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Review

Primary scalp alopecia: new histopathological tools, new concepts and a practical guide to diagnosis

The diagnosis of primary scalp alopecia remains one of the most challenging fields in dermatopathology. In this review, we would like to connect the established classification of primary alopecia into scarring (cicatricial) and non-scarring (non-cicatricial) with current concepts. We introduce a simplified pathway for the diagnosis of the most common causes of alopecia, including a discussion of tissue processing techniques and use of immunohistochemistry.

Keywords: non-scarring (non-cicatricial) alopecia, primary scalp alopecia, scarring (cicatricial) alopecia

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The diagnosis of primary scalp alopecia remains a challenge for both clinicians and dermatopathologists. New scalp grossing techniques have made this challenge easier, but there are still challenges in the utility of the currently established classification of alopecia (i.e. cicatricial vs. non-cicatricial).[1,2] In this manuscript, we will attempt to improve this classification by introducing new concepts on alopecia and a simple 2-step method for the diagnosis of the most common causes of alopecia based upon a review of the current knowledge and our experience in diagnostics of alopecia.

Tissue processing

Classical grossing of skin biopsies with vertical sections may be useful for cicatricial alopecia, but they are often not helpful for non-scarring processes, such as female pattern hair loss (androgenetic), which is the most common diagnosis of alopecia in women (Fig. 1).[3,4] Vertical sections show a limited number of follicular hair units (2–3 in a 4 mm punch biopsy),[5] and the cuts of the follicles are often tangential, since hairs often grow at an angle. Vertical sections also do not give information

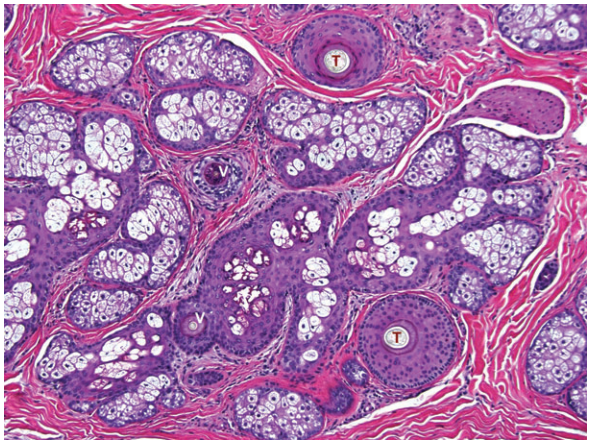

Fig. 1. Female pattern hair loss. Assessment of terminal (T) to vellus (V) hair ratio is possible only in horizontal sections.

concerning follicular density, hair cycle and hair miniaturization (terminal-to-vellus hair ratio), making interpretation of non-scarring, diffuse alopecia difficult.[6–8] Numerous studies have assessed horizontal vs. vertical sectioning, with variable conclusions.[7,9–12] In our opinion, all of the scalp grossing techniques work well only if the specimen is handled by experienced staff technicians. The key remaining problem, however, is the lack of implementation of proper grossing techniques by laboratories that are not familiar with alopecia.

New grossing techniques, which provide both horizontal and vertical sections, have dramatically improved histopathological diagnoses. Headington revolutionized histopathological evaluation of alopecia in 1984 by emphasizing the value of a follicular morphometric approach by transverse sectioning the scalp biopsy.[5] Surprisingly, the transverse sectioning of the scalp, similarly to the Headington technique, had already been proposed in the 19th century.[13] Following the technique proposed by Headington and later by Whiting[9], a single transverse section is performed 1 mm below the epidermal surface. Both cut sides of the specimen are embedded down in the cassette, so that the levels become progressively more superficial in one half of the specimen and deeper in the other half.[14] Improving this technique, Frishberg & Sperling proposed trisection or quadrisection, rather than the standard bisection, thereby allowing an even more thorough analysis of the tissue segment. To accomplish this, a 4 mm scalp biopsy specimen is cut transversely into three or four disks, and the deep surfaces are inked with their inked side placed down in the cassette.[15] In our opinion, these techniques were not universally adopted because pathologists were alarmed by the inability to easily analyze the surface epidermis. Elston proposed the performance of two biopsies, with one specimen being sectioned horizontally and the other specimen being sectioned vertically submitting half for direct immunofluorescence and half for vertical sections within the same block as the specimen for horizontal sections.[14,16] The inconvenience with this approach was the requirement of two scalp biopsies.

An interesting solution to the limits of the aforementioned techniques was the Tyler technique, which involves first vertically sectioning the entire specimen, followed by transverse sectioning of one half. Thus, one half of the specimen shows transverse sections and the other half vertical. As with aforementioned transverse sectioning techniques, serial cuts produce levels both towards the subcutaneous fat and the epidermal surface (Fig. 2).[8,17] The limitation of this technique is that fewer follicles are visualized transversely, limiting the evaluation of hair density, terminal-to-vellus hair (T : V) ratio and catagen/telogen (CT) hair count.

