of the hair shaft infundibulum, but the hair bulb area still remains entirely unaltered. Although the in some areas of the body the number of hair bulbs may be decreased [50], but in general, nude mice hold on the equal number of hair bulbs as normally hairy mice [51]. Dermal papilla fibroblasts and keratinocytes of the hair follicle matrix are also basically unaltered [51].

A loss-of-function mutated single gene, designated Whn nude gene expression analysis was conducted by using the Beta-Galactosidase activity in the morphogenesis of hair follicle and cycle: According to [16] Lee et al., during the anagen stage Whn expression was profoundly expressed in the precursor cells, hair cortex, ORS; in catagen follicle expression was terminated in the degenerating parts of the follicles, but remained and expressed surrounding the club; in telogen Beta-Galactosidase was expression level was very low and it was in the isthmus. Subsequently following the next hair cycles no significant differences was observed compared to the first hair cycle [16]. The nude hair follicle generally lacks a hair cortex containing the most of the hair's pigment melanin [32]. Therefore only little Beta-Galactosidase activity was detected in the differentiating hair shaft. In all other expression domains gene transcription was not affected significantly [16].

3. The physiological and genetic processes associated with nude hair follicle disorder

The hair follicle abnormalities in nude mice phenotype in the skin results from abnormal keratinization, possibly reduced synthesis of keratin protein. As the hair is the highly keratinized tissue and the final product is postnatally observed both in mouse and human. Research found that several kinds of hormonal changes may also as a cause of the nude phenotype; as this evident found in female nude mice with decreased concentration of progesterone, estradiol, prolactin, and thyroxin [51, 52].

The FOXN1 protein is evolutionarily highly conserved [53] and its analogue in *Drosophila* spp. possesses essential functions throughout the development [54, 55]. Foxn1 like transcription factor genes have been conserved in single copy throughout an animal of the large phylum *Chordata* [56]. Naturally occurring mutations in human and mouse Foxn1 genes shows very identical genomic structures with the location, phase, number, and sizes of introns as well as

also shows similarity index 85% for Foxn1 proteins [14]. DNA-binding domain and transactivation domain are similar. The mouse Foxn1 gene is localized on chromosome 11 [13, 14, 57–60] and is composed of nine exons, of which exon 1 is non-coding [14]. Altogether, six different spontaneously arisen allelic mutations in the mouse nude gene, all characterized by the lack of fur development and thymic agenesis. The original nude mouse phenotype (Foxn1nu) is caused by a single base pair deletion in exon 3. This deletion leads to a frameshift mutation and a premature stop codon, resulting in a protein that is predicted to lack the DNA-binding domain of the Foxn1 protein [12]. The 'winged-helix' structure of the Forkhead protein domain contains N-terminal DNA-binding domain and a C-terminal transcription activating domain [33]. Separation of both domains leads to a loss of function, while function is regained after they are linked noncovalently, representing that structural integrity and physical proximity of both domains are necessary for transactivation [43]. Human and mouse FOXN1 proteins have 85% sequence homology [53]. Very recently a human nude mutation was described [18]. Phenotypically, the affected person resembles the murine defect, i.e. she lacks hair and a thymic shadow upon X-ray examination, has dystrophic nails, and showed immunological abnormalities that have been overcome by bone-marrow transplantation. On the molecular level the defect is characterized by a homozygous nonsense mutation (R255X) in exon 5 of the WHN gene resulting in the absence of DNA-binding and transactivation domains. Forkhead factors mostly bind to DNA as monomers [61], however cases of homodimers [62] and heterodimers [63] have also been documented. Forkhead proteins also interact with non-transcription factor proteins such as coactivators, co-repressors, enzymes and other proteins. Furthermore, some Forkhead proteins are also subject to many posttranslational modifications such as phosphorylation, acetylation, methylation, and ubiquitination [64]. These post-translational modifications affect binding affinity and specificity of their target Forkhead proteins, their nuclear localization and even stability of some of these transcription factors. Finally, Forkhead proteins act as effector molecules for several signaling pathways, converting extra-cellular signals to changes in gene expression [64].

The FOXN1 protein is expressed exclusively in epithelial cells. This is in line with observations from hair reconstitution grafting assays: If wildtype dermal papilla cells are recombined with nude keratinocytes, hair follicles of the nude phenotype develop, suggesting that *Foxn1* activity is specific to epithelial cells [45]. Expression of the *Foxn1* express gene, the subsequent *Foxn1* mRNA, and the FOXN1 protein can be detected as early as day 13 of gestation in the developing nasal region [16]. On the 16th day of gestation, *Foxn1* is expressed in the suprabasal epidermis. It cannot be found in the hair bud, the first stage of hair express follicle development, but becomes detectable in a conical region above the bulbar matrix. Within the more mature hair follicle and in all anagen hair follicles, *Foxn1* is transcribed in the supramatrical region, in the hair shaft, and in the inner and outer root sheath [16]. FOXN1 is possibly involved in regulating the balance between epithelial cell growth and differentiation [16, 45]. This is in line with several observations that keratinocytes from nude mice have an increased propensity/tendency to differentiate abnormally and that the FOXN1 protein can specifically suppress the expression of differentiation-responsive genes in keratinocytes [25, 45]. Even in the hair follicle, expression of the *Foxn1* gene and its subsequent translation appear to correlate with the onset of terminal differentiation, although (FOXN1) has occasionally been found in some proliferating cells of the basal epidermis, the outer root sheath, and the hair

follicle matrix [16]. During hair follicle regression (catagen), *Foxn1* expression is lacking in the regressing epithelial compartment but remains in keratinocytes surrounding the developing club hair and is retained in some cells of the isthmus region during telogen [16]. Epithelial cell proliferation is enhanced and their terminal differentiation is disrupted in the absence of FOXN1, leading to epidermal thickening and persistent anagen [41]. *Foxn1* targeted genes are believed to (i) promote the differentiation of *Foxn1*-expressing keratinocytes and (ii) stimulate cell proliferation of neighboring cells via a paracrine mechanism [41]. Changes in gene expression keratinization are indeed associated with FOXN1 malfunction. Recently, the gene for a novel serine protease was shown to be overexpressed in nude mouse skin. However, its upregulation is probably an indirect consequence of the differentiation defect in the nude mouse hair follicle rather than a direct effect of FOXN1 signaling. The role of this novel gene in skin physiology and pathology has not been clarified to date [65]. *Foxn1* mRNA and the mouse ortholog of human acidic hair Keratin gene 3 (*KRTHA3*, hereafter *mHA3*) mRNA are coexpressed in hair follicles, nails, and papillae of the tongue, as it is critical for reliable prediction of gene function. In nude mice *mHa3* expression is completely absent in pelage hair follicles, indicating that *Foxn1* malfunction leads to a loss of expression of keratin genes [48, 65].

Although the transcriptional control of Foxn1 expression has not yet been completely elucidated, however Wnt glycoproteins regulatory signal is critical to regulate thymic function in the epithelial Foxn1 expression in both autocrine and paracrine fashions [66]. Therefore, genes including WNT, SHH, signal transducer and activator of transcription 3 that may be able to initiate anagen onset. Also some other genes that can extend to maintain the anagen stage, such as FGF7 and WNT are the potential candidates. In addition, activating genes that can increase the size of the hair follicle, such as SHH, or inhibiting genes that control anagen catagen transformation: such as FGF5 might be the key to success for future gene therapy research. Finally, it would be very useful if there is a complete loss of hair follicles, restoring the formation of entirely new follicles as it is similar to the occurrence during hair follicular embryogenesis [67].

4. Past, present and future therapeutic approach: from gene therapy to pharmacology

To design therapeutic approaches, the most effective way to restore hair would be to reactivate the miniaturized hair follicles and set back to normal cycle. As our understanding gene therapies might be designed to improve alopecia on the basis of polygenic approach.

In 1995, Hoffman et al. first demonstrated topically applied selective gene therapy on targeted mouse hair matrix cells with a liposome-entrapped lacZ reporter gene [68]. Topical administration of highly selective nature liposomes composition (containing the vector) for targeting the hair follicle are very effective and safe way at anagen onset and to increase the number of follicles [69, 70]. On the other hand, intradermal injection can also be considered to introduce plasmid DNA or viral vectors into the superficial dermis of hair-bearing skin. In a study, adenovirus vector was transfer the murine SHH DNA into the skin of postnatal mice and the authors concluded that localized overexpression of SHH in postnatal skin initiates the onset of anagen and thus acts similar to a biologic switch with no evidence of any pathologic abnormalities [71].

Based on its therapeutic effect the effectively deliver of a gene to the hair follicle, it must be either by an in vivo or an ex vivo method. The in vivo method is simple and direct, but has been shown to have only transient expression, usually; a plasmid or viral vector delivers the gene directly into the follicular keratinocytes. This can be done using a topical application of lipoplexed deoxyribonucleic acid (DNA), a liposome mixture containing the vector, or intradermal injection of vectors.

In contrast, in the ex vivo method, genes are introduced during the tissue culture. This method could give long-term gene expression because keratinocyte stem cells and progenitor cells can be manipulated and targeted. However, this is more technically demanding [72].

Various studies been shown that uncontrolled activation of SHH in the epidermis can cause basal cell carcinoma, and overexpression of Beta-catenin can cause either trichofolliculomas or pilomatrixomas [73]. Considering that hair restoration is a cosmetic procedure, therefore, highest diligence must be taken for the patient, to ensure any means of hair restoration is safe both locally on the scalp and systemically.

Certain clinically relevant pharmacological agents and drugs may also moderately overcome the absence of functional Foxn1 gene. As these agents may play active role for the development of new therapeutic tools to patients with hair growth disorders due to the absence of functional Foxn1.

Nude mice is very useful model system for studying a range of biological processes of skin and hair biology and various research reported that; cyclosporin A (CsA) [74, 75], keratinocyte growth factor (KGF) [76] and AS101 [77] are prospective therapeutic implements. Cyclosporin A (CsA), an immunosuppressive metabolite [78] induces macroscopically visible hair growth in nu/nu mice and isolated cultured Foxn1nu/nu mice vibrissae. Topical, oral, or subcutaneous administration of Cyclosporine induced hair growth, which was dose-dependent [74, 75, 79]. The underlying mechanism of Cyclosporin A on nu/nu hair follicles is still obscure. Since CsA stimulates hair growth in nu/nu speculate that, CsA partially restoring the inherited structural hair shaft disorders based on abnormal keratinization in absence of functional Foxn1. Recombinant keratinocyte growth factor (KGF) also known as FGF7 [80] been reported to stimulates hair growth in nude mice while injected intraperitoneally or subcutaneously. An increase in keratinocyte proliferation and normalization the morphology of nude hair follicle was observer under KGF treatment, probably by upregulating the expression of certain hair keratins [76, 81, 82]. It has been reported that the synthetic tellurium immunomodulator AS101 also activates the expression of KGF and able to induce hair growth in nu/nu mice via activation of the ras-signaling [77] pathway. Consequently, synthetic analogs of vitamin D3 (calcitrolis), which is also known to stimulate keratinocyte terminal differentiation, also stimulate hair growth in nu/nu mice associated with increased mRNA levels of hair keratin gene mHa1, mHa7, mHa8, and mHb3 [83]. The above mentioned pharmaceutical agents underlying mechanisms still remain obscure, however a putative molecular regulation of hair shaft keratinization have been shown to rescue of the nude phenotype with a proposed schematic presentation by Mecklenburg et al. [40] with the modification from Botchkarev [84].

Now new hair research has motivated on ethnopharmacognosy, a plant-derived natural product or their derivatives deliver tremendous prospects to discover novel therapeutic agents to replace synthetic drugs. As the chemically synthesized drugs and synthetic compounds is

known for their adverse side effects on human body. Various study reported that hundreds of plants or natural substances are prospective to stimulate hair growth. In particular, only little candidate plant plays vital role to enhance hair growth whose efficacy are now under investigation. Therefore, some plants those were traditionally acclaimed and purported in oriental medicine such as *Asiasari radix* [85], *Chrysanthemum zawadskii* [86], *Panax ginseng* [87, 88], and *Eclipta alba* [89, 90] exert to promote hair growth on C57BL/6 mice and rat.

Study has demonstrated that, *E. alba* might be considered an effective modulator to overcome defects in keratinocyte differentiation in the hair follicle of nude mice by stimulating the proliferation of epidermal basal cells and cells in the hair matrix [1, 91]. Based on these fruitful findings, the use of such a stimulatory agent may deliver a novel approach for the management of various forms of alopecia and may have clinical implications for hair loss.

5. Conclusions

For intractable alopecia, until now lot of research attempts have been made to develop or improve therapeutic approaches. While the prevalence of hair loss is ever increasing in the society but their effective treatment options are still limited. Therefore, future research endeavor is challenged for the modulation of the nude phenotype and need to definitively illuminate the target genes and regulate the function of Foxn1; a novel insights for the control of keratinocyte differentiation in epidermis. Researchers need to pay their attention to investigate the genes and signaling pathways underlying hair follicles to develop targeted therapies through comprehensive transcriptome analysis by using next-generation sequencing (NGS). At last, future research it is advised to exploit to correspondence all the novel findings for the development of innovative strategies and for the management of keratinization disorders by regulating Foxn1 targeted genes.

Conflict of interest

No conflict of interest.

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Chapter 3

Medical Treatment of Alopecia

Abdul Aziz Khalid

Additional information is available at the end of the chapter

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Abstract

Alopecia means partial or complete loss of hair from a part of the body where it exists naturally. It affects both men and women, and its treatment depends upon its cause, age of onset, and clinical presentation. It is divided into scarring and non-scarring alopecia. Scarring alopecia includes pseudopelade of Brocq, central centrifugal cicatricial alopecia, folliculitis decalvans, acne keloidalis nuchae, lichen planopilaris, frontal fibrosing alopecia and discoid lupus erythematosus, traumatic i.e., injury, radiation and post-operative scarring alopecia and certain neoplasms. Common causes of non-scarring alopecia are androgenic alopecia, alopecia areata, telogen effluvium, anagen effluvium, trichotillomania, traction alopecia, pressure-induced alopecia, alopecia due to iron deficiency, thyroid disease, and polycystic ovary syndrome. Topical remedies available are minoxidil 2 and 5%, topical & intralesional steroids, topical sensitization, anthralin, retinoids, tacrolimus, garlic, ketoconazole and prostaglandin analogs. Among systemic treatments, finasteride, steroids, immunosuppressant like azathioprine, methotrexate, sulfasalazine, zinc sulfate and iron are widely accepted. The phototherapies, photo-chemotherapies, platelet rich plasma (PRP) therapy, pharmacogenetics and hair transplant are new remedies for alopecia. It is concluded that minoxidil, finasteride, PRP, and hair transplant are the most widely being used modalities for alopecia.

Keywords: alopecia, scarring, non-scarring, minoxidil, finasteride, steroids, PRP

1. Introduction

Alopecia means partial or complete loss of hair from a part of the body where it exists naturally. It affects both men and women. According to cause it can be androgenic, autoimmune, traumatic, genetic, metabolic, and neoplastic. Clinically, it is divided mainly into two categories i.e., scarring and non-scarring alopecia. Proper history taking, the onset of disease, family history both paternal and maternal, past medical and surgical history, allergies to

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medicine, Physical examination, hair count, pull test, pluck test (trichogram), histological, serological and immunofluorescent data will lead us to proper diagnosis of the cause and its treatment plan. Biopsies provide diagnosis, the degree of hair shedding, type of hair, anagen to telogen ratio and provide information regarding the potential for hair regrowth. Many treatment modalities are available for treating alopecia i.e., medical and surgical. Medical treatments include minoxidil 2% and 5%, topical & intralesional steroids, topical sensitization/immunotherapy, anthralin, retinoids, tacrolimus, garlic, ketoconazole and prostaglandin analogs. Among systemic treatments, finasteride, dutasteride, spironolactone, flutamide, cyproterone acetate, sulfasalazine, corticosteroids, immunosuppressant like azathioprine & cyclosporine, methotrexate, biological agents, photochemotherapy, low-level light therapy, zinc, vitamins & supplements are widely being used. Platelet-rich plasma (PRP) therapy and hair transplant are very popular treatments now a days.

2. Topical treatment

Most common topical treatments are minoxidil, topical & intralesional steroids, topical sensitization/immunotherapy, anthralin, topical retinoids, tacrolimus, garlic, ketoconazole and prostaglandin analogs.

2.1. Minoxidil

Chemically, minoxidil is 2,4-diamino-6-piperidinopyimidine 3-oxide and it is being used as a topical agent for the treatment of androgenic alopecia since 1987. Mechanism of action is not exactly known, but it shortens telogen phase, extends anagen phase and is a hair growth stimulator. Scalp sulfotransferase converts minoxidil into minoxidil sulfate which is the active form of the molecule [1]. There are certainly proposed mechanisms of minoxidil actions are vasodilation, potassium channel opening, antiandrogen, angiogenesis, the release of growth factors, stimulation of dermal papilla, and immunosuppression [2, 3]. Topical minoxidil is available in spray and foam forms in two strengths i.e., 2 and 5%. Its half-life is 4.2 h and effects occur after 8 weeks and maximum after 4 months. It should be applied twice a day for at least 4 h to get good results. Micro-needling can be associated to enhance its efficiency. It will give good results if the age of alopecia is less than 5 years and hair follicles are not deeply miniaturized. Currently, Topical minoxidil is being used in patients with AGA, AA, hair transplant, scarring alopecia, hereditary alopecia/hypotrichosis, chemotherapy-induced alopecia, and monilethrix.

Adverse effects include allergic contact dermatitis; minoxidil induced telogen effluvium, skin irritation, and scaly changes in the scalp, isolated itching, localized or generalized hypertrichosis and hypotension. It is not advised in pregnant or breastfeeding mother.

2.2. Platelet rich plasma therapy

Platelet-rich plasma (PRP) therapy is a new medical technique widely being used in hair restoration especially in cases of androgenic alopecia and alopecia areata. To prepare PRP,

40–60 ml patient's blood is centrifuged for 10–20 min and 2–4 ml platelet rich portion is separated from the poor platelet plasma portion. It is injected locally in the areas of alopecia as multiple points in the dosage of 0.1 ml/cm^2 with 27–30 gauge needles with 4–6 mm length. It is repeated at 3–4 weeks intervals.

After activation, platelet alpha-granules release multiple growth factors; platelet-derived growth factor (PDGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), and interleukin-1 [4]. PRP acts on stem cells at bulge area and stimulates the growth of new follicle and neovascularization. No major side effects reported yet.

2.3. Topical and intralesional steroids

2.3.1. Topical corticosteroids

Topical corticosteroids are available in many formulations. These are divided into following categories by WHO according to their potencies [5]:

(I) Ultra high: clobetasol propionate 0.05%, diflorasone diacetate 0.05%.

(II) High: amcinonide ointment 0.1%, betamethasone dipropionate ointment 0.05%, desoximetasone cream/ointment 0.025%, fluocinonide cream ointment or gel 0.05%, halcinonide cream 0.1%.

(III) Betamethasone dipropionate 0.05%, betamethasone valerate 0.1%, diflorasone diacetate 0.05%, triamcinolone acetonide 0.1%.

(IV) Moderate: desoximetasone cream 0.05%, fluocinolone acetonate 0.025%. fludroxycortide ointment 0.05%, hydrocortisone valerate 0.2%, triamcinolone acetonide cream 0.1%.

(V) Betamethasone dipropionate 0.02%, betamethasone valerate 0.1%, fluocinolone acetonide 0.025%, fludroxycortide0.05%, hydrocortisone butyrate 0.1%, hydrocortisone valerate 0.2%, triamcinolone acetonide lotion, 0.1%.

(VI) Low: Betamethasone valerate 0.05%, desonide 0.05%, fluocinolone acetonide 0.01%.

(VII) Dexamethasone sodium phosphate cream 0.1%, hydrocortisone acetate 1%, methylprednisolone acetate 0.25%.

Topical corticosteroids promote maximum local action and minimum systemic side effects. The main mechanism of action is immunosuppression and reduction of local inflammation around dermal papilla [6]. Corticosteroids suppress the T-cell mediated immune action on the hair follicles.

Topical steroids are indicated in alopecia areata, alopecia areata incognita, lichen planopilaris, discoid lupus erythematosus, central centrifugal cicatricial alopecia, pseudopelade of brocq, frontal fibrosing alopecia.

Treatment should be continued up to 3 months to see results in case of alopecia areata. Topical steroids are less effective in cases of alopecia totalis and alopecia universalis. A local

telangiectasia and atrophy are seen in cases of high and ultra-high potent steroids and with long-term use of topical steroids.

2.3.2. Intralesional steroids

Intralesional steroids in alopecia areata are widely being used sine 50 years. They are the first-line treatment therapy if alopecia areata is involved less than 50% of scalp area. Following preparations are used:

- Triamcinolone acetonide: Concentration being used is 3–10 mg/ml and administering dose is 0.1 ml/cm^2 area.
- Triamcinolone hexacetonide
- Hydrocortisone acetate: Concentration being used is 25 mg/ml and administering dose is 0.1 ml/cm^2 area.

