DEPARTMENT OF DERMATOLOGY
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Overview of Alopecia: A Dermatopathologist's Perspective

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A Dermatopathologist can be an invaluable resource to the clinician.

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Abstract

Hair loss affects men and women of all ages and frequently has significant social and psychologic consequences. An awareness and understanding of the various microscopic tests that can be performed is helpful in making an accurate diagnosis. A Dermatopathologist can be an invaluable resource to the clinician.

Introduction

Hair loss (alopecia) can be caused by many factors ranging from genetics to the environment. While androgenetic alopecia is by far the most common form of alopecia, other forms including those related to underlying diseases are also frequently encountered. This review will highlight the roles of microscopic evaluation in the management of alopecia with Table 1 summarizing its importance.

Tests

Although not enough can be said for the importance of history and physical examination, the various tests that require microscopic evaluation can provide additional information to aid the clinician in making an accurate diagnosis.[1]

Hair Pull Test, Hair Pluck Test and Trichogram

Hairs, whether they are pulled, plucked or spontaneously shed, can be examined under the microscope without any special processing. A hair pull test can give a rough estimate as to how much hair is being lost. Gentle traction is placed on a small portion of hair by sliding the fingers down the hair on three separate areas of the scalp. The extraction of less than three hairs is considered a negative pull test, whereas extraction of greater than six is considered a positive test.[2] Before performing the test, the physician should remember to ask if the patient recently washed their hair since the results of the hair pull test may not be as accurate if the patient recently washed their hair. A forceable hair pluck is performed by grasping approximately 50 hairs with a rubber-tipped surgical clamp and quickly jerking the hairs from the scalp. Hairs may be placed on a slide for visualization (trichogram). Tape or clear nail polish may be used to keep the hairs in place. A few drops of immersion oil can be placed on the hairs and a second glass slide or cover slip is placed on top. Taping the slides together may help the ends of the hair to lie flat especially if the hair is curly.[3] Although a trichogram may be performed by the clinician, a dermatopathologist can be utilized to help with interpretation.

In the normal situation, the only hairs that can be gently and easily pulled from the scalp are those that are in the telogen phase of the hair cycle. If another type of hair is easily removed from the scalp, it can be

Table 1
Contribution of Microscopy to Alopecia Management
- Accurate Diagnosis
- Helps in Determining Prognosis
- Helps in Determining Treatment

regarded as an abnormal situation. Under the microscope, telogen hairs have completely cornified which prevents bending relative to the long axis, a process that can be seen in anagen hairs. Telogen hairs have an expanded, club shaped bulb. Anagen hairs have a pigmented bulb, characteristically in the shape of a triangle that results from the hair tearing away from the hair papilla. Due to plucking, the outer root sheath or both sheaths remain in the dermis and can give the plucked anagen hair a dysmorphic (bent) appearance. Plucked catagen hairs resemble telogen hairs characteristically shows a translucent epithelial sac surrounding the club.[3]

Biopsy

Biopsy, a Greek-derived word (bio-life; opsia-to see) loosely translated as "view of the living",[4] is used to support the clinical findings in alopecia by narrowing or making a diagnosis. It can also serve as an indicator of prognosis – Is there a chance of hair recovery and re-growth? Is there inflammation that can be treated and hopefully slow or halt the progression of disease? It is important for the clinician to never underestimate the importance of dialogue with the dermatopathologist as it is only through this partnership that the patient can really be helped.

Although the number of biopsies performed and selection of the most fruitful site is somewhat dependent on the disease process, typically the best place to biopsy is the leading edge of the process, where there is some hair loss and some erythema. It is generally not recommend to biopsy an area with complete hair loss, as there is limited information that can be gained by performing a biopsy in this area.

A 4 mm punch biopsy oriented in the direction of hair growth is advised.[5] A 4mm punch biopsy is recommended because hair counts that are performed on horizontal sections are standardized. Performing a smaller punch biopsy can lead to the misperception on histology that the number of follicular units are diminished.[3] Additionally, as a dermatopathologist I cannot stress the importance of orientating the biopsy in the direction of hair growth such that the hairs are cut transverse rather than tangential on horizontal sectioning. It is equally important for the clinician to remember to handle tissue gently. Although often unintentional, crushing of tissue with tissue forceps during the procedure or rough handling of tissue can destroy the histological features, rendering accurate microscopic assessment difficult.