We have employed the novel HoVert (Horizontal & Vertical) technique, introduced in 2011, which provides both horizontal and vertical sections from a single biopsy. In our experience, this procedure has improved and simplified diagnostics, mostly because fewer level sections are necessary.[18] A single 4 mm punch biopsy is appropriate if a single disease process is suspected, but additional biopsies may be necessary if there is more than one disease process at play.[19] Important to note, the HoVert technique is ideal for specimens obtained with the 4 mm punch tool used in the United States. However, the 4 mm punch tool used in Europe is smaller and HoVert sectioning is more difficult. In our experience, one solution is to send the specimen through overnight fixation before sectioning, so the tissue is firmer and easier to section precisely. Alternatively, clinicians can seek out a larger punch tool. The HoVert technique involves trisecting the specimen, once just below the epidermis and once at the dermal–subcutaneous junction. If the biopsy is too thin (i.e. little subcutis present), the specimen should be bisected rather than trisected (the specimen is not sectioned horizontally at the dermosubcutaneous junction). The HoVert technique allows both a vertical examination of the interfollicular epidermis, follicular ostium, superficial infundibulum and papillary dermis, and a transverse examination of the superficial and deep reticular

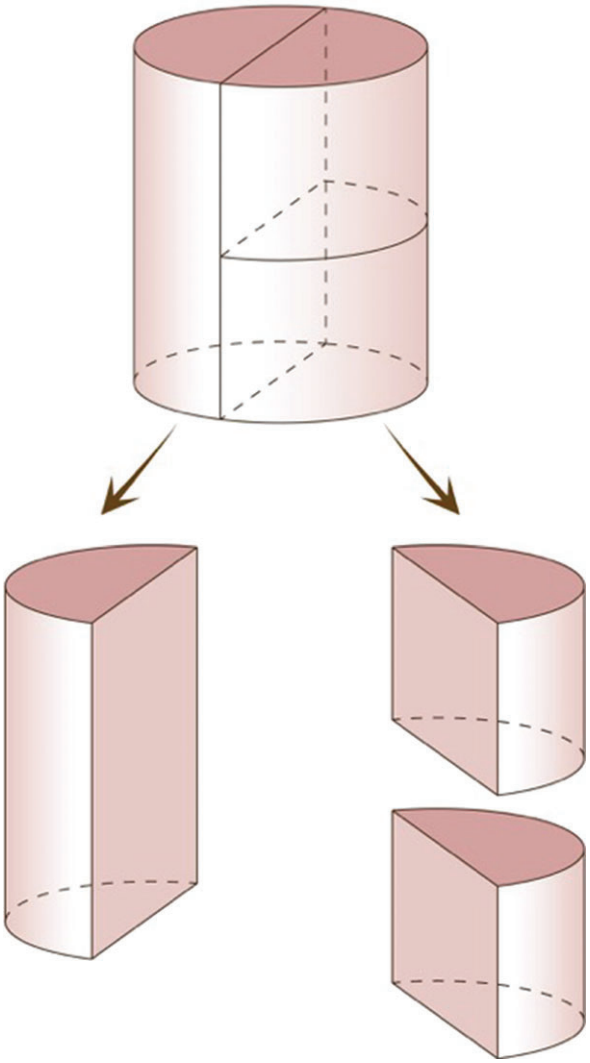

Fig. 2. The Tyler technique. This technique involves vertical sectioning of the specimen, followed by transverse sectioning of one half. The two resulting half circles are embedded facing one another, fresh cut side down.