It is injected into and just beneath the dermis. For an eyebrow and face, lower concentrations 2–3 mg/ml should be used and repeated after every 3–6 weeks. Hair growth is appreciated in 4–6 weeks. Local atrophy is reported with high concentrations of triamcinolone acetonide. Severe cases of alopecia, alopecia totalis, alopecia universalis, rapidly progressing alopecia areata and cases of greater than 2 years' duration of the current episode respond poorly [7].

2.4. Topical sensitization/immunotherapy

Sensitizers are the chemicals which can initiate an allergic response and immunity on exposure to the body. An inflammatory response is mounted by the immune system upon further exposure. Happle, in 1980, described the mechanism of this inflammatory response in the treatment of Alopecia areata that topical sensitizers redirect the inflammatory response in AA away from the hair follicles and direct it towards themselves. This theory is unacceptable in all cases as regrowth has been observed at distant sites or even at opposite sides from sensitizer application, called as castling phenomenon.

Topical sensitizers should have following characteristics:

- It should be capable to produce immunomodulation.
- It should not be present in the normal natural environment as this may cause damage to a person in his/her daily life exposures.
- It should not have cross-reactivity with other substances.
- It should be a safe chemical.

Followings are the common sensitizers being used:

- Dinitrochlorobenzene (DNCB)
- Squaric acid dibutylester (SADBE)
- Diphencyprone (DPCP)

There are a variety of mechanisms of action of topical sensitization/immunotherapy:

- A decrease in CD4 to CD8 lymphocytes count from 4:1 to 1:1 is seen [8].
- The concept of 'Antigenic Competition' was proposed by Happle et al., that an allergic reaction is generated which activates suppressor T cells that non-specifically inhibit the immune reaction against a hair follicle. Topical Immunotherapy reduces the abnormal expression of class I and II MHC molecules [8].
- Delayed-type hypersensitivity reactions to unrelated antigens occurring at remote sites are reduced significantly.
- Topical sensitizers attract a new population of T cells into the treated areas and thus helping in clearance of putative follicular antigen.
- Theory of 'Cytokine Inhibitor' was proposed by Buckley and Vivier that there was a possible interference of contact allergens with the pre-existing pro-inflammatory cytokine, and their continued production by follicular keratinocytes [8].
- The decrease in the raised interferon Y levels and increases in mRNA expression of interleukin 2, 8, 10 and tumor necrosis factor-alpha in the lesional skin are seen.

Topical sensitizers are usually used in severe cases of Alopecia areata involving more than 50% of total scalp having large patches or alopecia totalis. Overall topical sensitizers are well tolerated. These are usually applied at the scalp. The initial concentration of DPCP is 0.001–0.1% and is applied with a cotton-tipped applicator to an area of at least 10 cm^2. The patient is advised not to wash the area and avoid sunlight for first 48 h. The application is repeated weekly to induce mild contact eczema and concentration is adjusted according to response. It can take more than 5–7 days to see the significant eczematous response which indicates that sensitization has taken place. Total hair growth rates are 77% with DPCP and 64% with SADBE [9].

Side effects are persistent dermatitis, painful cervical lymphadenopathy, generalized eczema, blistering, contact leukoderma and urticarial reaction. Fever, arthralgia and yellowish discoloration of gray hair are noted with DNCB [10].

2.5. Anthralin

Anthralin (1,8-dihydroxy-9-anthrone), also called as dithranol, is a natural anthraquinone derivative obtained from the Araroba tree in Brazil as an old antipsoriatic agent but later on found effective in alopecia areata too. It inhibits the proliferation of keratinocytes, prevents the action of T-cells, and improves cell differentiation through blocking DNA synthesis and mitochondrial dysfunction [11]. Anthralin suppresses the release of IL-6 and TNF-alpha from monocytes. A cosmetic response was seen in 25% of patients with severe alopecia areata treated using 0.5–1% anthralin cream in an open study [12]. In another study, only 11% cosmetically acceptable response observed in 51 patients of alopecia areata treated with a combination of 5% minoxidil and 0.5% anthralin [13]. Anthralin should be used with high enough concentration and in daily repeated applications to get a mild irritant reaction to get

effective results. Severe irritation and staining of clothes and skin are most common side effects.

2.6. Topical retinoids

Retinoids are a group of medicines derived from vitamin A. Common topical retinoids are tretinoin, isotretinoin, adapalene, alitretinoin, tazarotene, and bexarotene. Initially, retinoids were used for the treatment of acne but now a day due to their role in the induction of T-cell apoptosis, they are being used in autoimmune alopecia like alopecia areata as we know perifollicular and intrafollicular monocyte infiltrates contain primarily activated CD4+ and CD8+ T-cells.

Hanson and colleagues note that topical bexarotene yielded significant hair regrowth when used to treat patients with follicular mucinosis of folliculotropic Mycosis Fungoides [14]. Retinoids regulate transcription signaling through the Retinoic Acid Receptor (RAR)-y, RAR-B and RAR-a thus inhibiting proliferation and normalizing differentiation [15]. Topical retinol also increases blood flow to hair follicles and encourage new blood vessel formation [16]. A 55% response in alopecia areata is seen with 0.05% topical tretinoin as compare to 75% with topical betamethasone dipropionate and 35% with 0.25% dithranol paste [17].

Yoo et al. investigated the combined effects of minoxidil and retinol on human hair growth in vitro and on cultured human dermal papilla cells (DPCs) and epidermal keratinocytes (HaCaT) and this combination promoted hair growth, hair shaft elongation than minoxidil alone [18].

Common side effects are irritation, dryness, stinging sensations, redness, swelling, peeling, blistering and sunburn in the treated area and can aggravate eczema. Retinoids are contraindicated in pregnancy and breastfeeding as negative animal studies are not always predictive of human response.

2.7. Tacrolimus

Tacrolimus is a calcineurin inhibitor, and is being used for an immunosuppression since its discovery in 1987 from a Japanese soil bacterium, *Streptomyces tsukubaensis* [19]. It is also known as Fujimycin or FK506. The name tacrolimus is derived from "Tsukuba Macrolide Immunosuppressant".

Topical tacrolimus is mainly used in scarring alopecias like lichen planopilaris (LPP), central centrifugal cicatricial alopecia, discoid lupus erythematosus (DLE), frontal fibrosing alopecia, pseudopelade of Brocq.

Calcineurin, a serine–threonine phosphatase is activated via calmodulin by intracellular calcium which is increased by activation of the T-cell receptor in T-cell. Calcineurin then dephosphorylates the nuclear factor of activated T-cells (NF-AT), which moves to the nucleus of T-cell and increases the activity of genes coding for IL-2 and related cytokines [20].

Tacrolimus by binding itself with an intracytoplasmic protein, FK506, blocks the dephosphorylation of the nuclear factor of an activated T-cell thus preventing entry of nuclear factor into the nucleus and limits lymphocyte proliferation, which is the predominant feature of scarring alopecias [21].

Topical tacrolimus is available if different potencies i.e., 0.1, 0.03 and 0.3%. Regrowth of terminal hair was noted in the outer region of a DLE patient with a monotherapy of topical 0.1% tacrolimus for 2 months [22]. Mild to moderate lichen planopilaris (LPP) involving less than 10% of the scalp is best treated with tacrolimus [23].

The adverse effects of topical tacrolimus are uncommon but some reported are peeling and burning at the area of application. Systemic absorption is undetectable even after topical use of months [24].

2.8. Garlic

Garlic (*Allium sativum*) is a natural product species belonging to genus onion. The raw garlic is composed of 59% water, 33% carbohydrates, 6% protein, 2% dietary fiber and less than 1% fat [25, 26]. It is also rich in calcium, vitamin C, phosphorus, sulfur, zinc, and selenium. Besides its use in alopecia areata (AA), androgenic alopecia (AGA), and scarring alopecias, it is also used for the treatment of cardiovascular disorders, cancers, and the common cold.

Topical garlic is very effective for alopecia areata in children [27]. The combination of topical garlic gel and betamethasone valerate cream was found more effective than betamethasone valerate cream monotherapy in the treatment of localized alopecia areata [28].

Garlic, as well as being good for our body, can be really good for our scalp and hair. Followings are the possible proposed mechanisms of action:

- It provides nourishment to hair follicles especially if the nourishment is blocked by dihydro-testosterone (DHT) or bad diet.
- Garlic can stimulate the flow of blood to the scalp, thereby nourishing the hair, and thus encouraging hair to grow and strengthen. Garlic is also good at adding body to hair, as well as giving hair a nice gloss.
- It contains antiseptic properties and was used as antiseptic in World War II [29].
- It has antifungal properties and anti-inflammatory properties.
- Garlic reduces platelet aggregation [30].
- Hair, nail, and skin have high levels of sulfur and garlic is a rich source of sulfur.

Apply garlic gel/crushed garlic clove and rub it into the area of hair loss 60 min before you go to sleep and wash the area in the next morning. Common side effects include irritation at the site and with systemic absorption, bad smell from mouth and breath may occur.

2.9. Ketoconazole

Ketoconazole is an imidazole antifungal being used mainly in seborrheic dermatitis and lichen planopilaris and has anti-inflammatory properties too. Some hypothesize that ketoconazole plays a role in the local disruption of the DHT pathway. These anti-androgen effects may explain the side effect of gynecomastia in male patients taking oral ketoconazole. Combinations with minoxidil and oral finasteride have shown comparable hair regrowth in both groups than patients without ketoconazole shampoo.

2.10. Prostaglandin analogs

Prostaglandin analogs are synthetic drugs which bind to specific prostaglandin receptor sites on cells to initiate the certain type of cellular activities. Most common are bimatoprost, latanoprost, travoprost, and tafluprost. Initially, they were used to treating glaucoma and eyelashes hypertrichosis and pigmentation notices in lashes and periocular area. They are being used for the treatments of androgenic alopecia, alopecia areata, chemotherapy-induced alopecia, vitiligo and hypopigmented scarring [31]. The extension of the duration of anagen phase and the induction of telogen follicles into anagen phase are supposed mechanisms of action of prostaglandin analogs [32]. The bimatoprost 0.03% lotion used in a mice study and demonstrated a significant proportion of hair regrowth in 14 days [33]. Increased follicular growth rate, number of anagen follicles, and the total number of hair were seen in a study of bimatoprost on cultured scalp cells in patients with androgenic alopecia [34]. Blume-Peytavi et al. demonstrated a significant increase in hair density with 24 weeks use of latanoprost 0.1% at frontotemporal androgenic alopecia [35].

Bimatoprost and latanoprost were used by Ross et al. and Rosoborough et al. respectively in cases of alopecia areata but did not display encouraging results even after use of 4 months. Ochoa et al. suggested some benefit in less extensive cases of alopecia areata [36]. Besides local erythematous reaction, no adverse events are reported yet.

3. Systemic treatment

As we know, there are many varieties of alopecia ranging from non-scarring alopecia like AGA, AA, alopecia associated with iron deficiency, chemotherapy and thyroid diseases to scarring alopecia like pseudopelade of Brocq, central centrifugal cicatricial alopecia, folliculitis decalvans, acne keloidalis nuchae, lichen planopilaris, frontal fibrosing alopecia, discoid lupus erythematosus (DLE), traumatic (injury, radiation and post-operative scarring alopecia) and certain neoplasms so, in many cases only topical treatments are not so much fruitful thus systemic remedies reserve their place in the treatment of alopecia. Available systemic treatments include anti-androgens (finasteride, dutasteride, spironolactone, cyproterone acetate, flutamide), systemic steroids, sulfasalazine, immunosuppressants (azathioprine & cyclosporine), methotrexate, biological agents, photochemotherapy, low-level light therapy, excimer laser & excimer light, vitamins & supplements, pharmacogenetics, wigs, hairpieces, and camouflages.

3.1. Finasteride

Finasteride, 17B – (N-tert-butylcarbamoyl)-4-aza-5a-androst-1-en-3-one, is a synthetic and rostane steroid and is analog of androgen steroid hormones like testosterone and DHT. It is a selective and competitive inhibitor of the two isozymes of 5a-reductase, type II and III, present in certain tissues like prostate, seminal vesicles, epididymis, skin, and hair follicles and responsible for the conversion of testosterone in into DHT [37, 38]. The type II 5a-reductase isozyme is also responsible for two-thirds of circulating DHT. Its half-life is 5–6 h in an adult, metabolized in liver, and metabolites are eliminated 57% in the feces and 40% in urine [39].

Finasteride has been shown to increase the ratio of anagen to telogen hairs. It is effective in increasing more hair weight than hair count [40]. Five-year results with 1 mg oral finasteride per day in men with balding vertex showed significant greater hair count than placebo after 1 year, hair growth peaked at 2 years and still stayed above baseline for 90% of patients after that [41]. A randomized placebo-controlled trial with finasteride 1 mg/day in men showed increased hair growth in men with modified Norwood-Hamilton grades II, II vertex, III or III vertex throughout the second year of the study [42].

Approximately 2% of men reported one or more sexual side effects like decreased libido, erectile dysfunction and ejaculation disorder. It is found that rate of these side effects became indistinguishable from a placebo after 2–4 years and usually got better with time. Gynecomastia and mastalgia are reported in 0.4% of patients. Some cases of depression anxiety and suicidal thoughts are also reported with finasteride.

3.2. Dutasteride

Dutasteride is a 5a reductase which inhibits all three forms of 5a reductase i.e., type I, II and III in certain parts of the body like prostate and scalp leading to 98% reduction in DHT levels [43]. It is being used for the treatment of androgenic scalp hair loss in South Korea since 2009 and in Japan since 2015 but in the USA it is often used off-label. In addition to inhibition of 5-alpha-reductase, it also inhibits neurosteroidogenesis from testosterone which contains antidepressant, anxiolytic and pro-sexual effects thus sexual dysfunction and depression have been seen with 5-alpha-reductase inhibitors like dutasteride [44]. It is three times more potent than finasteride in preventing 5 alpha-reductase type II and more than 100 times in type I [45]. Its approved a daily dose is 0.5 mg/day, half-life 4–5 weeks, metabolized in the liver and excreted mainly in feces. It is contraindicated in pregnant women. Side effects include decreased sperm count, decreased semen volume and reduction in sperm motility, gynecomastia, menstrual changes, and acne.

3.3. Spironolactone

Spironolactone is a synthetic steroid being used in the treatment of hypertension and different cardiovascular disorders since long time and found having antiandrogen properties. Spironolactone does not affect 5-alpha-reductase but acts by competitively blocking androgen receptors in prostate and scalp. It also weakly inhibits testosterone synthesis. It is used in

patterned hair loss and the women treated for 1 year with spironolactone 50–300 mg/day showed less hair loss than the untreated group [46]. Side effects are dose-dependent and mainly include menstrual irregularities, postmenopausal bleeding, breast tenderness or enlargement and fatigue. The combination with oral contraceptives reduces its hormonal side effects. No evidence of increased incidence of breast cancer found in women treated with spironolactone [47].

3.4. Cyproterone acetate

Cyproterone acetate, a potent progestin, is an androgen receptor blocker which has anti gonadotrophic effects too. Effects of cyproterone acetate are more prominent in women with hyperandrogenism. Commonly dosages being used are 50–100 mg/day for the first 10 days of each menstrual cycle while for postmenopausal women; it can be continuously given with or without estrogens. Side effects include weight gain, breast tenderness, and loss of libido, depression, and nausea. Feminization of male fetus is seen if cyproterone acetate is used during pregnancy or if a woman becomes pregnant while taking it.

3.5. Flutamide

Flutamide, a non-steroidal antiandrogen, inhibits androgen uptake and nuclear binding of androgen within the target tissue. Premenopausal women with female pattern hair loss (FPHL) got good results with flutamide but in postmenopausal women, results were not more than placebo with 1 mg/day dose [48]. Most common side effects are hepatotoxicity and teratogenicity.

3.6. Systemic steroids

Systemic steroids are used in non-scarring alopecia like Alopecia areata and in scarring alopecia like variants of follicular lichen planus i.e., lichen planopilaris, frontal fibrosing alopecia and DLE. With prednisone 0.5–1 mg/kg/day for 1–6 months, more than 25% hair regrowth was seen [49, 50]. There are certain regimens other than daily dosages for systemic steroids like mini pulse and pulse therapy. In a study, patients receiving prednisolone 200 mg once a week (pulse therapy) for 3 months showed better hair regrowth at 6 months than placebo [51]. Mini pulse therapy, tried in many skin diseases like alopecia areata and vitiligo, employs administration of high dose oral corticosteroids on two consecutive days every week with a 5 days' gap between the two pulses.

3.7. Sulfasalazine

Sulfasalazine was approved in the USA in 1950 and was considered a first-line therapy in rheumatoid arthritis, ulcerative colitis, and Crohn's disease and being used in psoriatic arthritis, reactive arthritis, and alopecia areata. Sulfasalazine and its metabolites have immunosuppressive, antibacterial, anti-inflammatory effects and inhibit the cysteine-glutamate

antiporter (a system which imports the amino acid cysteine into cells) [52]. Around 90% of sulfasalazine is metabolized in the colon by bacteria into a sulfapyridine and mesalazine and then most of the sulfapyridine is absorbed and further metabolized and eliminated in urine. The starting dose is 500 mg twice daily and gradually increased up to 1 g three times a day. In one study, 23% of patients of alopecia areata showed a good response with sulfasalazine therapy [53].

3.8. Immunosuppressants (azathioprine & cyclosporine)

Alopecia areata, a common non-scarring alopecia and scarring alopecia like follicular lichen planus are T-cell mediated autoimmune processes; therefore, immunosuppressive therapies are widely used in the treatments of alopecia. Most common immunosuppressants are azathioprine and cyclosporine.

Azathioprine was made in 1957 and was the most effective and safe medicine being used to prevent rejection following organ transplant, autoimmune diseases including rheumatoid arthritis, pemphigus, systemic lupus erythematosus, Behcet's disease, vasculitis, autoimmune hepatitis, atopic dermatitis, myasthenia gravis, reactive lung diseases, alopecia areata, and follicular lichen planus. Azathioprine inhibits purine synthesis, needed to produce DNA and RNA, which are necessary for the production of white blood cells, thus causing immunosuppression A prospective study of adult patients with recalcitrant alopecia areata universalis (unresponsive to oral corticosteroids) treated with oral 2.5 mg/kg/day for 6 months showed 52.3% mean global hair regrowth [54]. Side effects include nausea, vomiting, skin rash, acute pancreatitis and bone marrow suppression.

Cyclosporine lowers the activity of T-cells by preventing the mitochondrial permeability transition pore from opening and inhibiting calcineurin-phosphatase pathway. A study of adult patients (6 men, 1 woman) of alopecia universalis with oral cyclosporine 6 mg/kg/day for 12 weeks showed cosmetically acceptable hair regrowth in 50% of patients [55]. Side effects of cyclosporine are gum enlargement, increased hair growth, convulsions, peptic ulcer, pancreatitis, increased cholesterol, numbness in lips and high blood pressure.

3.9. Methotrexate

Methotrexate is an anti-metabolite agent being widely used in chemotherapy and immunosuppression. It is mainly used in cases of cancers of breast, head & neck, bladder, blood (leukemia, lymphoma), bones (osteosarcoma) and trophoblastic neoplasms. It is taken by oral or intravenous route in weekly doses and acts by blocking the body's use of folic acid. A study of 31 patients of alopecia areata with 10–25 mg/week methotrexate showed greater than 50% hair regrowth in 67.7% of patients [56]. In another study, Joly reported good results in 22 patients with alopecia areata, with or without systemic corticosteroids [57]. A further study of 31 patients of alopecia areata, with 10–20 mg/week methotrexate with and without 1 mg/kg/day oral prednisone taper, showed overall 71% response rate in 1–3 months and 36% of treated patients got complete hair regrowth in 6–18 months [58].

3.10. Biological agents

Biological agents are thought to act on tumor necrosis factor (TNF)-alpha and inter-cellular adhesion molecules 1 in the treatment of alopecia areata. Common biological agents are etanercept, efalizumab, alefacept, adalimumab, and infliximab. Different clinical trials are done but no promising results gained yet.

3.11. Photochemotherapy

Both systemic and topical photochemotherapy is done by psoralen plus UV-A (PUVA) in the treatment of alopecia areata. In a study, the initial response was 20–73% having relapse rate 50–88%. It interferes with the presentation of antigen to T-lymphocytes by depletion of Langerhans cells. The recommended dose is 0.6 mg/kg and after 2 hours affected area is exposed to UV-A for 20–30 min. In a study of PUVA in alopecia areata, hair regrowth is seen up to 70% [59].

3.12. Low-level light therapy

Many products are available in the market utilizing low-energy laser beams but only one device called Hairmax Lasercomb (Lexington International, Boca Raton, FL, USA) has got 510K FDA approval for use in hair loss as a medical device. Significant hair regrowth is seen with a light device in the treatment of male pattern hair loss [60].

3.13. Vitamins & supplements

There are many products available in the market as vitamins and supplements but not promising trials available. Commonly in use products are Saw Palmetto, biotin, zinc, and iron. Saw Palmetto inhibits the 5-alpha-reductase conversion of testosterone to DHT in the prostate [61]. A study of androgenic alopecia patients treated with Saw Palmetto showed increased hair growth [62]. Biotin is used to treat onychoschizia but no clinical trials are available in hair loss. Significant hair regrowth is seen with 30–50 mg/day zinc gluconate in patients of alopecia areata [63].