Additional biopsies may be required if there are more than one clinical presentation or if direct immunofluorescence (DIF) studies are desired. Biopsies obtained for DIF should be placed in the appropriate medium, such as Michel's solution, and it is generally not advisable to perform one punch biopsy and submit half of the specimen for routine processing and the other half for DIF studies. The importance of an adequate clinical history and description on the pathology requisition sheet cannot be emphasized enough and will enable the pathologist to provide the best clinicopathological correlation.

There are various manners a biopsy for alopecia can be processed. Visualization of the epidermis requires vertical section, making it appropriate for entities such a lichen planopliaris (LPP) and lupus erythematosus (LE). Vertical sections are limited, however, on the number and types of hairs visualized and thus have considerable sampling error.[6] Hair counts can only be performed on horizontal sections. Horizontal sections also allow for better evaluation of the hair cycle and the ability to see the entire hair follicle including the level / location of inflammation.[7,8] Ideally, it is best for the clinician to seek an experienced laboratory for processing of an alopecia biopsy since performing and interpreting horizontal sections is unfamiliar to many laboratories and pathologists.

The following sections will discuss a few alopecias with emphasis on special considerations for microscopic evaluation (summarized in Table 2).

Non-Scaring Alopecias

Androgenetic alopecia

Androgenetic alopecia (AGA) is also referred to as common balding, hereditary balding and male or female-pattern alopecia. It is extremely common and mediated by the effects of androgens on susceptible hairs. Aside from genetic factors, conditions that lead to the overproduction or exogenous administration of androgens can also cause or worsen androgenetic alopecia. The typical clinical pattern observed in AGA begins with recession of the frontal hair line followed by thinning of the vertex scalp with ultimate fusion of the two areas as the disease progresses.[9] In women there is widening of the central part of the frontal scalp with a pattern that has been likened to a Christmas tree. The sparing of the frontal hair line is also characteristic in women.[3]

Hair pull test can be useful in distinguishing AGA from telogen effluvium. Biopsy is typically helpful when the pattern of AGA is not typical or to exclude telogen effluvium or early scarring alopecia. In early disease, the ideal specimen should be 4mm punch biopsies of involved and uninvolved areas such that a comparison of follicular size and

Table 2
Special Consideration for Microscopic Evaluation of Alopecia

Alopecia	Special consideration(s)
Androgenetic alopecia (AGA)	Hair pull test - useful in differentiating AGA from TE Biopsy: Early disease: Two 4mm punch biopsies (lesional - frontal or vertex scalp and uninvolved - occiput scalp) processed horizontally so that hair counts can be performed Late disease: One 4mm punch biopsy from involved scalp processed horizontally so that hair counts can be performed
Telogen effluvium (TE)	Hair wash test / Hair pull test followed by trichogram - numerous clubbed hairs (if dysmorphic anagen hairs think of AA) Hair pull test followed by trichogram - telogen count must exceed 20% for diagnosis Biopsy: 4mm punch biopsy from edge of involved scalp processed horizontally
Alopecia Areata (AA)	Hair pull test followed by trichogram - telogen and pencil point shafts Biopsy: 4mm punch biopsy processed horizontally at the active / advancing margin of alopecia
Fungal alopecia	KOH preparation of scaly area with broken hairs Hair pull often not useful Biopsy: If clinical suspicion is high and biopsy is not diagnostic, still culture
Trichotillomania (TT)	Trichogram - to exclude hair shaft disorders Biopsy: 4mm punch biopsy of lesional area processed horizontally; helpful in establishing and convincing of diagnosis
Scarring alopecias	Biopsy: 4mm punch biopsy of the active boarder; clinical consideration can be given to submission of additional biopsies including biopsy for DIF

DIF - direct immunofluorescence

hair number can be performed. A single 4mm punch biopsy can be performed from the involved scalp if the disease is more advanced.[3]

Telogen Effluvium

When an abnormally large number of anagen hairs on the scalp enter the telogen phase, a telogen effluvium (TE) occurs. This may be caused by a variety of conditions - physiological, pathological, endogenous and exogenous resulting in stress on the follicle. The stressed follicle prematurely enters catagen which commits them to proceed to the telogen phase after which they are shed. The stress event typically occurs 3-4 months prior to a TE. Interestingly, the majority of TE cases likely do not come to clinical attention as an apparent reduction in hair density is only appreciated if a substantial number of hairs are lost. Additionally, since TE is reversible, the disease may be undetected clinically once the recovery phase begins, especially in cases where the patient is not seen by a physician prior to the recovery phase. If alopecia is present in TE, it diffusely affects the scalp. Complete hair loss is unusual in TE, however, if severe, it can effect non-scalp hair including the eyebrow hair and pubic hair.[10]