3.14. Pharmacogenetics

Pharmacogenetics is an emerging medical field which provides information about inherited genetic differences in metabolism, effects and adverse effects of a drug in genetically susceptible individuals. It is found that AGA has polychromosomal as well as polygenic origin associated with increased levels of androgen receptors and 5-alpha-reductase in both men and women. AR-DHT complex interacts with genomic DNA and initiates such cellular mechanisms that lead to AGA. By genetic studies, it is found that variations in the AR genes are present on the long arm of the X chromosome and polymorphisms of the AR gene are associated with hormonal and medical responses [64]. The repeat cytosine-adenine-guanine (CAG) nucleotide sequences in the exon 1 of AR gene are related with androgen sensitivity to the cell in men and women. Short CAG sequences are related with AGA. In pharmacogenetics,

there are two research points: Identification of potential therapeutic targets and individual variability in response to a specific drug. A strong correlation between the number of CAG repeats in AR gene and response to finasteride therapy is found administering finasteride 1 mg/day and variable follow up between 12 and 24 months. In this study, it was discovered that subjects with CAG repeat less than 21 showed greater response with finasteride than subjects having CAG repeats more than 23 [65]. In a study of chemotherapy-induced alopecia, a strong association in genetic variants near genes CACNB4 is found [66].

In the future, pharmacogenetics will help us to decide the treatments of an individual throughout its life about the drugs which can or cannot be used on the basis of metabolism, safety, and its effects. This analyzation of individual's DNA will be done only once and will provide information for the lifetime and as companies and laboratories that perform these testing can do so at low prices, the field of pharmacogenetics will gain great acceptance in the day to day medical treatments.

3.15. Hair transplant

In the hair transplant, follicular units are harvested from the safe zone at occiput and then implanted at bald areas. There are two popular methods i.e., follicular unit transplant (FUT) and follicular unit extraction (FUE). In FUT, a strip is harvested from safe zone and then follicular units are prepared by slivering the strip under a microscope. In FUE, follicular units are extracted one by one with 0.7–1.0 mm wide and 4–6 mm lengthy sharp or blunt punches which may be manual or motorized and then these follicular units are implanted at bald areas.

4. Conclusions

It is concluded that minoxidil, finasteride, and PRP are the best treatments for androgenic alopecia and alopecia areata while steroids, sensitizers, and immunosuppressants are mainly used in autoimmune alopecia. Hair transplant is the best option in cases of androgenic alopecia and stable forms of scarring alopecia. Pharmacogenetics is a newly emerging medical field which can change the future of medical treatments.

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Conflict of interest

I have no conflict of interest.

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Chapter 4

Pharmacological Treatment of Alopecia

Robert Gensure

Additional information is available at the end of the chapter

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Abstract

In this chapter, we will explore non-surgical treatments of alopecia. Unlike many other areas of medicine, pharmacological treatments for alopecia are relatively new. There are only two treatments which are approved by the Food and Drug Administration (FDA); the rest are drugs developed for other indications which have gained popular off-label use to promote hair growth. The reasons for this are many, including the designation of alopecia by the FDA as a cosmetic disease. This designation has restricted alopecia development programs to compounds with virtually no side effects. Unfortunately, it has also led to off-label use of far more dangerous compounds as alopecia treatments, without the benefit of controlled trials. There is a growing recognition that alopecia, particularly alopecia areata and chemotherapy-induced alopecia, are disorders which significantly alter the quality of life, similar to acne vulgaris and psoriasis, and merit treatment accordingly. There have also been several recent advances in our understanding of the hair cycle, revealing new targets for developing alopecia therapies. As a result, there is a more robust slate of programs for developing new pharmacological treatments for alopecia. In this chapter, we will review current pharmacological treatments for alopecia and selected treatments under development (i.e., those with significant preclinical or clinical data which have appeared in the published literature).

Keywords: alopecia, pharmacology, minoxidil, finasteride, glucocorticoid, diphenylcyclopropenone, DPCP, tofacitinib, ruxolitinib, bimatoprost

1. Introduction

While alopecia is a common problem, the lack of any direct health effects from hair loss has limited development of pharmacological solutions, mostly due to perceptions by regulatory authorities that there is no benefit to treatment and therefore there should be no side effects as well. Current therapies consist of repurposed drugs which were incidentally noted to have

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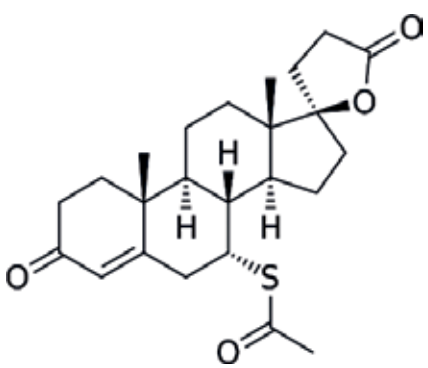
positive effects on hair regrowth and some therapies designed specifically to regrow hair. Most are applied topically to reduce the risk of systemic side effects, but this can be an inconvenient method of application if the hair loss is minimal, and often reduces the efficacy of the treatment. Insurers rarely provide coverage for such therapies. The resulting "benign but minimal efficacy" therapies have made little impact into the global problem of alopecia, and patients will often opt for surgical options with greater costs and risks, but promises of markedly improved results. In this chapter, we review current pharmacological treatments for alopecia and a selection of those under development (in late preclinical or clinical stage of development), including indications, mechanism of action, efficacy, and side effects.

2. Current pharmacological therapies for alopecia

Currently, there are only two Food and Drug Administration (FDA)-approved therapies for alopecia, minoxidil and finasteride. There are several other therapies commonly used in an off-label fashion as alopecia therapies.

2.1. Spironolactone

Spironolactone is a diuretic, which acts as an antagonist to aldosterone. Given the structural similarity of steroid hormones, spironolactone has also some limited androgen-blocking activity. While spironolactone is not specifically approved for use for hair loss, this weak androgen-blocking effect has led to the off-label use of spironolactone to minimize hair loss from polycystic ovarian syndrome (PCOS), usually in conjunction with oral contraceptive therapy [1]. As it is a weak androgen antagonist, it has not been found to be effective in treating androgenetic alopecia in males. Spironolactone is an oral medication taken twice per day.



Mechanism of action: spironolactone reduces hair loss by acting as a competitive antagonist at the androgen receptor. While technically, this could serve to block all forms of androgen-induced hair loss, the blockade is not potent enough to provide visible effects in androgenetic alopecia in males. There are observed effects in hair growth patterns, however, in females with

polycystic ovarian syndrome. Spironolactone can improve growth of scalp hair in this condition. Spironolactone can also reduce terminal differentiation and hair growth in androgen-dependent regions of the body (face, arms, back, and abdomen) and thus serve as an effective treatment for hirsutism.

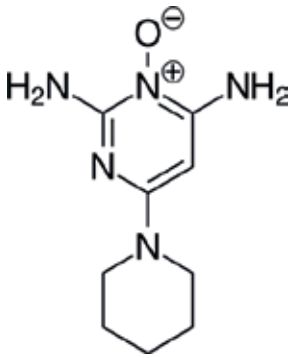
Side effects: spironolactone's primary clinical use is as a diuretic, and thus patients taking spironolactone for alopecia will experience an increased urine output. As spironolactone antagonizes the effects of aldosterone, potassium levels may become elevated and should be monitored while on therapy.

Clinical application: spironolactone's relatively weak effects at the androgen receptor make the treatment unsuitable as a therapy for androgenetic alopecia. Androgen levels are much lower in females with polycystic ovarian syndrome, and spironolactone therapy does result in some noticeable hair growth in some patients with this disorder. However, the overwhelmingly most prevalent indication for spironolactone therapy is not hair growth, but rather to prevent regrowth of hair in other androgen-dependent regions of the body as a therapy for hirsutism. Spironolactone is effective in reducing regrowth of hair in these regions, and, over time, can reverse terminal differentiation. However, as it does not induce shedding of existing hair, it can require up to 6 months for full effect and thus is often used as an adjuvant therapy to more traditional hair-removal techniques (shaving, depilating creams, laser therapy).

2.2. Minoxidil

Minoxidil (Rogaine) was originally marketed by Upjohn as a therapy for hypertension. It was noted during clinical trials that some male patients with androgenetic alopecia experienced regrowth of hair during the course of treatment. This led to an effort to repurpose the drug as a topical treatment for androgenetic alopecia, which was ultimately approved by the FDA for this indication under the branded name Rogaine. Minoxidil has been found to regrow hair in 40% of patients with androgenetic hair loss after 3–6 months of treatment [2]. Maintenance of this hair regrowth requires continued therapy. The treatment is most effective in younger patients with minimal hair loss. It is least effective if there is a broad region of hair loss.

Mechanism of action: minoxidil has no direct effects on the hair cycle; rather, it stimulates the vascular bed around the hair follicles and provides a more favorable environment for hair growth [3]. Specifically, the transition of hair follicles from a resting telogen phase to an active anagen phase, and maintenance of that anagen phase, depends on interactions between the bulb of the hair follicle and the vascular bed below. Minoxidil enhances these interactions by stimulating the proliferation of this vascular bed. Minoxidil does not directly stimulate hair follicles to transition to anagen phase, nor does it maintain hair follicles in this state. Minoxidil does not cause hair follicles to transition into terminal, or visibly pigmented hair.



Given this mechanism of action, it is not surprising that minoxidil has minimal effects in the setting of severe hair loss and has maximal effects when there are still ample normal cycling terminal hair follicles to support. As the treatment does not directly stimulate hair follicles to transition to the anagen phase or maintain them in this state, there are also reports that the resulting hair is thin, hypopigmented, and shorter than normal hair. The responses are not sustained, and without continued use, the hair is quickly shed.

Side effects: minoxidil was originally developed as an antihypertensive, and oral use could lower blood pressure and produce symptoms of dizziness, tachycardia, swelling, headache, and fainting. Topical application minimizes the risk of these systemic side effects. Other potential side effects include itchiness, rash, and allergic reactions.

While minoxidil is approved for use in androgenetic alopecia, only a small fraction of those with this disorder actually use minoxidil. The public is very aware of this treatment, likely the result of heavy marketing at the time of its release in 1988. The low-market penetrance appears to be the result of continuing costs in the setting of minimal efficacy for those who start therapy, or perception of minimal effects of treatment for those who do not.

2.3. Finasteride

Finasteride (Propecia) was developed by Merck as a therapy for androgenetic alopecia. The drug was developed as an oral antiandrogen, which acts by inhibiting the conversion of testosterone to its more potent form, dihydrotestosterone. The treatment has been shown to be effective in both androgenetic alopecia males; the treatment of alopecia for polycystic

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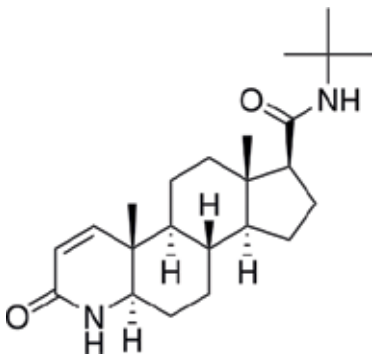
ovarian syndrome females appears to be less effective. Finasteride was approved for treatment of hair loss in the USA in 1997.



Mechanism of action: finasteride acts as an inhibitor of the enzyme 5-alpha reductase, which converts testosterone to the more potent form dihydrotestosterone. It is extremely effective and can lower levels by dihydrotestosterone by up to 70% in the bloodstream [4]. However, it does not inhibit or reduce testosterone levels, and some androgen effects on hair follicles remain with treatment. Androgens bind to testosterone receptors in stem cells in the bulge of the hair follicle. The complex binds and depletes intracellular beta-catenin. This inhibits the transition of the hair follicle from the telogen to anagen phase. By reducing levels of dihydrotestosterone, the most potent androgen, finasteride, can reverse these changes, allowing hair follicles to resume normal cycling with effects of visual hair growth. In regions of androgen-dependent hair growth (face, arms, abdomen, back), finasteride can reduce hair growth.

Side effects: as finasteride reduces androgen potency, it can lead to sexual dysfunction in males. These effects include a decreased sexual desire and impotency. These effects may be temporary as tolerance develops to the medication.

Finasteride is effective in reducing hair loss in males with androgenetic alopecia, resulting in approximately 30% increases in hair growth [5]. The effects are sustained as long as treatment is continued, and the hair loss resumes if treatment is stopped. As testosterone is still available to activate androgen receptors, the response is not complete. In women with polycystic ovarian syndrome, the effects on stimulating hair growth are more modest. The more pronounced effect on women with this disorder decreases in hirsutism. Similar to minoxidil, the only other FDA-approved therapy for androgenetic alopecia, finasteride, is used by only a small fraction of individuals for whom it is potentially indicated. The limited use appears to be related to limited overall effects on hair growth, requirement for continued therapy to sustain these gains, and the side effects of sexual dysfunction.

2.4. Oral contraceptives

Oral contraceptives are used in women with polycystic ovarian syndrome primarily as a means to regulate normal cycles. The treatments can sometimes complicate scalp hair loss and hirsutism further, as the progestins may have androgenic side effects. The use of oral contraceptives with reduced androgenic, and even some anti-androgen effects, together with the suppression of ovarian androgen production, can result in net improvements in scalp hair growth. Oral contraceptives are designed for use with polycystic ovarian syndrome, including Yasmin, Yaz, and Ocella (drospirenone/ethinyl estradiol).

Mechanism of action: in normal cycling females, the ovaries produce approximately 50% of androgen, the rest being produced in the adrenal glands. With PCOS, the anovulatory cycles reduce ovarian aromatization of androgens to estrogens, resulting in an increased ovarian androgen production. Oral contraceptives reduce FSH and LH stimulation to the ovaries, effectively reducing ovarian androgen production (but with adrenal androgen production unaffected). These net increases in androgen production, together with increases in serum estrogen levels, can slow or reverse some hyperandrogenic effects in PCOS, including scalp hair loss, hirsutism, and acne. Estrogen itself can improve scalp hair growth as well, having direct effects to make the hair thicker and more plusher. While some progestins, that is, norethindrone, can have some androgenic side effects which limit these effects, oral contraceptives designed for use with polycystic ovarian syndrome contain the progestin drospirenone, which has less androgenic effects. In fact, drospirenone has anti-androgen and anti-mineralocorticoid effects similar to spironolactone and can aid further in inducing regrowth of scalp hair and reduction of hirsutism.

Side effects: side effects of oral contraceptives relate mostly to the dose of estrogen. Estrogen can increase the risk of migraine headaches and deep vein thrombosis. Doses of estrogen in oral contraceptives designed for use in polycystic ovarian syndrome are lower, such that risks of these complications, while increased from pre-treatment levels, should not exceed those of normal cycling females. The progestin drospirenone has an increased risk for deep venous

thrombosis and blood clots beyond that from the estrogen component of the oral contraceptive, which has resulted in warnings to discontinue therapy with surgery [6].

Oral contraceptives are most effective at treating hirsutism and acne from PCOS. Improvements in scalp hair growth can be obtained from a reduced ovarian androgen production, direct effects of estrogen on hair growth, and with some oral contraceptives, the inhibition of remaining androgen effect via competitive blocking of the androgen receptor. Oral contraceptives do not reduce adrenal androgen production, and as such, the effects on scalp hair growth are incomplete. Oral contraceptives provide no benefit for other forms of hair loss.

2.5. Glucocorticoid therapy

Glucocorticoid therapy is used for the treatment of autoimmune alopecia, specifically alopecia areata/totalis/universalis [7]. It has no effect on androgenetic alopecia. Glucocorticoids are injected subcutaneously directly into the sites of hair loss and can require large number (i.e., >80) injections if hair loss is extensive. The response is low, with 30–50% of individuals showing a response in different patient series. This therapy is not approved by the FDA for this indication.

Mechanism of action: glucocorticoids are powerful anti-inflammatory components when given at high doses. Pulsed high-dose oral administration has been shown to be effective [8], but to minimize systemic side effects, the preferred route of administration is direct injection to the affected areas. Topical therapy has been found to be less effective [9]. Glucocorticoids do not directly alter the hair follicles, but the anti-inflammatory effects reduce the autoimmune disruption of anagen hair follicles which is characteristic of alopecia areata. Response rates are higher in milder forms of alopecia areata; it is likely that senescence of hair cycling and the autoimmune response, which is often seen with alopecia totalis and universalis, limits the efficacy of glucocorticoid therapy in the more severe forms.

Side effects: side effects of glucocorticoid injection result from systemic absorption, which is minimized but not eliminated with subcutaneous injection. Glucocorticoid administration can result in increased appetite and weight gain, peripheral muscle wasting, immune suppression, and osteoporosis. Chronic use can result in skin thinning and adrenal suppression, with the resulting risk of adrenal crisis with illness. In children, chronic administration can result in short stature.

Glucocorticoid injections provide a modest hair growth response in alopecia areata with an overall low risk of systemic side effects. Although not approved by the FDA for this indication, it is currently the primary therapy for alopecia areata. While side effects are minimized, the process of receiving multiple scalp injections is difficult, and many patients will decline glucocorticoid therapy for this reason alone.

2.6. Cyclosporin A

Cyclosporin A is an immune-suppressant drug used primarily to prevent the rejection of transplanted organs. As an immune suppressant, it has been used for the treatment of

autoimmune hair loss, specifically in alopecia areata/totalis/universalis. Cyclosporin also has direct effects to promote the cycling of hair follicles, making it more effective and potentially complementary to other immune-suppressant therapies. However, cyclosporine has severe side effects, including an increased risk of serious infections and cancer, hyperglycemia, and diabetes mellitus. As a result, it is not commonly used as an alopecia therapy.

Mechanism of action: cyclosporin is a power anti-inflammatory agent, which acts by blocking the transcription of cytokine genes in activated T-cells [10]. Cyclosporin complexes with cyclophilin and inhibits phosphatase activity of calcineurin, which in turn alters the activation of NFAT transcription factors. Cyclosporin also blocks antigen recognition pathways by preventing the activation of the JNK and p38 pathways.

Cyclosporin also acts directly on hair follicles, promoting transition from telogen to anagen phase and promoting hair growth [11]. This combined mode of therapy makes cyclosporin ideal as a therapy for alopecia areata. Cyclosporin can be used in combination with glucocorticoid therapy to improve responses [12]. The effects of cyclosporine do not persist if the therapy is discontinued.

Side effects: side effects from cyclosporine therapy are severe. The immune suppression is significant and can increase risks of a serious bacterial infection and certain cancers, particularly lymphoma. Monitoring for immune function is recommended on therapy. Cyclosporin also causes hyperglycemia and can cause diabetes mellitus.

While cyclosporine is theoretically an excellent choice for several forms of hair loss, including alopecia areata and androgenetic alopecia, the side-effect profile results in very limited use in clinical settings. As a telling example of this, the patient in the cited case report [12] is now deceased from pneumonitis.

2.7. Janus kinase (JAK) inhibitors (tofacitinib and ruxolitinib)

Janus kinase inhibitors have been developed primarily as an immune-suppressant therapy for rheumatoid arthritis. Primary research into the immune-signaling mechanisms for alopecia areata revealed that janus kinases are integral to these pathways, and janus kinases inhibitors have been shown to promote hair growth in alopecia areata. While there are specific janus kinase inhibitors in development for this indication, there is significant off-label clinical use of janus kinase inhibitors approved for rheumatoid arthritis for the therapy of alopecia areata. These agents have the highest reported response rates in the most severe forms, alopecia totalis and alopecia universalis. An initial report indicated that 75% of patients responded with at least 50% improvement in hair growth by SALT score after 12 months of therapy, although the study employed a small number of subjects [13]. A larger series show a more modest 33% of patients with >50% increase in SALT score at 6 months, which is still superior to other therapies [14]. While JAK inhibitors are effective, they are also expensive, and the hair growth is not maintained once the treatment is discontinued. They do not appear to be effective in other forms of alopecia, even to the point that treatment with a JAK inhibitor revealed unknown androgenetic alopecia in a patient who had alopecia totalis from early in life [14].

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Mechanism of action: there are three janus kinases, designated JAK-1, JAK2, and JAK3. Their primary substrates are the various STAT proteins, and they play a critical role in signaling of immune regulatory factors. JAK-2 has additional clinical importance as the primary signaler for growth hormone, through linking with STAT-5b; mutations in this STAT protein have been shown to cause growth hormone resistance and short stature. JAK inhibitors used for applications of hair growth target JAK-1 and JAK-3, which in turn inhibit STAT3. Inhibition of JAK pathways inhibits interferon-gamma signaling and inhibits signaling in CD8 + NKG2D+ T-cells, which have been shown to cause alopecia areata in mouse models [15]. There may also be some direct effect to promote hair cycling [16].

Side effects: JAK inhibitors have been found to cause elevated liver function studies and serious bacterial infections in trials for rheumatoid arthritis and psoriasis [17]. Studies in alopecia areata have not shown these side effects, but the total number of patients in those studies was much lower. Given the similarities in dosing and duration of treatment, it is likely that these same side effects will be observed with larger numbers of patients treated.

While JAK inhibitors provide excellent efficacy for hair growth in alopecia areata, the high cost, latency to effect (6–12 months), lack of durable effect, and side-effect profile are all potential concerns and limit current clinical use. In addition, JAK inhibitors do not appear to be effective in other forms of alopecia. Importantly, there are JAK inhibitors under development, including topically applied compounds, which may provide a more favorable side-effect profile and expand the use of these compounds in clinical practice.

2.8. Bimatoprost

Bimatoprost (Latisse) is a prostaglandin analog which was discovered to promote eyelash growth. It has been approved by the FDA for this indication. Its mechanism of action is to promote the entry of hair follicles into the anagen phase, and given this, there was promise that bimatoprost could be used as a more general treatment for various forms of hair loss.

However, clinical trials have been disappointing, and it appears that the effect is restricted to the eyelash region.

Bimatoprost was developed for therapy for open-angle glaucoma, under the trade name Lumigan. However, in clinical trials, it was noted to have a remarkable effect to increase the growth of eyelashes. It was repurposed for this indication and has been approved by the FDA and currently marketed for regrowth of eyelashes [18]. It appears to be effective across a variety of conditions, including chemotherapy, alopecia universalis, and normal individuals desiring thicker eyelashes [19]. It is applied topically to the eyelash region, and growth of eyelashes occurs rapidly.

Mechanism of action: bimatoprost is a prostaglandin F2a analog which inhibits prostamids. There are known links between prostaglandins and hair cycling, including prostaglandin D2 [20], and it appears that bimatoprost grows eyelash hair by transitioning hair follicles into the anagen phase. This results in rapid regrowth of eyelashes.

Side effects: as bimatoprost is applied topically to eyelash area directly, there are minimal reports of systemic side effects. Local side effects include conjunctivitis and blurry vision. There are additional off-target benefits, a decreased intraocular pressure, based on its original development as a therapy for open- angle glaucoma.

While bimatoprost is very effective at promoting the growth of eyelashes, its effects are minimal for promoting hair growth outside the eyelash region, with phase 2 trial showing a similar or only slightly superior efficacy to minoxidil. The reasons for this are not clear, but it does appear that the interaction between prostaglandins and hair cycling differs based on the site and/or type of hair follicle. Eyelashes are very different from scalp hair, producing a thicker shaft that only grows to a specific length; thus, it is not surprising that the regulation of the hair cycle for these follicles might be different as well. Unfortunately, this limits the clinical application of bimatoprost to therapy for eyelash shedding.

2.9. Diphenylcyclopropenone (DPCP)

DPCP is a sensitizing agent which is used in the treatment of alopecia areata. The agent is topically applied weekly and left in place for 6–24 h. Approximately 50% of patients treated with DPCP respond with regrowth of hair after 6 months of treatment [21]. The response is durable, with 60% of responders showing a continued hair growth 12 months after receiving therapy. DPCP is not effective in treating other forms of alopecia.

Mechanism of action: DPCP induces a contact dermatitis, which is thought to redirect the immune reaction from the hair follicles, allowing regrowth of hair. This includes decreasing the CD4/CD8 ratio and decreasing levels of interferon-gamma at the site of application. The patient must first be sensitized to DPCP prior to therapy on the scalp. The response develops slowly, requiring up to 6 months of treatments. However, once the response develops, it persists after treatment is discontinued.

Side effects: DPCP treatments are expected to cause a contact dermatitis, with redness and itching. More severe reactions, including swelling, burning, urticaria, and blistering, can occur.

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Other side effects include fever, arthralgia, more widespread eczema, erythema multiforme, hyperpigmentation, and hypopigmentation (vitiligo) [22].

DPCP treatments provide response rates in alopecia areata as high as JAK inhibitors, at a lower cost and with a less severe side-effect profile. However, the contact dermatitis, which is required for a therapeutic response, is irritating to the patients. Hair growth takes months to develop, and dermatitis can prevent patients from wearing wigs during the treatment phase.

3. Pharmacological therapies for alopecia under development

There are many therapies for alopecia in various stages of development. A partial list of the major categories of compounds is subsequently provided, including those with significant clinical data or established mechanism of action.

3.1. JAK inhibitors

Aclaris is developing JAK inhibitors specifically for therapy for alopecia areata. A-201 is an oral product, and A-301 is a topical product. The mechanism of action is likely to be similar to tofacitinib and ruxolitinib, described earlier. Topical application may improve the safety profile by minimizing systemic exposure and may improve efficacy by delivering higher doses to the skin regions where hair growth is desired. Aclaris is also developing its JAK inhibitors as therapy for androgenetic alopecia, although studies with other JAK inhibitors suggest that this will be ineffective.

Concert pharmaceuticals are also developing a JAK inhibitor specifically for therapy for alopecia areata. CTP-543 is a deuterium-modified analog of ruxolitinib and is believed to have a similar mechanism of action. Deuterium may alter the compound's pharmacokinetics. Leo Pharmaceuticals is developing LEO-124249, a topical JAK inhibitor for the treatment of alopecia areata. Incyte Corporation is developing a topical preparation of ruxolitinib for the therapy of alopecia areata.

Tigo GmbH is developing interferon-gamma receptor antagonists as a therapy for alopecia areata. These would act upstream in the same signaling pathway targeted by JAK inhibitors.

3.2. Androgen receptor antagonists

Androscience Corporation is developing an androgen receptor antagonist ASCJ-9 for the treatment of androgenetic alopecia. The compound is also being developed for acne vulgaris and wound healing. It is a small molecule which is topically applied and increases degradation of the androgen receptor. Valeant Pharmaceuticals is developing CB-0301, an androgen receptor antagonist, for the therapy of androgenetic alopecia.

3.3. Vitamin D analogs

Berg L.L.C. is developing BPM 31543, a topically applied analog of 1,25-dihydroxyvitamin D. It is being developed specifically for the therapy of chemotherapy-induced alopecia. Patients

with VDR receptor mutations have total body alopecia. However, it is the unoccupied VDR that is required to activate the hair cycle, apparently by forming complexes with beta-catenin and LEF-1. There is no hair loss observed in patients with 1-alpha-hydroxylase deficiency, who make no 1,25-dihydroxyvitamin D. It is therefore unclear how 1,25-dihydroxyvitamin D analogs would prevent hair loss.

3.4. Parathyroid hormone analogs

Parathyroid hormone antagonists can promote hair growth by preventing hair follicle transition from anagen to catagen phase. ICI Pharmaceuticals was developing parathyroid hormone antagonists as a therapy for chemotherapy-induced alopecia, but the project was discontinued because of lack of efficacy. Parathyroid hormone agonists can promote hair growth by stimulating hair follicles to transition from telogen to anagen phase. BiologicsMD is developing parathyroid hormone agonists as a therapy for several forms of alopecia, including alopecia areata, androgenetic alopecia, and chemotherapy-induced alopecia.

3.5. TGF-beta receptor antagonists

Auxagen is developing a small molecule compound that suppresses TGF-beta, inhibiting the transition of hair follicles from anagen to catagen phase. The compound is topically applied. The company reports that this product is more effective than minoxidil at promoting hair growth.

3.6. Anti-fibrogenic factor

Birch Biomed Inc. is developing a combination therapy for alopecia areata. Fibrostop 2 is a kynurenic acid cream which is administered topically. Fibrostop 2 is being developed as a therapy for psoriasis, alopecia, and scars.

3.7. Neurotrophic activator

BRIM Biotechnology, Inc. is developing a peptide compound that acts to enhance cell proliferation. The compound, BRM-421, also has ophthalmologic applications and is proceeding to clinical trials for these indications.

3.8. Stem cell signalers

Histogen is developing a mixture of biologics called hair stimulating complex. This product includes KGF, VEGF, and follistatin, compounds which signal stem cells in the body and are critical for hair follicle formation and stimulation of existing hair follicles. The compound activates stem cells in the bulge and promotes anagen transition of hair follicles.

Rivertown Therapeutics is developing RT1640 for the treatment of androgenetic alopecia. RT1640 is a mixture of three molecules which is applied topically and stimulates hair growth

—the compounds are minoxidil, cyclosporin A, and a proprietary compound RT1640. The compound is applied topically.

3.9. RNAi

There are several companies, including OliX Pharmaceuticals, Quark Pharmaceuticals, and RXi Pharmaceuticals developing RNAi-based treatments for alopecia. There is little information at this time regarding specific compounds or targets.

3.10. Stem cell therapy

RepliCel Life Sciences is developing a method of autologous cell therapy which utilizes dermal sheath cup cells to treat androgenetic alopecia.

3.11. Histone deacetylases

TetraLogic Pharmaceuticals is developing remetinostat for the therapy of alopecia areata. The compound is suberohydroxamic acid phenyl ester (SHAPE), which acts by inhibiting histone deacetylases. It is topically applied and is also being developed for the therapy of cutaneous T-cell lymphoma and plaque-type psoriasis.

3.12. Interleukin antibodies

AstraZeneca is developing Tralokinumab for the treatment of alopecia areata. Tralokinumab is a human monoclonal antibody that inhibits interleukin 13, which is an important cytokine for developing hair loss in alopecia areata. Novartis is developing secukinumab for the treatment of alopecia areata. Secukinumab is a human monoclonal antibody that inhibits interleukin 17A, which is also an important cytokine for developing hair loss in alopecia areata. Secukinumab is also being developed for severe plaque psoriasis, psoriatic arthritis, rheumatoid arthritis, ankylosing spondylitis, and uveitis.

3.13. Stem cell therapy

Stem cells play a critical role in hair follicle regeneration and in the hair cycle. Stem cells are located in the bulge region of the hair follicle, and under control of Wnt signaling, they proliferate and migrate down the hair follicle shaft to transform the follicle into the anagen phase. Efforts have been made to treat various forms of hair loss, including androgenetic alopecia and alopecia areata, with stem cell injections. There are reports of improvements of hair growth with these techniques [23], and there are some hair growth centers which utilize these techniques.

3.14. Platelet-rich plasma therapy

Rather than infusing stem cells, one can also infuse plasma, which is rich in factors which can stimulate stem cells. Platelet-rich plasma (PRP) is a plasma concentrate which has been used

for regenerative purposes in a variety of settings, including wound healing, cartilage damage, and scarring. Injection with autologous PRP has been shown to increase hair growth in androgenetic alopecia [24], presumably by stimulating stem cells in the bulge region to regenerate hair follicles and induce anagen transition.

3.15. Adipose-derived stromal vascular fraction (SVF)

Adipose-derived stem cells, also referred to as stromal vascular fraction (SVF), are another potential source of stem cells and/or stem cell-stimulatory factors which can be used to promote hair growth. This was discovered incidentally by observing effects after the transplant of autologous fat on hair growth. Early trials of injection of SVF show photographic evidence of hair regrowth and quantifiable (23.3%) increase in hair counts [25] in patients with androgenetic alopecia.

4. Conclusions

While there are only two FDA-approved pharmacological treatments for alopecia, there are a wide variety of compounds used off-label and an even larger number of compounds in various stages of development. This reflects a large and growing interest in developing effective therapies for alopecia. However, most of these compounds in clinical use suffer from the same limitations, which are poor efficacy and lack of durable effect. Several, particularly the immune modulators, are also hampered by being associated with severe side effects. Development programs have been hindered by the designation of alopecia as a cosmetic disease, which restricts programs to compounds with virtually no side effects. Advances in basic science have led to an improved understanding of the hair cycle, revealing new targets for drug development. As a result, there is a robust slate of development programs, which show promise for the development of more effective treatments for alopecia in the future.

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Conflict of interest

Robert Gensure is a consultant and has an equity stake in BiologicsMD, a biotechnical company which is developing alopecia therapies.

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Chapter 5

Cicatricial Alopecia

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Additional information is available at the end of the chapter

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Abstract

Cicatricial alopecia represents a group of disorders sharing a final pathway of destruction followed by replacement with fibrous tissue of the hair follicle unit. Cicatricial alopecia is classified into two categories, namely primary cicatricial alopecia, in which the hair follicle is the sole target of a progressive inflammatory process in a group of diverse skin or systemic diseases, and secondary cicatricial alopecia, referring to the hair follicle destruction as a result of a nonspecific disruption of the dermis. Permanent hair loss may also occur in the late phases of some nonscarring alopecias that are called "biphasic alopecias." Based on the pathological characteristics, the lesions of primary cicatricial alopecia are divided into lymphocyte-predominant subgroup, neutrophil-predominant subgroup, or mixed subgroup. In principle, the primary goal of the treatment aims to attenuate the progression of the inflammatory and the scarring processes at the earliest phase of the disease. In clinical practice, the lymphocyte-predominant lesions are treated with immunosuppressive agents, whereas the neutrophil-predominant lesions are treated with antimicrobials or dapsone. As the efficacy of medication treatment against the cicatricial alopecia varies significantly, autologous hair transplantation is recommended to patients who have a relatively stable primary or a secondary cicatricial alopecia.

Keywords: cicatricial alopecia, hair follicle unit, inflammatory progression, hair transplantation, follicular unit extraction

1. Introduction

Alopecia, also known as baldness or hair loss, refers to the partial or complete loss of hair over the body, although the hair loss over the head may be the most concerned issue among a large number of people who pursue medical assistance. Hair loss often causes the most

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severe psychological distress in both sexes at the onset of symptoms and negatively affects self-image and self-esteem of the patient. The mechanisms underlying the alopecia are not fully understood, but the pathological progression results in the disintegration of the follicular unit and a permanent loss of ability to produce hair fiber [1]. In contrast to androgenic hair loss, the most common cause of alopecia, cicatricial (scarring) alopecia represents a group of disorders in which the common final pathway is the destruction followed by the replacement with fibrous tissue of the hair follicle unit [2]. Cicatricial alopecia leads to permanent damage of the stem cells in the hair follicle bulge; therefore, the end result is usually a permanent or irreversible loss of hair leaving the effacement of follicular orifices, the replacement of follicles with fibrotic stelae, and the fibrosis or hyalinization of surrounding collagen in the bald skin. The causes of cicatricial alopecia are categorized as primary or secondary [3]. In primary cicatricial alopecia, the follicle unit is the sole target during the pathological progression of the disease. In secondary cicatricial alopecia, the hair follicle destruction occurs as a result of a nonspecific disruption of the dermis, as in the thermal burns or blistering disorders. Permanent hair loss may also occur in the late phases of some nonscarring alopecias that are called "biphasic alopecias."

Pathologically, the causes of primary cicatricial alopecias are a diverse group of diseases that share common characteristics of skin inflammation. It is noted that the inflammatory processes confine within the scope of the hair follicular units although the cellular or molecular mechanisms involved are currently ill defined. Primary cicatricial alopecias should be considered as a trichologic emergency because this disease will progress to permanent hair loss if the management of the inflammatory processes is delayed [4]. Nevertheless, the treatment options are poorly defined and often limited in effect [2]. The secondary cicatricial alopecias can be caused by various cutaneous inflammatory processes or by physical trauma, which damages the skin and skin appendages.

Cicatricial forms of alopecia account for about 3.2% [5] of all trichologic consultations and the frequency of cicatricial alopecia is about 5.0–7.3% of all the hair loss cases [2]. The majority of affected European adults were females (female:male ratio = 2.6:1), and the primary cicatricial alopecia is more common than the secondary (primary:secondary cicatricial alopecia ratio = 4:1) [5]. A survey also reported that the prevalence of primary cicatricial alopecias is higher in women than in men in regions of Middle East, and the possible associating factors include social culture, scarf wearing, and treatment delay. Among the diseases that may cause primary cicatricial alopecia, pseudopelade of Brocq (PPB) (40.6%) was the most frequent one followed by lichen planopilaris (LPP) (12.6%), and folliculitis decalvans (FD) (11.2%). In another report, however, discoid lupus erythematosus (DLE) (33.9%) was the most common cause followed by pseudopelade of Brocq (PPB) (24.1%) and lichen planopilaris (LPP) (22.3%) [6, 7]. Histopathologically, the majority cases of primary cicatricial alopecia are characterized by lymphocytic infiltrate.

The efficacy of the treatments for various forms of cicatricial alopecia depends on the clinical diagnosis that requires quality inspection of individuals during the physical exam and laboratory tests. The following items are routinely utilized by many clinics:

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Physical exam

A. Gross inspection of hair: (a) generalized, patterned, or focal hair loss and (b) density of hair, presence of broken hairs, vellus (thin, downy premature hair) vs. terminal hairs (thick, strong mature hair).

B. Inspection of scalp: (a) absence of follicular ostia and scar tissue and (b) papules, pustules, scaling, and perifollicular erythema.

C. Diagnostic procedures: (a) hair pull test: useful for telogen effluvium; performed by grasping a small portion of hair and gently applying traction while sliding the fingers along the hair shafts. Evaluation of results: normal: 1–2 hairs removed; abnormal: ≥6 hairs removed; (b) direct microscopic inspections of hair shaft: exclamation point hairs: distal end broader than proximal end; seen in alopecia areata. Evaluation of the results: anagen hairs: elongated, distorted bulb with attached outer root sheath; telogen hair: club-shaped bulb; (c) "Hair growth window": repeatedly (weekly) shaving a small area of involved scalp to demonstrate normal regrowth; and (d) scalp biopsy: useful for the diagnosis of scarring alopecias [8].

Laboratory tests

Total and free testosterone and dehydroepiandrosterone sulfate serum concentrations. This test is useful for diagnosis and differential diagnosis of androgenetic alopecia.

Evaluation on tissue biopsy

Two skin biopsies each with more than 4 mm in diameter are required. The biopsy punch should be aligned with the hair shaft angle and should reach the deep subcutis when performing tissue punch. Under a microscope, the density and ratio of each type of the infiltrating inflammatory cells need to be recorded. In addition, some special staining of tissue sections is required that usually including staining of elastic tissue, Gram stain, periodic acid-Schiff stain, and colloidal iron stain for mucin. Tissue cultures or immunofluorescence studies are often required as well [9].

Principle of treatment

Spontaneous regrowth of hair in case of cicatricial alopecia hardly ever occurs. The primary goal of the treatment aims to attenuation of the progression of the inflammatory and the scarring processes at the earliest phase of the disease. The monitoring of disease activity by frequent clinical evaluation in combination with dermatoscopy observation should be scheduled. A practical way to assess the treatment responses is the evaluation through dermatoscopy. Bear in mind however, there is so far no clear consensus regarding the successful treatments in terms of their efficacy to cure cicatricial alopecia, and the clinical improvement on many subtypes of the disease has not been fully supported by evidence-based trials. Therefore, the treatment of cicatricial alopecias is selected in the absence of precise information on the expected outcome and the current treatment failure is common.

As a general rule, lymphocyte-predominant subgroup of primary cicatricial alopecias is treated with immunosuppressive agents. Neutrophil-predominant subgroup of primer cicatricial alopecias is treated with antimicrobials or dapsone. Systemic therapy is usually combined with topical or intralesional corticosteroids or topical calcineurin inhibitors. Adding anti-inflammatory shampoos is often recommended. The treatment effects may take 6 months or more to appreciate, and disease may resume upon discontinuation of systemic and/or topical therapy. The effectiveness of maintenance therapy is still elusive [2, 10].

Surgical treatment of stable cicatricial alopecia includes hair transplantations, excision of affected area, flap surgery, or scar reduction with tissue expansion.

2. Pathology of cicatricial alopecia

Although cicatricial alopecia includes a group of diverse diseases, there are currently several pathological features that are shared among some of the different subtypes of hair loss of this category. Understanding of such pathology traits would help understand the treatment strategies. From the clinical points of view, the pathological pathways of primary cicatricial alopecias merge into a few inflammatory processes that eventually result in the disintegration of hair follicular units. By consensus, four main categories are proposed based on the types of immune cell infiltrates during the pathogenesis of primary cicatricial alopecia (**Table 1**) [4].

Lymphocytic cicatricial alopecia
Chronic cutaneous lupus erythematosus
Lichen planopilaris (LPP)
Classic LPP
Frontal fibrosing alopecia
Graham-Little syndrome
Classic pseudopelade (Brocq)
Central centrifugal cicatricial alopecia
Alopecia mucinosa
Keratosis follicularis spinulosa decalvans
Neutrophilic cicatricial alopecia
Folliculitis decalvans
Dissecting cellulitis/folliculitis (perifolliculitis abscedens et suffodiens)
Keratosis follicularis spinulosa decalvans (KFSD)
Mixed cicatricial alopecia
Folliculitis (acne) keloidalis
Folliculitis (acne) necrotica
Erosive pustular dermatosis
Nonspecific cicatricial alopecia
Sebaceous gland abnormalities (primary or secondary)

Table 1. Immune cell infiltrates and their corresponding clinical diseases.