Patients may present to the clinic with a mound of hair, usually collected from the shower drain or hair comb (referred to as the "wash test") that can be evaluated microscopically.[11] A hair pull test followed by microscopic evaluation will reveal several clubbed hairs. If a hair pluck is performed, telogen hair counts should exceed 20% for a diagnosis of TE to be made.[3] Of note, the diffuse variant of alopecia areata can be a deceptive clinical mimic of TE.[12] Lack of stressor, dysmorphic anagen hairs on hair pull test and 4mm punch biopsy processed horizontally can be helpful. An important point to remember is that TE can unmask early androgenetic alopecia. Thus, if this is a clinical concern for AGA paired biopsies form the frontal and occipital scalp can be performed.[3]

Alopecia Areata

Alopecia areata (AA) is another common non-scarring alopecia. Aside from the scalp, alopecia areata can affect hair in any area of the body. The pathogenesis is believed to be a multifactorial autoimmune process of unknown etiology. The clinical presentation of AA can be quite varied, with the degree of involvement ranging from a small, well-defined, circular, smooth bald patch to total scalp hair loss (alopecia totalis) to total body hair loss (alopecia universalis).[13] Because the inflammation in AA is deep in the skin, there are typically no visible changes, such as erythema, to the patch of alopecia. Additionally, there is typically no surface change that is appreciated. "Exclamation point" hairs (short hairs that taper as they approach the scalp) are characteristic and found most often at the edge of the active / advancing margin of alopecia. The hair pull test in AA reveals many telogen and pencil point shafts (hairs with tapered ends).[3] 4mm punch biopsy at the active / advancing margin of alopecia can be helpful in quantifying the degree of inflammation and distinguishing AA from other alopecias.

Dermatophyte-induced alopecia (tinea capitis)

Tinea capitis is an infectious form of alopecia caused by pathogenic fungus infecting the superficial portions of the skin and hair shaft. Worldwide, the fungus *Microsporum audouini* is a very common cause of tinea capitis, but increasingly *Trichophyton tonsurans*, a large-spore endothrix infection, can also cause tinea capitis, especially in the United States and Latin American countries. The disease is caused by invasion and subsequent proliferation of the fungus within the hair shaft leading to hair fragility and breakage leaving a bald patch of skin.[13]

Although many clinical presentations are possible, including mild scaling within an alopecic plaque to intense erythema, induration, pustules and purulent discharge, the classic presentation is a bald, scaly patch where the follicular ostia are filled with keratinous debris.[13] "Black dots" may be seen resulting from the residua of the broken, pigmented hair. An important clinical clue that can be helpful in distinguishing tinea capitis from other forms of alopecia is the presence of post-auricular and cervical lymphadenopathy.[3]

Diagnosis can be made by scraping the scalp in the area of the broken hairs for potassium hydroxide (KOH) preparation. An attempt to visualize abnormalities of hairs obtained by hair pull test is often not useful, as the long hairs obtained are not those infected. Interestingly, infected hairs can be difficult to find on biopsy, thus if the clinical suspicion is high, fungal culture may yield better results.[3]

Trichotillomania

Trichotillomania (TT) is recognized by the DSM IV as an impulsive behavior disorder. It is also linked to many mood and anxiety disorders. The typical age of onset is 12-13 years of age with most patients being selective with the areas of hair pulling.[12] Common areas of involvement include the scalp, eyebrows, eyelashes, body hair, facial hair, and pubic hair. The alopecic areas are typically sharply demarcated and unusually shaped. Any hairs that are present are of varying length. There is no scale or erythema; however, excoriations may be present.[13]

The role of light microscopy lies in excluding other hair disorders, including those of the hair shaft that can cause hair fragility. Biopsy can be crucial for establishing the diagnosis and convincing the patient, especially since patients frequently deny hair plucking.[3] Of note, it has been reported biopsies taken within eight weeks of onset of TT have the highest yield.[17]

Scarring Alopecias

Scarring alopecia, also known as cicatricial alopecia, refers to a collection of hair loss disorders including entities such as lichen planopilaris, central centrifugal cicatricial alopecia folliculitis decalvans, and dissecting cellulitis. Scarring alopecia may also be part of a systemic condition such as lupus erythematosus. While many forms of scarring alopecia exist, they all share a common premise of potentially permanent and irreversible destruction of hair follicles with replacement of scar tissue. Most forms of scarring alopecia tend to first occur as small patches of hair loss that may progress over time Inflammation causing redness can be an associated finding. The loss of follicular openings or ostia is a key clinical finding that differentiates scarring from non-scarring alopecia.[18] Because the scarring alopecias can progress to permanent hair loss, the importance of early diagnosis and treatment strategies to forestall permanent hair loss is of greatest significance with biopsy playing a crucial role.