Several lines of evidences in basic and clinical research have indicated that the local inflammatory destruction of the pilosebaceous unit leads to the permanent damage of the follicular stem cells in the bulge region and several associated hypotheses are suggested as following [1]: Autoimmune-mediated hypothesis: The autoantigens/epitopes that could elicit the autoimmune response are formed in the permanent region of the follicle including the stem cells in the bulge area; (2) immune privilege breakdown and Langerhans cell distribution hypothesis: MHC class I, β2-microglobulin, and MHC class II immune reactivity are significantly upregulated in the bulge region, and this reaction may be triggered by aberrant distribution of Langerhans cells; (3) bulge stem cell destruction hypothesis: The lost of Ck15 positive bulge stem cells is one of the evident histopathological traits of primary cicatricial alopecia; (4) hair Follicle Epithelial-Mesenchymal Communication Inhibition Hypothesis: As the induction of life-long cyclic transformations of hair follicles is epithelial-mesenchymal interaction dependent, inflammatory interruption in the communication between the epithelial stem cells and the hair follicle mesenchyme would damage the integrity of hair follicles [2]. (5) Peroxisome proliferator-activated receptor-γ (PPAR-γ) deletion hypothesis [2]: PPAR-γ, which is highly expressed in human sebaceous glands, plays roles in lipid homeostasis, sebocyte maturation, peroxisome biogenesis and anti-inflammatory effects. Dysfunction of PPAR-γ is believed to result in follicular inflammation and the pathogenesis of the primary cicatricial alopecia [11]; (6) Sebaceous Gland Dysfunction Hypothesis: The aberrant gene expressions in sebaceous gland lead to the abnormalities in the sebum secretion and hair growth; (7) "No Danger" Signal CD200 Deletion Hypothesis: In this hypothesis, the interruption of the ligand-binding signal between CD200 and its receptor is speculated as the trigger of local inflammatory reactions [12]; (8) Genetic Mutation of Keratin Hypothesis [2]: keratin gene mutation that associated with pathogenesis of alopecia microacanthosis leads to the disintegration of hair follicles.

As for secondary cicatricial alopecia, **Table 2** lists the lesions commonly seen in clinics that eventually result in reversible or irreversible hair loss.

Infection
Fungal (tinea capitis)
Bacterial
Viral (e.g., herpes zoster)
Immunologic
Sarcoidosis
Necrobiosis lipoidica
Morphea
Graft-versus-host disease
Malignancies
Alopecia neoplastica
Lymphoproliferative
Exogenous factors
Radiation
Burns
Drugs

Dermatoses
Psoriasis
Bullous disorders
Cicatricial pemphigoid
Epidermolysis bullosa
Hamartomas
Organoid nevus
Miscellaneous
Lipedematous alopecia

Table 2. Etiology of secondary cicatricial alopecia.

Another form of alopecia that is characterized by "biphasic pattern" also involves the scarring processes pathologically. Clinical observations suggest that this form of alopecia may be irreversible. Examples of the clinical diseases that can be categorized in this form are known as "Late-stage nonscarring alopecias" including alopecia areata, patterned hair loss, and traction alopecia, etc. Recent report indicated that the occurrence of biphasic alopecia can also be iatrogenic [13].

3. Clinical features of cicatricial alopecia

According to the clinical course, physicians tend to classify all forms of cicatricial alopecias into unstable group, meaning there is an increased recurrence even with the treatment, and stable group, referring to the forms of cicatricial alopecia that have a low recurrent rate after treatment. The formal form usually includes lichen planopilaris, pseudopelade of Brocq, and discoid lupus erythematosus, whereas the latter form includes isolated traumas, burns, infection, and prior surgery-induced alopecias.

The clinical treatment options for cicatricial alopecia are summarized in **Figure 1**.

3.1. Characteristics of primary cicatricial alopecia

Several factors may have associated with the distribution of primary cicatricial alopecia throughout the world. For example, lymphocytic form dominants among North and South American and Iranian patient groups, whereas neutrophilic form is the dominant one among Chinese patient group [14, 15]. The occurrences of dissecting cellulitis and acne keloidalis nuchae may have close association with genetic background and are seen more frequently in patients of African descent. Sex is another factor believed to be related with the prevalence of primary cicatricial alopecia. Folliculitis decalvans, dissecting cellulitis, and acne keloidalis nuchae are more common in males, while central centrifugal cicatricial alopecia, lichen planopilaris, discoid lupus erythematosus, and pseudopelade of Brocq have a female predominance. In addition, discoid lupus erythematosus, dissecting cellulitis, and acne keloidalis nuchae are frequently seen in younger patients, whereas central centrifugal cicatricial alopecia, lichen

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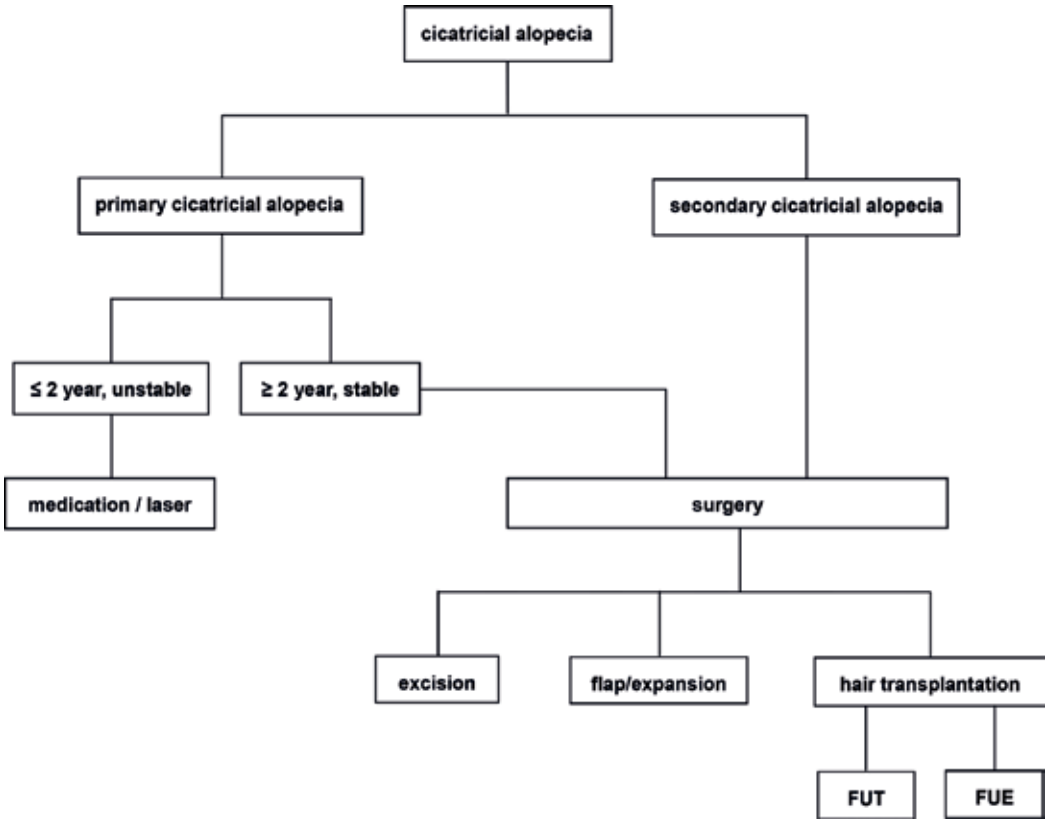

Figure 1. Schematic diagram of treatment options.

planopilaris, and folliculitis decalvans tend to be distributed among older patients. Lastly, there are correlations between certain forms of cicatricial alopecia and the patterns of the baldness. For example, unifocal-ragged border of bald area may associate with central centrifugal cicatricial alopecia and discoid lupus erythematosus, multifocal-interconnected pattern may often be seen in lichen planopilaris and folliculitis decalvans, and multifocal-separated pattern may be one of the characteristics of clinical feature of dissecting cellulitis.

3.2. Lymphocytic cicatricial alopecia

3.2.1. Chronic cutaneous lupus erythematosus (classic discoid lupus erythematosus)

3.2.1.1. Clinical features

This is one of the major forms of cicatricial alopecia among European patients and typically affects women with age between 20 and 60 years old [9, 16]. It presents with erythematous scaly papules with characteristic follicular plugging (**Figure 2**). The lesions evolve into hypo- or depigmented atrophic plaques with peripheral hyperpigmentation, overlying telangiectasias, and loss of follicles. Systemic lupus erythematosus (SLE) associates with 20% of cases [17], and the detections of antinuclear antibodies are positive in approximately 20% of cases [16].

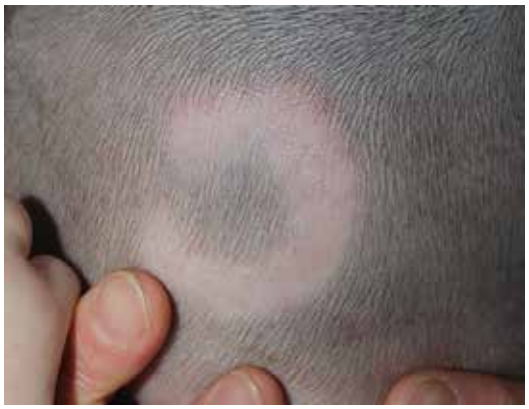

Figure 2. Alopecia at occiput region in a patient with cutaneous lupus erythematosus.

3.2.1.2. Pathological features

Interface dermatitis with epidermal atrophy or hyperplasia in the hypertrophic variant of chronic cutaneous lupus erythematosus; ortho-hyperkeratosis with areas of parakeratosis and follicular plugging; an inflammatory lymphocytes infiltrate with admixed plasma cells distributed around the superficial and deeper dermal vasculature as well as adnexal structures and scattered within the interstitium; early destruction of sebaceous glands; replacement of the follicle with fibrous tissue in late stages; hair fiber granulomas on occasion; occasional inflammatory infiltrate into the subcutis with lymphoid follicle formation [18].

3.2.1.3. Featured stainings

Periodic acid-Schiff (PAS) stains showing thickened PAS-positive basement membrane zone; mucin deposition in reticular dermis or perifollicular scarring tissues; immunofluorescence staining positive signals of IgG, IgM, and complement C3 along the dermal-epidermal or follicular epithelial-dermal junction [19, 20].

3.2.1.4. Treatment

First-line systemic therapy: hydroxychloroquine (HCQ).

3.2.2. Lichen planopilaris (LPP)

3.2.2.1. Clinical features

The lesion appears at the scalp in about half of the cases. Some of the lesions may develop to mucous membranes [2]. Clinically, hair loss can be patchy or diffuse and presents with perifollicular erythematous papules and acuminate hyperkeratotic follicular spines (**Figure 3**). The disease is most active at the hairbearing periphery of the alopecic patch [21].

Lichen planopilaris and frontal fibrosing alopecia are slowly progressive with episodes of worsening. Systemic therapy is strongly recommended if symptoms or hair loss is persistent or progressive.

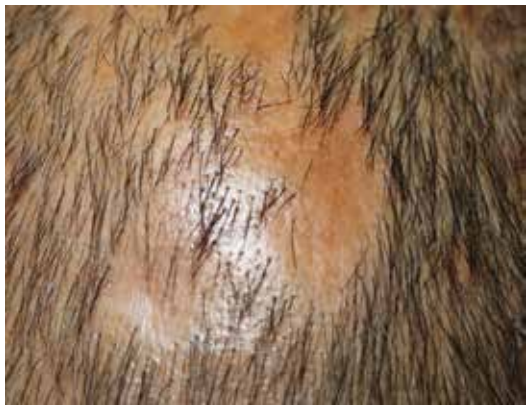

Figure 3. Scalp lesion of lichen planopilaris leads to alopecic patches.

3.2.2.2. Pathological features

Lichenoid band-like infiltrate in the infundibulum, isthmus, and variably the interfollicular epidermis regions; cytoid bodies, basal vacuolization, pigment incontinence, and Max Joseph spaces along the follicular epithelium; follicular plugging; concentric lamellar fibroplasia, loss of sebaceous glands and follicles [22, 23].

Vague mucin deposition in dermis; loss of elastic tissue staining of the upper third of the fibrous tract, and associated destruction of the elastic sheath in late-stage lesions.

Nonspecific IgM within cytoid bodies along the upper hair follicle on direct immunofluorescence observation [24].

3.2.2.3. Treatment

First-line therapy: systemic medications, tetracycline antibiotics, or HCQ. Tetracycline 500 mg twice daily and doxycycline hyclate 100 mg twice daily. The mechanism of action is thought to be due to the anti-inflammatory benefits of the tetracycline antibiotics. The medications are recommended to be limited to 6 months. The efficacy of HCQ in LPP is identical to that of tetracyclines [25].

3.2.3. Frontal fibrosing alopecia (FFA)

3.2.3.1. Clinical features

Frontal fibrosing alopecia, as described by Kossard, is a scarring alopecia that occurs most often in postmenopausal women [26]. It is clinically characterized by a progressive recession of the frontal and temporal hair lines with follicular hyperkeratosis, perifollicular erythema, and loss of follicular ostia [2]. More than half of the FFA patients also have eyebrow hair loss.

3.2.3.2. Pathological features

Histopathology shows a lichenoid reaction against miniaturized hair follicles.

3.2.4. Graham-Little syndrome

Patchy, progressive scarring alopecia of the scalp, nonscarring alopecia of axillary and pubic hair, and the presence of widespread horny follicular papules on the trunk and limbs are clinical traits of Graham-Little syndrome [2].

3.2.5. Classic pseudopelade (Brocq)

3.2.5.1. Clinical features

Classic pseudopelade is a rare, slowly progressive hair disorder of typically the most common form observed in middle-aged Caucasian women. It is presented footprints in the snow to round hypopigmented atrophic alopecic plaques resembling "footprints in the snow" [27].

3.2.5.2. Pathological features

Perifollicular lymphocytic infiltrate, with eccentric atrophy of the outer root sheath epithelium, prominent concentric lamellar fibroplasia, loss of sebaceous glands and follicles, and hair-shaft granulomas [15].

Elastic fibers are markedly thickened in pseudopelade of Brocq. Direct immunofluorescence usually negative but may reveal scant finely granular IgM along the basement membrane zone of the follicular infundibulum [28].

3.2.6. Central centrifugal cicatricial alopecia

3.2.6.1. Clinical features

It is commonly seen in young to middle-aged women of African-American descent with a prevalence of 3–6% [29–31]. It presents progressive alopecia over the crown and vertex that expands centrifugally without overt inflammation and with tufting and perifollicular hyperpigmentation.

3.2.6.2. Pathological features

Premature desquamation of the inner root sheath; perifollicular lymphocytic inflammation of the infundibulum and isthmus; eccentric atrophy of the outer root sheath epithelium, concentric lamellar fibroplasia, and hair fiber granulomas within fibrous tract remnants [32].

Preserved elastic sheath and thickened dermal elastic fibers. Direct immunofluorescence usually negative [33].

3.2.7. Alopecia mucinosa

3.2.7.1. Clinical features

Papules and plaques of hair loss with variable dysesthesia and anhidrosis on the eyebrows, scalp, trunk, and limbs. Presents in all age groups. May be benign or associated with lymphoma (especially mycosis fungoides).

3.2.7.2. Pathological features

Lymphocytic inflammation around perifollicle; variably atypical lymphocytes; restricted T-cell lineages seen in benign and malignant forms.

Direct immunofluorescence negative.

3.2.7.3. Treatment

Tetracycline antibiotics typically are helpful although not recommended for systemic therapy. Improvement may be seen within 2–6 months followed by dose reduction [34].

3.2.8. Keratosis follicularis spinulosa decalvans

3.2.8.1. Clinical features

An X-linked disorder of cornification with the onset of the alopecia in the teenage years. Acuminate keratotic follicular papules and pustules on the scalp, eyebrows, and eyelashes, keratosis pilaris on the trunk and extremities, photophobia, and corneal dystrophy as the clinical characteristics.

3.2.8.2. Pathological features

Follicular plug with compact hyperkeratosis and hypergranulosis; upper follicle lymphocytic infiltrate; concentric lamellar fibrosis, hair shaft granulomas, and fibrous follicular tract remnants.

3.2.8.3. Treatment

Etretinate, isotretinoin, oral antibiotics, and dapsone are reported medications [35].

In summary, lymphocytic primary scarring alopecias generally can be treated with tetracycline antibiotics, HCQ (hydroxychloroquine), or immunosuppressant medications such as corticosteroids, cyclosporine, and mycophenolate mofetil. Auxiliary drugs are also used specifically for some types of diseases. For example, pioglitazone or 5-alpha reductase inhibitors are used for LPP or FFA, respectively. Treatment response can be seen typically between 6 and 12 months, and gradual taper may result in sustained remission after discontinuation of oral therapy [7, 10].

3.3. Neutrophilic cicatricial alopecia

3.3.1. Folliculitis decalvans

3.3.1.1. Clinical features

Occurs primarily in young and middle-aged adults with a slight predominance in men. Multiple hairs emerge from a single follicular orifice in a pattern known as "tufted hair folliculitis." Erythematous follicular papules or pustules on the crown progress to pseudopelade-like round alopecic patches with pustules at the advancing margins and with

polytrichia. *Staphylococcus aureus* is commonly isolated from primary lesions. Dermoscope examination reveals low hair density and loss of follicular ostia, thinned shafts of the remaining hairs [24].

3.3.1.2. Pathological features

Follicular plugging and intra/perifollicular neutrophilic infiltrate of the upper and middle portions of the hair follicle; follicular rupture with infiltrates of lymphocytes, histiocytes, and plasma cells; neutrophilic abscess is common; hair shaft granulomas prominent; late-stage replacement of hair follicles with scarred fibrous tracts. Existence of plasma cells has diagnostic significance in advanced cases.

3.3.1.3. Treatment

First-line treatment: oral administration of tetracycline, doxycycline, minocycline, erythromycin, and clindamycin alone or in combination with rifampin. However, relapse is common after discontinuation of these antibiotics [36].

3.3.2. Dissecting cellulitis/folliculitis (perifolliculitis abscedens et suffodiens)

3.3.2.1. Clinical features

Commonly seen in younger men of African Americans with inflammatory plaques and nodules, often associated with formation of sinus tracts that exude a purulent discharge. The process commonly begins on the occiput or vertex and often progress to involve the entire scalp (**Figure 4**). Recurrent bacterial infections and disordered keratinization are important causes [19].

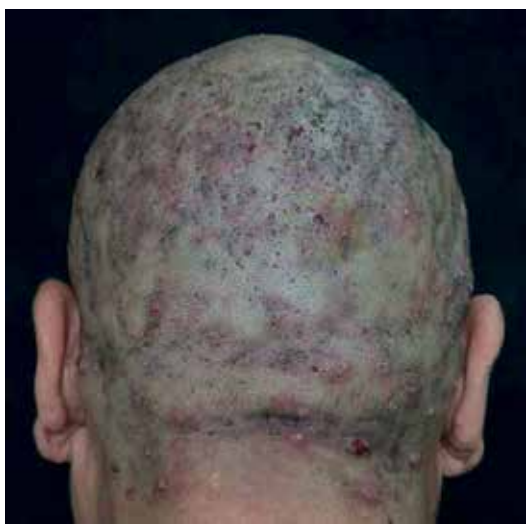

Figure 4. A patient with dissecting cellulitis/folliculitis presents with inflammatory plaques, nodules, and purulent discharges over the entire scalp.

3.3.2.2. Pathological features

Follicular plugging and collections of neutrophils at the follicular ostia; extensive abscess formation and extensive dermal fibrosis.

3.4. Mixed cicatricial alopecia

3.4.1. Folliculitis (acne) keloidalis

3.4.1.1. Clinical features

Most frequently in African postpubertal males with follicular erythematous papules and pustules that progress to hairless keloid-like nodules.

3.4.1.2. Pathological features

Neutrophilic or lymphoplasmacytic perifollicular infiltrate; thinned outer root sheath with reparative concentric lamellar fibroplasia; follicles are destroyed, hair-shaft fragments are extruded; hair-shaft granulomas or microabscess formation are prominent [2].

3.4.2. Folliculitis (acne) necrotica

3.4.2.1. Clinical features

Lesions present as crops of red-brown papules and papulopustules that undergo necrosis and become punched out, depressed scars after eventual healing.

3.4.2.2. Pathological features

Fragments of hair shaft; scattered neutrophils are present in the superficial dermis where follicular epithelium undergoes necrosis [7].

3.4.3. Erosive pustular dermatosis

3.4.3.1. Clinical features

Primarily affects elderly women and presents with extensive, boggy, crusted, erosive plaques on the scalp. It is thought to be triggered by local trauma sometimes months to years prior to the appearance of lesions or occur as a result of autoimmune disease [37].

3.4.3.2. Pathological features

Nonspecific findings in biopsy samples indicate the epidermis may be ulcerated or appear atrophic or hyperplastic. A mixed patchy dermal inflammatory infiltrate is seen. Suppurative folliculitis, intraepidermal, and subepidermal neutrophil infiltrates are common. Dermal fibrosis may focally replace the follicles or may diffusely involve the dermis, depending on the extent of inflammation. Scattered naked hairshaft granulomas are also seen in later stages.

3.5. Characteristics of secondary cicatricial alopecia

The causes of secondary cicatricial alopecia include even more diverse forms of trauma and inflammatory injuries. Of note, iatrogenic cause of hair loss such as with esthetic surgery is a concern among both patients and physicians. Currently, there is no effective treatment protocol for any subtypes of secondary cicatricial alopecia except to attenuate the development of inflammation processes initiated by topical tissue damages or infection. Medications used for these purposes include high-potency topical corticosteroids, immunosuppressants, and antibiotics. Nevertheless, permanent hair loss may eventually be the outcome, and the baldness

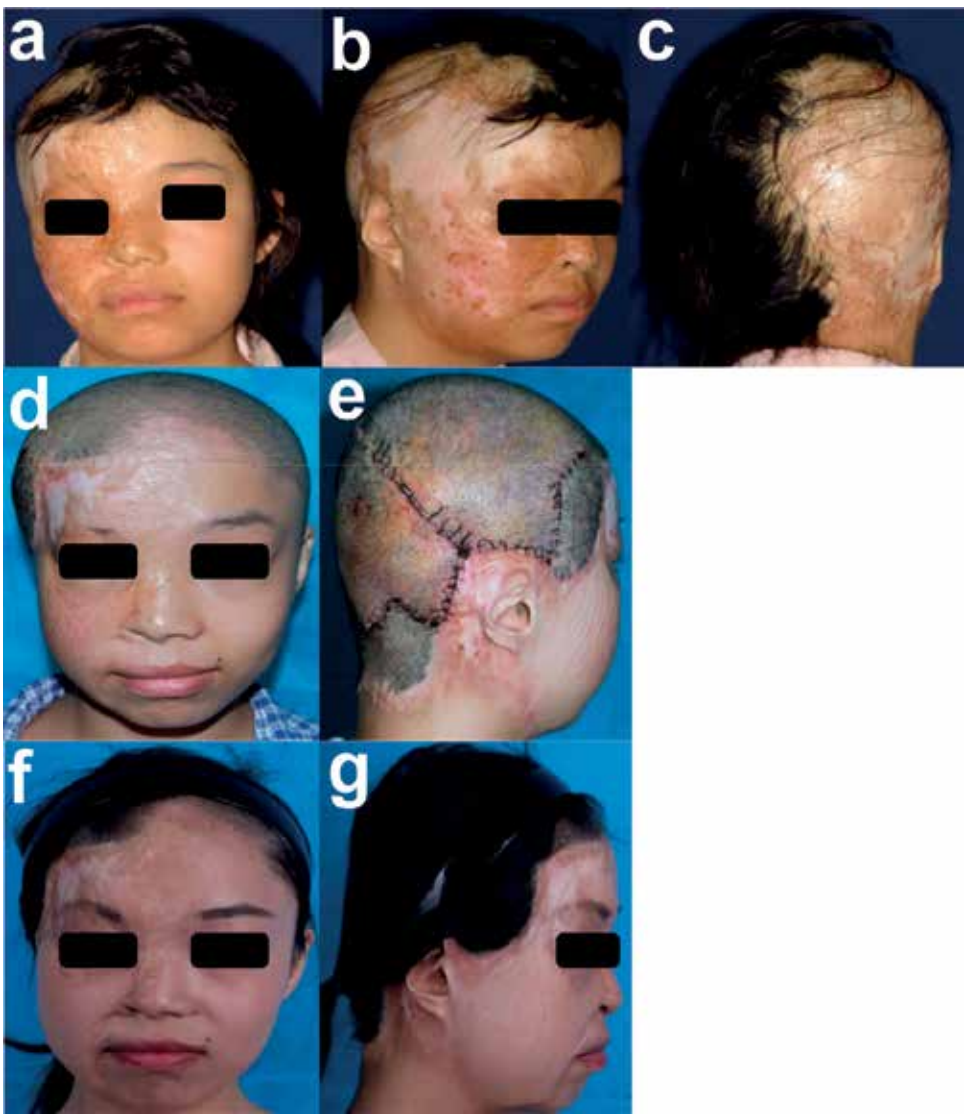

Figure 5. Reconstruction of secondary cicatricial alopecic region with expanded scalp flaps. (a–c) Photographs of a patient with thermal injury induced alopecia; (d, e) Photographs taken at post-operative day 7 after reconstruction of bald areas with expanded skin flaps; and (f, g) Follow-up photographs taken at month 29 post-operatively.

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would become evident if the area of the secondary cicatricial alopecia is extensive (**Figure 5**). Therefore, autologous hair transplantation and replacement of the bald skin with skin flaps that have hairs with satisfactory density are acceptable procedures for this form of alopecia [2].

3.6. Biphasic alopecias

The term "biphasic alopecia" includes some conditions where cicatricial alopecia becomes apparent in the late stages of an otherwise nonscarring form of alopecia. Alopecia areata, androgenetic alopecia, and traction alopecia may belong to this category. Alopecia areata can result in fibrosis in 10% of cases, while in advanced stages of androgenetic alopecia, vellus hairs also disappear and the alopecia becomes permanent [2]. We have noticed that esthetic filling with hyaluronic acid (HA) can induce biphasic alopecia that may leave permanent hair loss in the severely necrotic lesion region, whereas hair regrowth was found in possible less ischemic area (**Figure 6**).

3.7. The application of minoxidil

Minoxidil has demonstrated an antifibrotic action and believed to be efficient in the early course of some dermatoses leading to scarring alopecia, such as scalp burning disease. Mechanistically,

Figure 6. Hyaluronic acid filling in the left temple region results in the skin necrotic and ischemic lesion leading to an area of a permanent hair loss surrounded by regions with slow-restoration of hair regrowth [13]. (a) Complete hair loss was seen in the patient's left temple area after HA injection. A HA injection induced necrotic skin crust surrounded by bald skin area at day 22 is shown; (b) near healed prior necrotic skin and surrounding skin still showed no sign of hair regrowth at post-HA injection day 42 post-HA injection; (c) partial hair regrowth was seen in the bald area except the prior necrotic crust covered region at post-HA injection day 74; and (d) a view of long-term recover of hair regrowth surrounding a permanent bald area at post-HA injection day 209.

scalp sulfotransferase changes minoxidil into minoxidil sulfate, which is thought to be the active form of the molecule. The variations of sulfotransferase activity between individuals may explain the interindividual variations in minoxidil efficiency. Minoxidil acts by shortening telogen phase and thus causing the quiescent hair follicles to enter prematurely into anagen phase, resulting in telogen effluvium after the initiation of minoxidil therapy, although such effect of minoxidil may increase hair length and diameter. Some basic research results suggest that minoxidil can intervene on the potassium channels of the vascular smooth muscles and hair follicles, which may induce the following effects: (1) stimulation of the microcirculation near the hair follicles by inducing arteriolar vasodilation, which may cause hair growth. Minoxidil induces the expression of vascular endothelial growth factor (VEGF) which increases vascularization around the hair follicles, thus contributing to hair growth; (2) activation of the prostaglandin-endoperoxide synthase one which stimulates hair growth; (3) inhibition of the effects of androgens on the androgen-sensitive hair follicles; and (4) direct stimulating action on the hair follicles: Minoxidil may act as an 'epidermal growth factor' on matrix cells delaying their aging, thus prolonging the duration of anagen phase, probably via the activation of the beta-catenin pathway [38–40].The effect of minoxidil becomes evident after approximately 8 weeks post-treatment, and the maximal effect may be seen after 4 months. Most of the clinical responders to minoxidil have alopecia with the onset of less than 5 years, particularly among young adults, with their follicles not deeply miniaturized [41].

Minoxidil is well tolerated. However, propylene glycol contained in the liquid form (solution) of minoxidil may be the cause of some adverse effects reported by patients that include: (1) minoxidil-induced telogen effluvium: the shortening of telogen phase by minoxidil causes marked shedding; (2) skin irritation: with erythema, discomfort, and burn sensation; (3) scaly changes of the scalp: due to irritation or exacerbation of seborrheic dermatitis; (4) isolated itching; (5) allergic contact dermatitis: with erythema, eczematous skin reaction, and itching. Minoxidil and propylene glycol are the major allergens in allergic contact dermatitis. Patch testing may be helpful to reveal the causative agent. In case of allergic contact dermatitis to propylene glycol, minoxidil foam (i.e., not containing propylene glycol) may be used; (6) localized or generalized hypertrichosis: this effect seen during oral minoxidil treatment may also be observed with topical form. It is probably related to the prolongation of anagen phase. This effect seems to be more commonly encountered with 5% minoxidil than 2% minoxidil.

Percutaneous toxicity is exceptional after a conventional use of minoxidil which has no known antidote for minoxidil massive oral ingestion. Accidental oral ingestion of minoxidil results in mild vomiting and rarely requires hospitalization. However, cases of hypotension, tachycardia, and/or electrocardiographic changes after accidental ingestion have been reported. Refractory hypotension may be managed by intravenous fluids and vasopressor agents. Gastric wash and activated charcoal may be indicated to prevent systemic toxicity in massive accidental ingestion of minoxidil.

3.8. Hair transplantation

The hair transplantation replaces the lost hairs with autologous hairs harvested from donor sites. Based on clinical experience, another 2 years may be required before the recipient site fully supports the survival and growth of hair grafts after the disease is stabilised. For limited area of hair loss, select local skin flap. For large bald area, expanded scalp skin flap can be selected or select hair transplantation technique [42, 43] (**Figure 5**).

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For transplantation procedure, here we briefly introduce follicular unit extraction (FUE) technique. It is the treatment of choice in instances where there is good availability of donor hair and vascular circulation [44]. Briefly, in FUE surgery, there is no linear scar but instead each extraction site leaves a small round dot scar that can be hidden even with shorter hairstyles compared to a strip scar (**Figure 8**). The punches vary in size but generally are in the range of 0.75–1.2 mm in diameter attached to either a hand-held manual device, a hand-held mechanized drill device or an automated robotic device. The recommended density of the site in the recipient area is 15–20 FU/cm^2 in areas with low perfusion and 20–30 FU/cm^2 in areas with sufficient blood perfusion. The integrated grafts started to grow 4–5 months after the surgery and most entered the anagen phase approximately 1 year postoperatively (**Figure 7**). For hair loss from burn injury, successful treatment with hair transplantation was reported although the hair follicle graft survival rate in scar tissue can be varied significantly [43–45].

Advantages of FUE include the absence of suture wounds and linear scars, less bleeding, and less postoperative discomfort than follicular unit transplantation. In addition, it is suitable for patients with less scalp laxity and poor wound healing. Furthermore, scarring at each individual site may limit the possibility of harvesting hair in the same area in the future. Moreover, the hair transection rate is higher than in follicular unit transplantation and buried tissue can cause inflammation and cyst formation [44].

Figure 7. Autologous hair transplantation. (a) Burns induced hair loss in the temple region; (b) seven days after hair transplantation by FUE technique; and (c, d) photographs of hair regrowth at month 11 after hair transplantation.

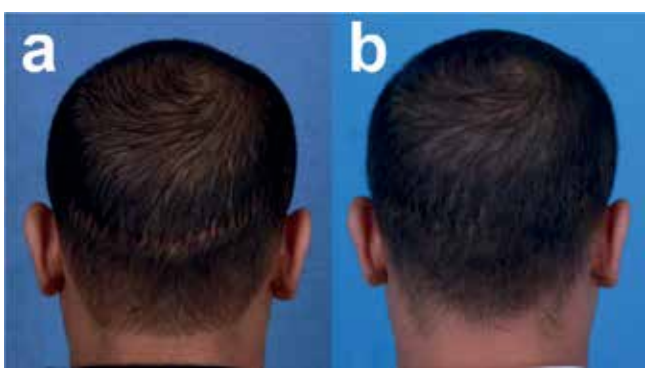

Figure 8. Linear scar at the donor site resulted from FUT procedure. (a) A linear scar with loss of hair is visible at the occiput donor region after FUT procedure and (b) the scar is invisible after hair implantation by a FUE procedure.

Disadvantages of this technique are that large harvesting sessions require the entire donor area to be shaved and the grafts tend to have less tissue around them and therefore more care is needed in handling them. Only with multiple or ill-planned procedures, there will be thinning out of the donor area.

Currently, the recipient holes or slits are made using either a punch, scalpel, different types of needles, or automated devices. A novel technique based on CO_2 laser tissue ablation was introduced in 1994 by Unger and David. However, one major possible disadvantage is the known thermal coagulation zone more or less present in all CO_2-based laser systems. Such thermal damage may raise concerns of reduced blood perfusion present in treated scar tissue [46].

The Er:YAG laser produces energy in the mid-infrared light spectrum at 2940 nm. This wavelength has 10–15 times greater water absorption than a CO_2 laser with an emission at 10,600 nm. Together with a pulse below the thermal relaxation time of skin, the Er:YAG laser allows "cold"-ablation with very little vaporization and desiccation. Minimal damage of the surrounding tissue combined with the precision of a laser should make this a valuable instrument for creating holes or slits for hair transplantation in cicatricial alopecia [46].

4. Conclusions

Cicatricial alopecia refers to a diverse group of local and systemic diseases in which hair loss is one of the outcomes. Although efforts have been made through medication treatments to counter the pathologic progression that would damage and replace the follicular unit with fibrous scarring, the efficacy of each treatment protocol may still lack of the supports through evidence-based studies, therefore, warranting further basic and clinical investigation. In contrast, hair transplantation has demonstrated more predictable and satisfactory outcome. Nevertheless, preparation of scalp recipient tissue with medication prior the surgery and post-surgery care with professional assistance are imperative for the survival and long-term maintenance of hair grafts.

Conflict of interest

The authors declare no conflict of interests.

Acronyms and abbreviations

Brocq	classic pseudopelade
CCCA	central centrifugal cicatricial alopecia
CK	cytokeratin
CO_2	carbon dioxide
FD	folliculitis decalvans

FFA	frontal fibrosing alopecia
FUE	follicular unit extraction
HA	hyaluronic acid
HCQ	hydroxychloroquine
KFSD	keratosis follicularis spinulosa decalvans
LPP	lichen planopilaris
MHC	major histocompatibility complex
PCAs	primary cicatricial alopecias
PPAR-γ	peroxisome proliferator-activated receptor-γ
PPB	pseudopelade of Brocq
PAS	periodic acid-Schiff
SLE	systemic lupus erythematosus
VEGF	vascular endothelial growth factor

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Chapter 6

Ethosomes: An Exciting and Promising Alcoholic Carrier System for Treating Androgenic Alopecia

Veintramuthu Sankar, Santhanam Ramesh and Karthik Siram

Additional information is available at the end of the chapter

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Abstract

Androgenetic alopecia (male-pattern hair loss) is characterized by the deposition of dihydrotestosterone at the pilosebaceous unit of the scalp. Oral administration of drugs (like finasteride) which can reverse androgenic alopecia causes undesired effects to the body. Targeting these drugs directly to the pilosebaceous unit of the scalp will enhance the pharmacological response at the desired site by reducing undesired systemic side effects. This chapter discusses about ethosomes, a specially tailored ethanolic vesicular carriers which can efficiently deliver various drugs with different physicochemical properties to and through the skin. The unique characteristics of the ethosomal carriers, their composition, preparation methods, and the mechanism of permeation, safety, and practical experience (finasteride and herbal extracts) have been discussed in detail.

Keywords: androgenic alopecia, ethosomes, finasteride, nanoparticles

1. Androgenic alopecia

Hair growth in the scalp is an androgen-dependent process. Many androgens target tissues in scalp hair follicles and are more responsive to dihydrotestosterone than testosterone. Two types of 5α-reductase enzymes, which convert testosterone to dihydrotestosterone, are present in humans. Scalp skin contains type 1 5α-reductase in the sebaceous glands and type 2 5α-reductase in the dermal papillae of hair follicles and connective tissue sheaths. Male-pattern hair loss, also referred to as androgenic alopecia, is a type of hair loss that occurs due to shrinkage of hair follicles by the influence of androgenic hormones. Type 2 5α-reductase promotes conversion of testosterone to dihydrotestosterone. When compared to the occipital

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scalp, the frontal scalp of young men with androgenic alopecia contains higher levels of dihydrotestosterone, a type 2 5α-reductase enzyme, and androgen receptors. The conversion and deposition of dihydrotestosterone from testosterone occur due to the action of an enzyme 5α-reductase, which is present at the pilosebaceous unit. After conversion to dihydrotestosterone, it binds to the androgen receptor, and this hormone-receptor complex activates the genes responsible for the shrinkage of hair follicles. Men with androgenic alopecia are found to have higher 5α-reductase, lower total testosterone, higher unbound/free testosterone, and higher free androgens, dihydrotestosterone. Cross talk occurs between androgens and the Wnt-beta-catenin signaling pathway that leads to hair loss. At the somatic stem cell level, androgens promote differentiation of facial hair dermal papillae but inhibit it at the scalp [1]. There are different stages in which hair loss takes place in the scalp as classified by Norwood.

2. Treatment options for treating androgenic alopecia

As hair loss is considered as a serious matter, many remedies have been evaluated to a great extent. Both males and females have issues regarding hair loss.

2.1. Hormonal treatment

The level of androgen plays an important role in the treatment of androgenic alopecia. The role of androgens in the etiology of androgenetic alopecia is considered to be a widespread aid in the treatment. Antiandrogens are usually delivered systemically in women and act by blocking the androgen receptors. They are contraindicated in men due to their feminizing action. Topical estrogens and antiestrogens have been used in both men and women [2].

2.2. Surgery

Surgery involving restoration of hair involves transplantation, scalp reduction surgery, or a combination of both. Hair transplantation is considered to be less invasive. Follicles that are not affected by miniaturization are redistributed over the scalp under local anesthesia. The result of hair transplantation is based on the texture of hair, the quality of hair, and also the number of transplanted hair in relation to the area to be covered or densified.

2.3. Combination therapies

A combination of medical and surgical therapy seems to be superior to surgery alone. A study revealed better clinical results for male patients treated with combination of finasteride 1 mg daily and hair surgery versus male patients treated with hair surgery alone, 12 months after follicular unit transplantation.

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3. Application of drug delivery in the treatment of androgenic alopecia

The skin is a multilayered structure composed of stratum corneum, the outermost and the tightest layer of the skin and below which lies the epidermis and dermis. This highly hydrophobic layer is composed of differentiated nonnucleated cells, corneocytes, which are filled with keratins and embedded in the lipid domain. Since the rate-limiting step for the skin absorption of most molecules is due to this nonviable layer, percutaneous permeation of molecules is believed to be governed by diffusion laws. The skin contributes to 4% of the total body weight. The extent of skin permeation of a compound may depend on the route of absorption. Application of novel drug delivery systems to target and enhance permeation of drugs through and to the skin has been on the rise from the past decades. Topical route of delivery system is a noninvasive method that enables the drug to reach the systemic circulation. There are three pathways which are involved in the transdermal permeation of drugs: (1) through the intercellular lipid zone in the stratum corneum, (2) through the skin appendages, and (3) through the keratin bundles in the stratum corneum.

4. Ethosomes

In the recent years, application of vesicles for the delivery of drugs has gained attention owing to their amphiphilicity, biodegradability, nontoxic nature, and possibility to modulate the pharmacokinetic profile, and their applications in the coming years might further escalate. Further, their structural characteristics like size, shape, surface charge, and composition can be tailored as per the need. Vesicular systems are uni- or multilamellar spheroidal structures composed of amphiphilic molecules assembled into bilayers. They are considered primitive cell models, cell-like bioreactors, and matrices for bioencapsulation. They can encapsulate both hydrophilic and lipophilic drugs and can release the drug in a sustained fashion.

The vesicular drug delivery systems are categorized based on the presence of lipid (conventional liposomes, deformable liposomes or transfersomes, ethosomes), polymer (polymersomes), surfactants (niosomes), and pharmacosomes, cubosomes, virosomes, and sphingosomes [3]. This chapter throws light on ethosomes.

Initially, because of interdigitation effect of ethanol on the bilayers, it was thought that the presence of ethanol would destruct liposomes. Thus, even if ethanol was used during the preparation of liposomes, it was removed [4]. But, Touitou et al. were able to show that the presence of ethanol did not show such effect and the existence of ethanolic vesicles has been confirmed by ^{31}P nuclear magnetic resonance, dynamic light scattering, transmission electronic microscopy, and scanning electron microscopy. Ethosomes are soft, malleable lipid-based vesicular delivery systems consisting of amphipathic phospholipids arranged in one or more concentric bilayers enclosing numerous aqueous compartments (**Figure 1**) [5]. As the name suggests, ethosomes consist of ethanol (up to 45%) along with phospholipid and water.

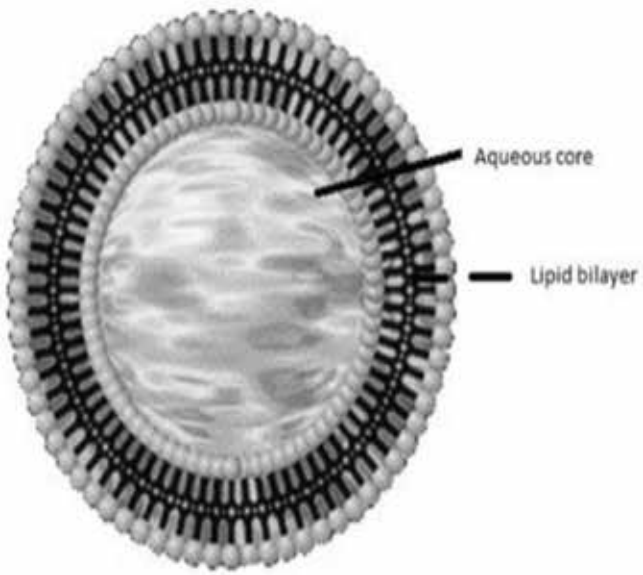

Figure 1. Structure of ethosomes (courtesy: www.pharmatutor.org).

Due to its amphiphilic nature, ethosomes can encapsulate and enhance the delivery of both hydrophilic and lipophilic drugs across the skin. When compared to the other vesicular drug delivery systems, ethosomes contain the highest amounts of ethanol (20–45%). In simpler terms, ethosomes can be described as liposomes with high ethanol content. The average size of ethosomes ranges between tens of nanometers and microns [4]. They can permeate across the skin dermal layers and deliver the drug to the deep skin or systemic circulation. The higher content of ethanol fluidizes both the ethosomal lipids and bilayers of the skin and facilitates permeation across the skin, thereby enhancing the availability of the drug to the blood.