Lichen Planopilaris

Lichen Planopilaris (LPP) is the most common cause of scarring alopecia and typically affects middle aged women, with an average of onset of 51 years, and without racial predilection.[19] Tumor necrosis factor-alpha, interferon-alpha and the peroxisome proliferator-activated receptor-gamma pathway have been implicated in the pathogenesis.[20-22] The follicular keratotic lesions result in inflammation and destruction of the hair follicle with secondary hair loss. Presentation may vary depending on the stage of the disease. In general, patients typically present with scaling, perifollicular erythema, and an area of atrophic scarring with peripheral follicular papules.[19]

Biopsy for routine processing (hematoxylin and eosin) and direct immunofluorescence testing can be helpful in nailing down the diagnosis and determining the stage of disease. The latter can be important to the clinician in guiding treatment and prognosis counseling. Direct immunofluorescence in LPP is associated with Civatte / colloid bodies and can be useful in distinguishing LPP from alopecia caused by lupus erythematosus.[3]

Lupus Erythematosus

Hair loss is lupus erythematosus (LE) is common. Although the most common pattern of hair loss seen in LE is the scarring type, LE may also cause non-scarring patterns. Both the non-scarring and scarring form of alopecia secondary to LE are discussed in this section since the nonscarring plaques of LE alopecia can progress to a scarring form if left untreated.[23]

The nonscarring form of alopecia secondary to LE presents clinically as patches of partial or total hair loss throughout the scalp. Mild erythema may be present.23 Interestingly; the residual hairs in the balding patch are almost all telogen hairs or dystrophic anagen hairs making trichogram useful in this situation. Biopsy is also beneficial in making the diagnosis, but should be used in

conjunction with serology as distinction from other causes of alopecia, such as alopecia areata and syphilitic alopecia can be difficult on histopathological grounds alone.3 Some dermatopathologists advocate performing two biopsies so that one can be processed horizontally and the other vertically, with the latter allowing visualization of the dermoepidermal junction. Certain laboratories, such as our own, use an alopecia protocol that allows for visualization of the epidermis vertically following horizontal evaluation.

Chronic cutaneous lupus erythematosus (CCLE) is the term used to encompass a variety of clinical expressions of cutaneous LE. The lesions of discoid chronic cutaneous lupus erythematosus are a cause of inflammatory scarring alopecia. Discoid lupus erythematosus (DLE) is more likely to occur in adults, predominately in women. Some patients are asymptomatic, while others can experience itching or tenderness. On physical examination, this disease presents with dilated keratotic follicular plugs, erythema, epidermal atrophy and alopecia. A common finding in darker-skinned patients is central hypopigmention with peripheral hyperpigmentation.25

Histological evaluation following hair pull test can shows an increased anagen count in active disease. Biopsy of the active edge of alopecia can help make the diagnosis. Performing an additional biopsy for direct immunofluorescence can also be helpful with granular IgG and C3 along the dermoepidermal junction seen in 70-90% of cases.[3]

Central Centrifugal Cicatricial Alopecia

Central centrifugal cicatricial alopecia (CCCA) is the most common form of scarring alopecia in African American patients, with adult females being three times more likely to suffer from this disease than males. The average of onset is the mid-30s. There is a close association of the disease with the use of various hair care practices. CCCA is predominately located in the vertex or on the crown and spreads in a centrifugal pattern. Common symptoms include pruritus and tenderness.[25] Pustules and crusting can also be found in some patients as it takes on what some consider the inflammatory variant of this entity, Folliculitis Decalvans.[3]

Biopsy is helpful in confirming the diagnosis. Importantly, horizontal sections should be requested as even a 4mm biopsy that is taken from the periphery of the alopecic area may contain only one or two "diagnostic" follicles since the involved follicles are selectively destroyed. The utility in performing a biopsy for direct immunofluorescence is variable, with direct immunofluorescence serving to exclude other entities.[3]

Conclusions

The approach to a patient with alopecia begins with a complete history and physical, but also involves the use of ceratin tests that require microscopic evaluation. A carefully selected, performed and interpreted trichogram or biopsy can be critical in rendering an accurate diagnosis.

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Disclosures

None reported.

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