4.1. Composition of ethosomes

The components of ethosomes are categorized under GRAS (generally recognized as safe) and usually contain ethanol, phospholipids, and water. Sometimes, they also contain propylene glycol, isopropyl alcohol, transcutol, and other surface modifiers to enhance the solubility of the drug(s) in the solvents and to enhance the permeation of drugs across the membranes. Some of the lipids which have been prominently used in the ethosomes are phosphatidylcholines and phosphatidylethanolamine containing compounds like Phospholipon 90, Soya phosphatidylcholine (S-75), and Lipoid S 100 [4].

Many experimental works have been carried out to study the effect of excipients on the size of the vesicles. It has been reported by Touitou et al. that as the amount of ethanol increases (20–45%), the size of the vesicles reduces (193–103 nm) and the amount of drug that permeates to and through the skin increases. An increase in the zeta potential to more negative values could be a possible reason for the decrease in the size of the ethosomes. They have also observed that up on increasing the amount of phospholipid (0.5–4%) the particle size of the ethosomes

increased (118–249 nm). Dayan and Touitou have shown that the nature of drug could also play a role in determining the particle size. They have observed a reduction in particle size (154–90 nm) upon increasing the concentration of trihexyphenidyl HCl (0–3%), an anti-M1 muscarinic activity used for treating Parkinsonism [6]. The researchers have attributed the reduced particle size to the surface activity of the compound.

4.2. Preparation of ethosomes

Ethosomes can be prepared in a simple manner and usually does not require state of the equipments using a cold method. However, few methods like, hot method, thin-film hydration technique, and transmembrane pH gradient methods have also been reported for the preparation of ethosomes.

4.2.1. Cold method

This is the most commonly followed simplest method for preparing ethosomes and does not require any special equipment, and the process can be easily scaled up. Briefly, the lipid and drug will be dissolved in ethanol (or mixture of ethanol and glycols), followed by addition of sufficient amount of water as a slow stream with constant stirring for sometime. Proper care should be taken to prevent the evaporation of ethanol, and the temperature during the whole preparation should be maintained at 30°C [5].

4.2.2. Hot method

This method is similar to the cold method except for the fact that the ethanolic mixture is heated to 40°C [7].

4.2.3. Thin-film hydration technique

The lipids will be dissolved using organic solvent in a round bottom flask, and the organic solvent is evaporated above the lipid transition temperature using a rotary evaporator. The thin film formed around the inner walls of the round bottom flask will be hydrated using ethanolic mixture and dispersed with a probe sonicator to obtain a suspension of ethosomes [8].

4.3. Advantages

1. Ethosomes enhance permeation of drugs across/through the skin in an efficient manner, thereby enabling the drug to reach the desired site in the skin or to the blood.
2. Ethosomes can deliver both hydrophilic and lipophilic molecules, peptides, and other macromolecules.
3. Higher entrapment efficiencies of drugs when compared to liposomes can be observed.
4. The components of the ethosomes are generally recognized as safe (GRAS) and approved for pharmaceutical and cosmetic use.

5. Excellent stability over long periods can be observed.
6. Alcohol in the ethosomes acts as natural preservative, and hence there is no necessity to add any other preservatives.
7. There is no necessity of using high-end instruments for producing ethosomes, and large-scale production is feasible.
8. The cost of manufacturing ethosomes is very cheap.
9. Good patient compliance can be observed.
10. The transport of drugs across the skin is not concentration dependent.
11. It has numerous applications in pharmaceutical, veterinary, and cosmetic segments [4, 9, 10].

4.4. Disadvantages

1. Allergic reaction can be observed if the patients are allergic to ethanol or to any of the components of the ethosomes [4, 9, 10].
2. Ethosomal carriers are pertinent only for transdermal application unlike other carriers (solid lipid nanoparticles, polymeric nanoparticles, etc.) which can be used for multiple routes.
3. As ethanol is inflammable, proper care should be taken during preparation, application, transportation, and storage.

4.5. Mechanism of permeation across the skin barriers

Ethosomes are widely explored for the delivery of the drugs across the skin. The fluidizing effect of ethanol on the lipid bilayers of the stratum corneum together with the softness of the ethosomal carrier gives them the capability to penetrate the perturbed SC lamellae more easily, thus promoting delivery of the actives into the deep layers of the skin and through the skin.

Touitou et al. proposed a hypothetical action mechanism of ethosomal system for its enhanced permeation across the skin. First, ethanol interacts and disturbs the organization of the stratum corneum lipid bilayer and enhances its lipid fluidity by reducing the melting point. The flexible ethosome vesicles can then penetrate the disturbed stratum corneum bilayers. During the process of penetration, these ethosomes fuse with the lipids present in the skin along its pathways and promote release of the drug at various points. When compared to other vesicles, occlusion slightly increases the penetration of ethosomes across the skin, which indicates that an osmotic gradient across the skin is not necessary. These data differ from that observed with elastic vesicles where permeation enhancement occurred only in nonocclusive conditions and points toward different mechanisms of action of the carriers [4, 9].

4.6. Applications of ethosomes

Ethosomes were found to highly enhance the permeation across the skin when tested against individual components of the system (ethanol, hydroethanolic solution, and ethanolic

phospholipid solution). More research has been concentrating on its application across the skin. Some of the applications of ethosomes are [3, 7, 9, 11–13]:

1. Ethosomes can be used as an efficient drug delivery system for the delivery of antibiotics to the bacteria localized within the deep skin strata to eradicate staphylococcal infections.
2. Ethosomal carrier systems can be used as a carrier for treating various dermal-based inflammatory infections.
3. Ethosomes can be used for the topical delivery of anti-psoriatic drugs.
4. It can be used for targeted delivery of drugs to the pilosebaceous units and hair follicles.
5. Ethosomes can be used for androgenic deficiency problems related to menopausal and postmenopausal syndromes.
6. Erectile dysfunction problems can also be treated using ethosomes.

4.7. Characterization of ethosomes

After preparing the ethosomes, it is highly necessary to characterize them on the basis of particle size, morphology, zeta potential, entrapment efficiency, physical state of the entrapped drug, and permeation studies across the skin.

The particle size can be measured by dynamic light scattering or photon correlation spectroscopy using Malvern particle size analyzer. Dynamic light scattering measures the average hydrodynamic diameter of particles by measuring the changes in the speckled pattern produced by the scattered light of the particles in Brownian motion. Additionally, the surface charge of the ethosomes can also be measured using Malvern particle size analyzer using a suitable probe (zeta dip cell). The zeta potential provides information about the stability of the ethosomes during storage. The morphology of the ethosomes can be visualized by atomic force microscope and transmission electron microscope. Using transmission electron microscope, 1D, 2D, and 3D imaging, measuring, modeling, and manipulating matter can be accomplished. It is based on the absorption of electron beam as it passes through ultrathin (<100 nm) samples. The AFM utilizes piezoelectric ceramics to move a specimen in nanoscale increments in the X, Y, and Z directions. The basic principle of atomic force microscope depends on the measurement of the interactive force between a tip and the sample surface using special probes made by an elastic cantilever with a sharp tip on the end.

The amount of drug entrapped in the ethosomes can be measured by separating the ethosomes from the free drug. By centrifuging the sample at high speed, heavier ethosomes form sediment at the bottom by leaving out the free drug in the supernatant. Estimating the free drug would give an idea on the amount of drug entrapped in the ethosomes. Using differential scanning calorimetry, the transition temperature of the vesicular lipid systems and the physical state of the entrapped drug can be identified. Using excised skin, the rate and the ability of the ethosomes to permeate the skin can be measured. Additionally, the mechanism by which the ethosomes permeate the skin can also be visualized by confocal laser scanning microscope.

4.8. Stability of ethosomes

The presence of ethanol and phospholipids induces high negative charge and can allow the formulations to remain stable over a long period of time. A number of studies have shown that ethosomes of trihexyphenidyl HCl were stable for at least 2 years. In another study, erythromycin ethosomes were found to be stable for 1 year. Even a marketed ethosomal formulation of acyclovir (Supra-Vir cream, Trima, Israel) has shown decent stability for 3 years. Finasteride-loaded ethosomes prepared in our laboratory have shown excellent stability for over 2 years. Hence, ethosomes offer a good stability as a drug carrier for a long period.

4.9. Safety of ethosomes

In vitro cell line studies carried out for ethosomes on skin cells (three T3 fibroblasts) did not show any toxicity. Histological observations also did not show any marked changes in the structure and thickness of the horny layer, and inflammatory cells were not observed. Both acute and continuous applications of ethosomal patch for 2 weeks in rabbits did not produce any skin irritation. Estimation of biochemical parameters in rat's blood (with regard to liver, kidney, and muscle function parameters) after 5 days of treatment with transdermal ethosomal ibuprofen gel also did not show significant differences among the treated group and the control group. Even skin tolerability tests by reflectance spectrophotometry using ethosomal systems on healthy human subjects did not induce skin erythema 12, 24, and 48 h after application. But, application of hydroethanolic solution using the same drug with an equal water/ethanol ratio to that of ethosomes resulted in significant skin erythema. Furthermore, application of various ethosomes (clindamycin and salicylic acid, acyclovir, or PGE1) to the skin of human volunteers in three clinical studies did not show any adverse skin reactions. Moreover, products formulated with ethosomal carriers have been in use for a number of years, without any reports on skin irritation or safety issues [9].

5. Book to bench experience on application of ethosomes for androgenic alopecia

5.1. Application of finasteride-loaded ethosomes

This section has been added to share our first-hand experience on developing ethosomes of finasteride in treating androgenic alopecia. As stated earlier, androgenetic alopecia is caused by the deposition of dihydrotestosterone at the androgen receptors present in the pilosebaceous unit. Finasteride is a potent 5-α reductase inhibitor which can prevent the conversion of testosterone to dihydrotestosterone. The conversion and deposition of dihydrotestosterone from testosterone occur due to the action of an enzyme 5-α reductase, which is present at the pilosebaceous unit. After conversion to dihydrotestosterone, it binds to the androgen receptor, and this hormone-receptor complex activates the genes responsible for the shrinkage of hair follicles. Hence, targeting specifically 5-α reductase

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present at the pilosebaceous unit can be a good option for treating androgenetic alopecia. Although finasteride is a clinically proven and FDA-approved type 2 5α reductase inhibitor, which can potentially inhibit the conversion of testosterone to dihydrotestosterone, its oral administration in males causes infertility. Thus, we have developed ethosomes of finasteride using a cold method (**Figure 2**) to target the pilosebaceous unit. Further, we have evaluated the ability of different permeation enhancers to enhance permeation across human frontal scalp skin and rat skin using Franz diffusion cell (**Figure 3**). The particle size of various formulations was found to be in the range of 105–227 nm (**Figure 4**). A maximum entrapment efficiency of ~90% was observed for this lipophilic drug. These ethosomes permeated well across both human frontal scalp skin and rat skin when compared to finasteride solubilized in pH 7.4 phosphate buffer saline. The values of transdermal flux across the skin showed that permeation enhancers (oleic acid, thymol, and isopropyl myristate) enhanced the permeation of finasteride across the skin. The most highlighting fact is its stability for 4 years in refrigerated conditions. We did not observe any increase in size during this period possibly due to the stabilization of the particles in the ethanolic medium [14]. Hence, ethosomes represent an exciting and the most promising drug delivery system for treating androgenic alopecia. As the preparation method is simple, ethosomes can also have good industrial viability, and we expect several ethosomal products to be marketed in the upcoming years.

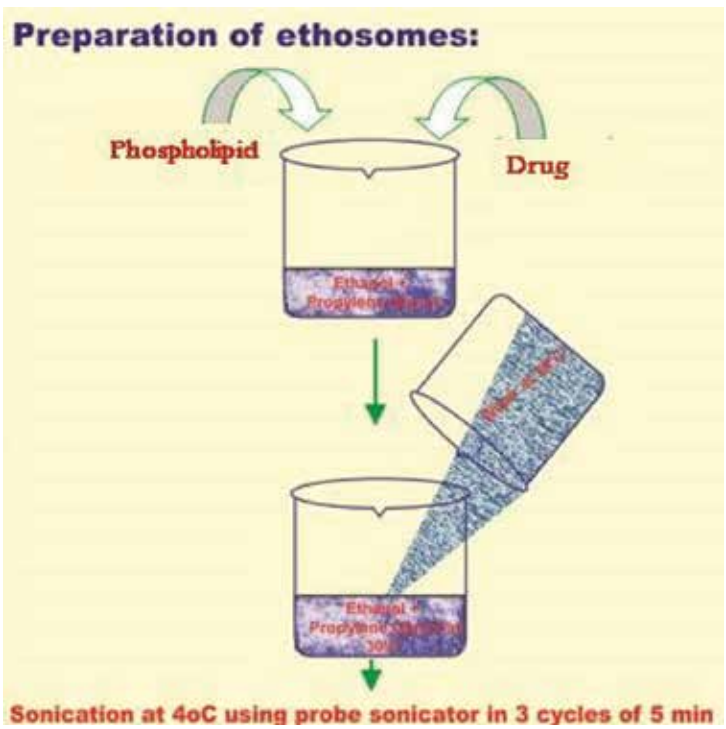

Figure 2. Preparation of ethosomes.

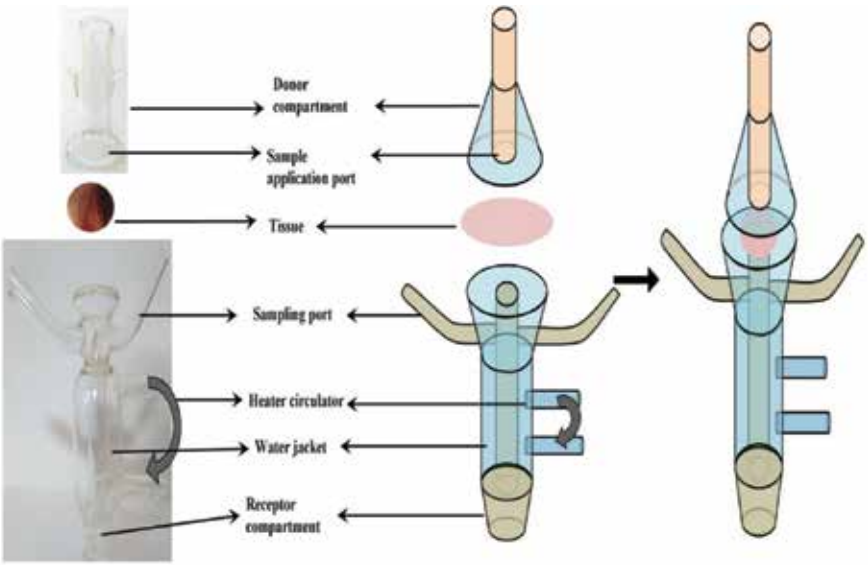

Figure 3. Schematic representation of Franz diffusion cell setup.

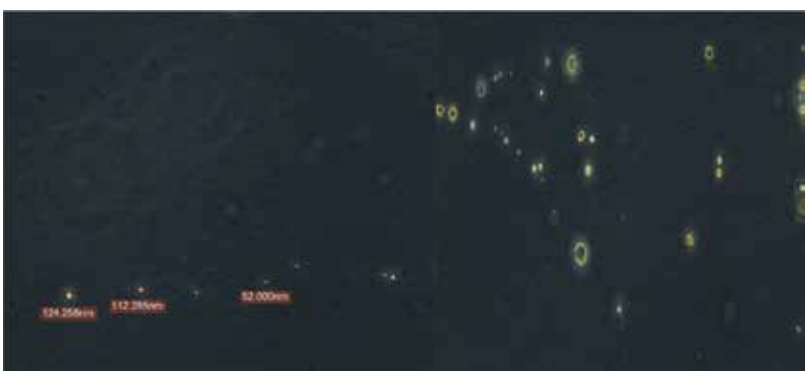

Figure 4. Phase contrast microscopic images of ethosomes (40,000×).

5.2. Application to ethosomes loaded with herbal extracts

Through this research work, an attempt was made to prepare ethosomes containing herbal extracts of *Phyllanthus niruri*, *Croton tiglium* and *Zingiber officinale*. In ancient Indian Ayurvedic scripts, these plants have been reported to combat androgenic alopecia. Initially, ethanolic extracts of *Phyllanthus niruri* (aerial parts), *Croton tiglium* (seeds), and *Zingiber officinale* (rhizomes) were prepared. After screening these extracts for preliminary constituents, the active components were identified using high-performance thin-layer chromatography and gas chromatography mass spectroscopic analysis. Ethosomes containing these herbal extracts were

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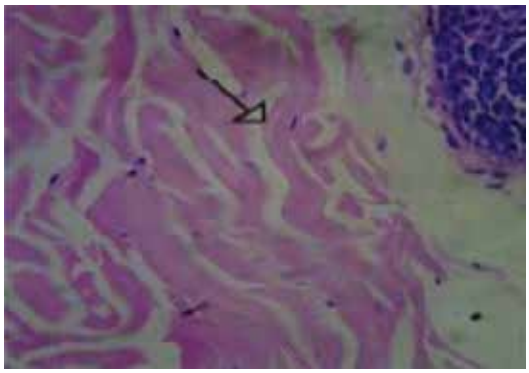

Figure 5. Histopathology of rat skin after treating with ethosomes.

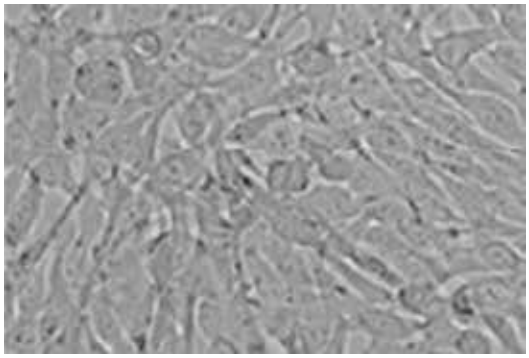

Figure 6. Viable HaCaT cells after exposure to ethosomes.

prepared using cold method. The particle size range of the ethosome formulations were in the range from 167 to 1524 nm. The histopathology of the rat skin after applying these ethosomes on the skin of rats did not show any changes in the morphology (**Figure 5**). Additionally, these ethosomes did not show any toxicity toward immortalized human keratinocytes (HaCaT) cell line. Currently, we are testing the effectiveness of these formulations in an animal model for their ability to treat androgenic alopecia (**Figure 6**).

6. Conclusion

Ethosomes have shown a tremendous promise in their ability to permeate skin. They can be safely used to deliver the drugs to and through the skin. Ethosomes can be an excellent potential carrier for the transportation of drugs to the pilosebaceous unit of the scalp, the main site for the deposition of DHT, for treating androgenic alopecia.

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Conflict of interest

There is not conflict of interest among the authors.

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Chapter 7

Anesthesia in Hair Transplantation

Wenceslao M. Calonge and Darya Louie

Additional information is available at the end of the chapter

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Abstract

Current hair transplantation techniques require a reliable anesthesia for long periods of time (2 h or more). They demand hemostasis of extended surfaces on wide-awake patients. A combination of anesthetic agents and local vasoconstrictors is needed. We present customary technical characteristics of these procedures as local nerve blocks (supratrochlear nerve, supraorbital nerve, zygomaticotemporal nerve, auriculotemporal nerve, retroauricular nerve, lesser occipital nerve, great occipital nerve) and tumescent field anesthesia. The ordinary drug combinations for premedication and procedure are presented. Special emphasis is done to discuss recommendations to cope with undesirable events that may arise during anesthesia (vasovagal syncope, anesthetic toxicity, anaphylactic and allergic reactions).

Keywords: anesthesia, loco-regional, nerve block, hair, graft, transplantation, implant, follicular unit, patient safety, supratrochlear nerve, supraorbital nerve, zygomaticotemporal nerve, auriculotemporal nerve, retroauricular nerve, lesser occipital nerve, great occipital nerve

1. Introduction

Hair transplantation techniques require a reliable anesthesia for long periods of time. Particular features of these procedures include hemostasis of extended surfaces and wide-awake, conscious patients, and a combination of anesthetic agents and local vasoconstrictors is needed. As a matter of fact, local scalp blocks are common practice in reconstruction of traumatic injuries as well as in neurosurgical interventions that involve intraoperative, functional assessments like deep brain stimulation and epilepsy surgery [1]. Another important feature is the obtainment of local tumescence to allow advantageous firmness of the donor and recipient areas [2, 3].

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Lidocaine is the preferred local anesthetic nowadays. It is a short-acting, amide agent and its onset takes place 2 min after injection. The effects are 1–2 h long but the concomitant use of adrenaline stretches them up to 4 h. A typical hair transplantation session may involve 2–3 injections of lidocaine with 2–3 h intervals. *Bupivacaine and ropivacaine* are long-acting, local anesthetics. Their price and cardiotoxicity are greater than those of lidocaine but they remain an interesting option when the anesthetic effect should be maintained long after the procedure is finished and they can be associated with lidocaine in the same injection point [4]. Additionally, some surgeons inject bupivacaine or ropivacaine at the end of procedures that demand long incisions to harvest a donor area.

Ester anesthetics (procaine and chloroprocaine) are known to trigger more allergic reactions though their systemic toxicity is lesser than for lidocaine. Their use is less popular than some 30 years ago. *Opioids* (morphine, fentanyl, and meperidine) are still used in some countries: Handling and storing them requires security measures to avoid abuse and trafficking. Their potential depression of respiratory automatism hampers a wider utilization.

Adrenaline (epinephrine) acts both on alpha and beta receptors but beta effects predominate in the long run. It increases systolic blood pressure, cardiac output volume, heart rate and coronary perfusion. It also increases myocardial consumption of oxygen. It may induce ventricular fibrillation.

The dilution of adrenaline is expressed as a ratio. The 1:1000 concentration means that there is 1 g/l or 1 mg/ml. Standard concentrations of 1:100,000 are equal to 0.01 mg/ml. When using combined 2% lidocaine with 1:100,000 adrenaline, the maximal amount of lidocaine will be achieved long before reaching the maximal dose of adrenaline. The first local effect of adrenaline is vasoconstriction. Systemic effects will only be visible after reaching a dose of 0.015 mg. These include palpitations, sweating, tachycardia and increase in systolic blood pressure up to 70 mmHg over the basal line.

It is worth to bring to mind that most local anesthetic agents act as vasodilators: They dilate local blood vessels which increases their absorption area and diffusion in the circulatory system. Therefore, local vasoconstriction at the areas of injection will result in a decreased risk of toxicity and a longer duration of the effect of the chosen anesthetic agent. Moreover, the reduction of perfusion entails a reduction in blood loss.

2. Contraindications and main risks for local vasoconstriction and anesthesia

Direct vascular traumatism may cause local ecchymosis and should be addressed by effective compression of the area. No large vessels are crossed in the commonest procedures. Needless to say, anti-inflammatory drugs and antiaggregation agents like salicylates should be avoided (when possible) during the days before the procedure.

Intravascular injection of the anesthetic mixture must be avoided through the usual cautions of anatomical knowledge and short “suction before injection” routine.

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Intranervous injection and damage are rare and may be prevented by the constant pressure flow of the anesthetic product itself that detaches the tissues. Unexpected movements of the patient and changes in angulation of the needle without a slight withdrawal may also lead to kerbed lacerations.

Overdosing is prevented by the previous estimate of doses according to the weight of the patient (**Table 1**) [5].

Contraindications to the utilization of vasoconstrictors include untreated hypertension, episodes of angor, recent myocardial infarction, severe bradycardia, auriculoventricular block (second or third degree), accelerated idioventricular rhythm, untreatable arrhythmias as well as concurrent treatment with amiodarone, procainamide, flecainide, quinidine or disopyramide. Hyperthyroidism warrants the presence of a trained anesthetist during the intervention. Patients taking tricyclic antidepressants should never receive noradrenaline or levonoradrenaline.

Vagal reaction is a repeated event. Some general measures help to reduce their numbers. Excessive lighting and temperature of the operation room must be avoided at all costs. Some patients may appreciate a first visit before the procedure takes place in order to identify it as a familiar, less hostile environment. Many surgeons make the patients lay flat for the simple nerve blocks and slowly make them sit in the final position. This is simpler when comfortable, hydraulic, bendable stretchers or operation tables are used. A disproportionate needle size may cause bigger pain, so the gauges usually have 25–30 gauge diameters. The smaller the caliber, the lesser the pain, and some teams favor the use of 32G needles. As always, an exaggerated syringe volume may cause higher pressure at the point of injection. A small vibration (by rhythmic tapping or by special devices) is well known to detour attention of the patient and make the injection more bearable. Verbal contact must be maintained all along the procedure. All injectable products should be as close as possible to body temperature: a cold injection may unchain a vagal reaction. Application of eutectic mixture of local anesthetics (EMLA) that usually includes lidocaine and prilocaine as patches or cream on the

	Maximum dose	Duration of effect	Maximum dose with adrenaline	Duration of effect with adrenaline
Amides				
Lidocaine	4 mg/kg	30 min–2 h	7 mg/kg	Around 3 h
Bupivacaine	2 mg/kg	2–4 h	3 mg/kg	3–5 h
Ropivacaine	5 mg/kg	2–6 h		
Mepivacaine	4 mg/kg	1.5–3 h	7 mg/kg	2–4 h
Prilocaine	7 mg/kg	30 min–2 h	8 mg/kg	Around 2 h
Esters				
Procaine	5 mg/kg	20–30 min	7 mg/kg	30 min
Chloroprocaine	11 mg/kg	15–30 min	14 mg/kg	30 min

Table 1. Comparison of maximum doses and duration of effect between common local anaesthetic drugs.

intended injection sites 45–60 min before injection is advisable for all patients, especially when they have declared aichmophobic events in the preoperatory anamnesis. Earphones or even movies may help to distract attention. Premedication (as 5–20 mg of oral diazepam) is more and more common.

Allergic reactions are extremely rare. They involve true hypersensitivity and the intensity of the reaction is dependent on the dose of the reagents. There is a marked susceptibility in asthmatic patients. A particular culprit is sodium metasulfite, a preserving additive for adrenaline.

2.1. Attitude in case of anesthetic toxicity

Early signs of toxicity of ester anesthetics include a metallic taste sensation in the mouth, numbness of tongue, muscle trembling, visual alterations and shivering [6]. Later signs are loss of consciousness, convulsions, coma and respiratory arrest. Metabolic acidosis, hypoxia and hypercarbia may be triggered by convulsions before respiratory arrest appears. In the face of central nervous symptoms of toxicity, the patient must be put in the Trendelenburg position and must receive supplemental oxygen. Hyperventilation may prevent the onset of seizures but once the patient gets unconscious, it seems advisable to intubate to secure an open airway. Seizures are controlled by 5 mg bolus of intravenous diazepam, to be repeated every 5–10 min in case there is no response. In case of severe bradycardia, small doses of intravenous atropine (0.5 mg) should be sequentially administered.

Opioids may induce inappropriate euphoria, nausea, vomits and respiratory depression, bradycardia and bronchospasm. Treatment includes intravenous naloxone titrated in 0.1 mg doses every 2 min until reaching a 10 mg total dose.

Benzodiazepines also may induce respiratory depression. This side effect is potentiated by opioids. This adjuvant phenomenon prevents the two families of drugs from being widely used together. Intravenous flumazenil is the common antidote for benzodiazepines and it should always be available in a practice that applies oral premedication.

2.2. Attitude in case of anaphylactic shock

Anaphylaxis is a life-threatening allergic reaction with cardiorespiratory involvement that sets in minutes. It must be remembered that cutaneous signs are not the main sign of anaphylaxis. Sudden wheezing or coughing, complaints of throat tightness or voice changes should trigger suspicion of respiratory involvement. By diminishing circulation of blood, anaphylactic shock itself averts the full treatment of the anaphylaxis. Fortunately, true anaphylaxis is rare but it is the main reason for establishing a venous line before starting these long procedures. Whenever a venous line is not established, adrenalin injectors must be available. All personnel in the operating facilities, including technicians and secretarial staff, should be trained in basic life support. On a precise spot of the premises, the basic reanimation drugs must be conspicuous and ready. Most times, adrenaline will be used when the situation corresponds to impending shock. The first line of actuation includes 0.2–1 mg of subcutaneous adrenaline (0.2 –1 ml in a standard 1:1000 solution). Most severe reactions prompt an intravenous, slow injection of 0.1 mg of adrenaline over 5 min.

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When precociously detected, a mild, allergic reaction may be jugulated by the combined intravenous use of an antihistaminic (typically 50 mg of diphenhydramine) and a corticoid (typically 100–200 mg of hydrocortisone). These can prevent the mast cells and basophils to degranulate, thus inhibiting the release of chemical mediators as histamine, prostaglandins, leukotrienes and tryptase. Antihistaminic drugs are also useful in the treatment of biphasic and protracted types of anaphylactic reaction. The patient must be kept under observation for a minimum of 4 h. Additional treatment may include intravenous fluids and supplemental oxygen. Other vasopressor drugs and intubation are more specifically delivered in emergency rooms.

2.3. Attitude in case of vasovagal syncope

In vasovagal syncope, the patient undergoes short unconsciousness as a result of abrupt descent of blood pressure. It is more frequent when the patient is in standing or sitting position. Some patients may show short nonperiodic jolts. Common prodromal symptoms include copious sweating, nausea, ear buzz, transient aphasia and vision disturbances (like "seeing white" or "clouded"). Some people with recurrent episodes of vasovagal syncope can somehow anticipate them and increase venous return by muscle contraction of their legs, elevation of both hands over the head or lying in prone position with lower limbs on a cushion.

The main risk of such an event is injury from falling. Once the patient has lost consciousness, full supine position without elevation of the head is enough to regain normal, awakened state. Trying to sit or stand up immediately after such an event may result in a new loss of consciousness, and sweating or nausea may persist for several minutes.

3. Premedication

Premedication is more and more common for these procedures though it entails that the patient must attend the premises 30–45 min before entering the operation room.

Oral diazepam in a dose of 5–20 mg is the usual choice. A (desirable) side effect of diazepam consists of elevating the threshold for convulsive effects of lidocaine. The utilization of benzodiazepine has important legal and safety implications and the patient must be formally instructed to avoid driving, handling of dangerous tools and signing legal contracts for 24 h after administration. Barbiturates are no longer in use for this indication.

4. Other intraoperatory cautions

Verbal contact should be maintained and the patient is always instructed to unambiguously verbalize painful sensations and bizarre feelings before the procedure starts. Recurrent assessment of cardiac frequency, blood pressure and pulse oximetry seem advisable, at least every

30 min [7, 8]. Small, commercial devices for the assessment of cardiac frequency and pulse oximetry are relatively inexpensive.

Though many surgeons may boast of never having unexpected events, an intravenous line should be placed once the premedication has done its effects.

As for the blocks, patches of EMLA are an interesting option in aigmophobic patients 45 min prior entering the operation room. They can be administered at the same time as the oral premedication.

In case the procedure is carried out in a small practice away from hospital premises, an evacuation plan with safe medical transport must be always established. Paramedical crew should find no major obstacles for quick transfer and evacuation.

5. Technique of local anesthesia

Different areas of the scalp correspond to different sensory nerves [9–12]. These include the supraorbital and supratrochlear branches of the ophthalmic nerve (V_1), the zygomaticotemporal branch of the maxillary nerve (V_2), the auriculotemporal branch of the mandibular nerve (V_3), the lesser occipital nerve and the retroauricular nerve from the third (and second) cervical roots as well as the greater occipital nerve from the second cervical root (**Figure 1**).

The first phase of anesthesia involves several peripheral nerve blocks by exclusive anesthetic injection WITHOUT adrenaline. The second phase involves anesthetizing and making tumescent the whole operative field by a mixture of adrenaline and anesthetic agent.

5.1. Supratrochlear nerve block

The supratrochlear nerve emerges medial to the supraorbital notch and it lies in the medial third of the upper orbital rim. It supplies sensory innervation to the medial area of the upper eyelid, the root of the nose and to the frontal scalp and forehead. It must be remembered that surgery on one side of the forehead requires a block of the contralateral supratrochlear nerve due to overlapping territories [13]. For a supratrochlear block, the main landmark lies on top of the angle formed by the eyebrow and the nasal spine. At this point the nerve is in contact with the bone (**Figure 2**). After the injection, firm pressure is applied for better anesthetic spread and prevention of ecchymosis.

Complications of this block (and the following one) are very rare but they include hematoma, intravascular injection and eye globe injuries.

5.2. Supraorbital nerve block

The supraorbital nerve emerges through a foramen on the middle of the orbital rim. The foramen is palpable as a clear step in osseous continuity. The supraorbital nerve exits with its vessels through the *foramen supraorbitalis* and continues superiorly between the *levator palpebrae*

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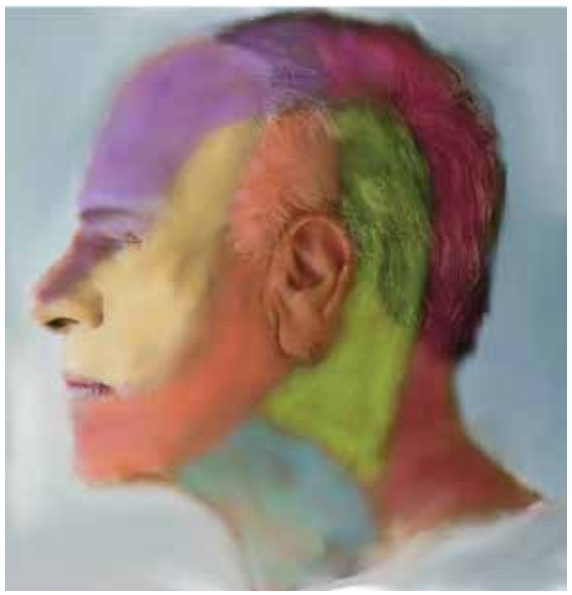

Figure 1. Surface areas that correspond to the sensitive territories of the lesser occipital and retroauricular nerves (green), the greater occipital nerve (pink) and the three divisions of the trigeminal nerve: ophthalmic (violet), maxillary (orange), and mandibular (red).

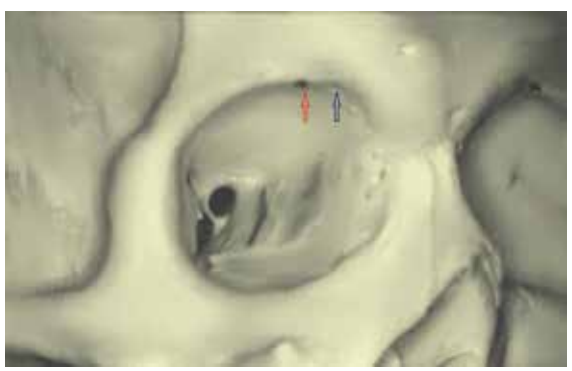

Figure 2. The foramen supraorbitalis (red arrow) lies at the junction of the middle and inner thirds of the superior orbital rim. It marks the emergence of the supraorbital nerve and the supraorbital artery. Mild pressure to the nerve causes some pain. The needle is inserted over the eyebrow level and advanced until touching the periosteum. After injecting 1 ml of lidocaine, the needle is redirected medially and 1 ml of lidocaine is injected along the way. Finally, 1 ml is injected again at the final site that corresponds to the supratrochlear nerve (blue arrow).

superioris and the periosteum. *The foramen supraorbitalis* is easily palpable by following the orbit rim 2 cm from the midline in adults and it loosely corresponds to the same sagittal plane as the pupil when facing the patients head. Previous LeFort fractures may modify reference points. A 25–30 gauge needle is intradermally introduced 0.5 cm under the inferior edge of the eyebrow and is directed medially and cephalad. Once the needle tip is near the supraorbital notch, after

test aspiration, local anesthetic solution (0.5–1 ml) can be injected in an extended subcutaneous wheal. Injection *into* the foramen must be avoided (**Figure 2**).

It is worth noting that the supratrochlear nerve can be blocked immediately following supraorbital nerve block, without removing the needle, by directing the needle about 1 cm toward the midline and injecting an additional 0.5 ml of local anesthetic.

5.3. Zygomaticotemporal nerve block

One of the two branches of the zygomatic nerve (branch of the maxillary division of the trigeminal nerve) is the zygomaticotemporal nerve. It receives sensitive innervation from the temporal area and a small portion of the forehead (**Figure 3**). The nerve lies lateral to the orbital rim in the fossa temporalis [14]. It is usually approached through an injection in the middle third of the upper aspect of the malar branch, targeting the orbital rim and delivering 1 ml of 2% lidocaine while advancing the needle in a 45° angle. The bony rim of the orbit should never be reached to avoid migration inside the orbit. At the final point, 1 ml is carefully injected.

5.4. Auriculotemporal nerve block

The auriculotemporal nerve is a branch of the mandibular division of the trigeminal nerve. It receives sensitive innervation from the posterior area of the temple and the superior two-thirds of the anterior surface of the pinna [15, 16]. It crosses through the parotid gland and continues anterior to the auditory canal escorting the superficial temporal artery. It intersects the zygomatic arch near the surface (**Figure 3**).

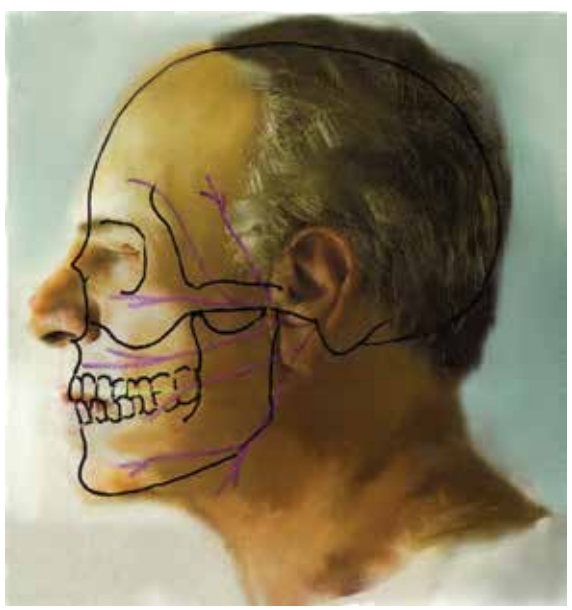

Figure 3. Surface references for the main branches of the trigeminal nerve.

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The auriculotemporal nerve is blocked by injecting above the posterior portion of the zygoma, anterior and superior to the tragus and behind the superficial temporal artery that must be avoided. A total of 1–2 ml of 2% lidocaine is delivered after delicate aspiration.

5.5. Lesser occipital nerve and retroauricular nerve block

The lesser occipital nerve is a small branch from the ventral primary rami of the second and third cervical roots. It receives sensitive innervation from the lateral occipital zone and the upper earlobe (**Figure 4**). It runs posteriorly at 45° to the vertical, parallel to the posterior border of the sternocleidomastoid. A subcutaneous injection along the hairline behind the ear is the way to block both nerves [17].

5.6. Greater occipital nerve block

The greater occipital nerve provides cutaneous innervation to the major portion of the posterior scalp from the inion to the vertex. It stems from the second cervical nerve root that comes out between the atlas and the axis. It runs between the *obliquus capitis inferior* and *semispinalis capitis* before piercing the latter muscle. After piercing the aponeurosis of the *trapezius,* it

Figure 4. Reference points for the lesser occipital and retroauricular nerves (red arrow) and the great occipital nerve (blue arrow).

becomes subcutaneous distally to the superior nuchal line. Typically, it lies 4 cm lateral to the inion and medial to the occipital artery. The pulsation of the occipital artery is easy to palpate (**Figure 4**). There is considerable variation in its position (1.5–7.5 cm from the inion) [18, 19] and some anesthetists advocate the use of ultrasound probes for safe localization [20].

A 25 G is driven at 90° toward the inion. After aspiration, 1–3 ml of local anesthetic is injected and pressure should be maintained over the site of injection to soak the nerve and to achieve hemostasis once the needle has been withdrawn. Numbness up to the top of the head is a sign of an effective block.

5.7. Field anesthesia

Once the regional blocks have been performed, the surgeon can proceed to the anesthesia of the operative field in a "crown" fashion. Some surgeons advocate only using this kind of anesthesia without nervous blocks (or just the supraorbital and supratrochlear block). Now, a significant, desired outcome is the tumescence of the donor and recipient areas. Adrenaline would prevent hemorrhage and is an integral part of the anesthetic mixture. The injections cause a dissection of the subcutaneous layer providing hemostasis and a firm, stable working plane. However, the onset of the effect is longer than for the blocks and may take about 10–15 min. Anesthesia is achieved by several injection points 1 cm caudally to the hairline or its intended location and each injection point may be used as a departing point for several anesthetic tracts. The number of injection points is highly variable and depends on the preferences and experience of the surgeon. The same applies to the composition of the injected mixture. A common "recipe" employs 50–100 ml of 2% lidocaine and 2–5 ml of adrenaline 1:1000 in 1 l of 0.9% saline (but the whole amount of the mixture is not needed). Some "recipes" include 10 ml of 8.4% sodium bicarbonate to diminish the irritating effects of the low pH of the mixture [2, 3, 21]. Many surgeons add bupivacaine to achieve a lasting effect or even corticoids to reduce the swollen, traumatic appearance of the scalp in the days after the procedure.

5.8. Intravenous sedation

There are remarkable differences in attitudes toward this kind of anesthesia and they vary according to regions and training [21, 22]. The presence of a standby anesthetist to cope with unwanted side effects seems unnegotiable to most surgeons. Nowadays, a combination of midazolam and fentanyl is the preferred option.

5.9. Inhalatory anesthesia

Inhalatory anesthesia is not justified for elective hair transplantation but rare indications include pediatric patients that undergo procedures after burn injuries. Though cumbersome, some clinics use nitrous oxide sedation under supervision of an anesthesiologist or a trained nurse.

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Edited by Muhammad Ahmad

Being the editor of the book *Alopecia*, I feel delighted to work with the world-leading publisher, IntechOpen. The current book has chapters emphasizing a variety of alopecias. The administration of newer drugs may treat hair loss by a variety of mechanisms. All the clinical variants of alopecias are discussed in detail.

The book will help dermatologists, students, hair transplant surgeons, and physicians related to hair loss problems, giving them the opportunity to understand basic pathophysiological, clinical, and medical management options. The basic idea of the book is to diagnose alopecia correctly.

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