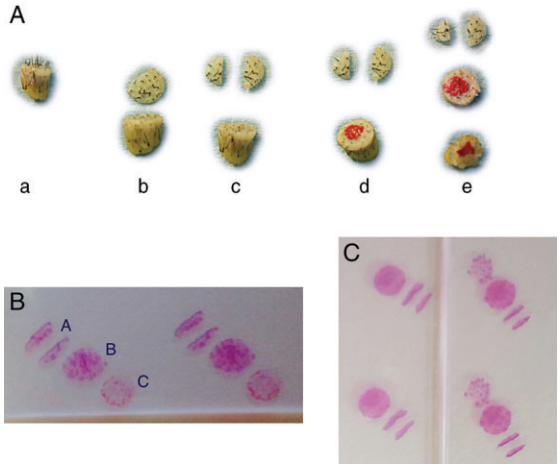

Fig. 3. The HoVert (Horizontal Vertical) technique. A) HoVert grossing. a) A 4 mm scalp biopsy specimen is processed. b) The biopsy specimen is transected horizontally (transversely) approximately 1 mm below the skin surface to create an epidermal disc and a lower portion. c) The epidermal disc is bisected and embedded vertically. d) The upper surface of the dermal/subcutaneous portion is inked. e) The dermal/subcutaneous portion is bisected horizontally (transversely) at the dermosubcutaneous junction. The upper surface of the newly formed subcutaneous portion is inked. The two inked surfaces are embedded down. B) The HoVert technique provides both horizontal and vertical sections from a single biopsy. a) Two vertical sections allowing visualization of the entire interfollicular epidermis, the dermoepidermal junction, the papillary dermis and superficial portion of the hair follicles. b) Horizontal section at the superficial reticular dermis, allowing visualization of all the follicular units and the superficial reticular dermis. c) Horizontal section at the dermosubcutaneous junction allowing visualization of all the hair follicles, the deep reticular dermis and the upper subcutis. C) If the biopsy is too thin, the specimen should be bisected (left slide) rather than trisected (right slide). The procedure for the bisected HoVert is the same as the trisected Hovert, apart that the dermal/subcutaneous portion of the specimen is not sectioned horizontally at the dermosubcutaneous junction.

dermis and the subcutis of a single punch biopsy (Fig. 3).[18] Of note, HoVert does not allow some of the tissue to be available for direct immunofluorescence, which may aid in the diagnosis.

Immunohistochemistry has classically been limited to experimental and research settings.[20] However, with our recent studies,[21–23] we have identified a few new indications for immunohistochemistry in specific diagnostic impasses. In our experience, obtaining a few unstained sections with the initial hematoxylin and eosin (H&E) sections allows for this application, since diagnostic changes in alopecia may be quite focal and since refacing the tissue block may produce sections without diagnostic features.

CD3, CD123 and treponema immunostains can be useful in alopecia diagnosis:

CD3 immunostain in subacute and chronic alopecia areata

Diagnosis of acute alopecia areata is easy because of the presence of the peribulbar 'hive-of-bees' lymphocytic infiltrate. However, subacute (and chronic) alopecia areata often do not show this peribulbar infiltrate and a distinction from pattern hair loss may not be possible because both disorders are characterized by follicular miniaturization and an increased CT hair count. CD3+ T lymphocytes within the empty follicular fibrous tracts, which are often not obvious on H&E sections, make the distinction between pattern hair loss and alopecia areata possible.[21]

CD123 immunostain in lupus alopecia

Clusters of plasmacytoid dendritic cells are found in skin biopsies from patients with

systemic lupus erythematosus (LE), Jessner's lymphocytic infiltrate (lupus tumidus) and subcutaneous LE.[24–27] The presence of clusters of at least five CD123+ plasmacytoid dendritic cells helps distinguish alopecic LE from lichen planopilaris (LPP) in challenging cases, which may have only a superficial lymphocytic infiltrate and an unaffected interfollicular epidermis.[22] A stain for interstitial mucin, such as colloidal iron stain, may also be quite helpful, though the normal scalp has more interstitial mucin than most other cutaneous sites.

Treponema immunostaining in syphilis

The presence of plasma cells in the inflammatory component and psoriasiform epidermal changes should raise the question of syphilis. H&E features of syphilitic alopecia are described further in this manuscript.

Of note, though the loss of cytokeratin 15+ follicular bulge stem cells is important in the disease progression of lichen planopilaris,[28,29] the loss of this immunostaining does not appear to have any diagnostic value because it is observed within affected follicles in other cicatricial alopecias.[23]

Current alopecia classifications and their limitations

Alopecia is classically divided into cicatricial (scarring) and non-cicatricial (non-scarring). Cicatricial alopecia may be either primary or secondary in nature. Secondary scarring of localized areas of the scalp may result from trauma, burns, radiation dermatitis, cutaneous malignancies, sarcoidosis, scleroderma, necrobiosis lipoidica, cutaneous tuberculosis and other infections of bacterial, mycotic or viral origin. In these circumstances, the follicle is affected in a non-specific manner. Primary cicatricial alopecia reflects a disease specifically involving the hair follicle.[14,30–34]

For the pathologist, primary cicatricial alopecia represents a heterogenous group of hair disorders all characterized by having, as final outcome, the destruction of the follicular and sebaceous epithelium and its replacement by fibrous tissue (follicular scarring). In contrast, in non-cicatricial alopecia the follicular and sebaceous epithelium are preserved and the hair loss is potentially reversible.[30,35] Primary cicatricial alopecia results in the destruction of the bulge zone, which corresponds to the non-cycling portion of the follicle and the location of the follicular stem cells. Destruction of the bulge zone therefore results in an irreversible hair loss.[35–38] In acute (rapid) cicatricial alopecia the follicular and sebaceous epithelium are destroyed, but the hair shafts remain 'naked', often surrounded by a granulomatous infiltrate. In chronic (biphasic) alopecia (see below) the whole follicular unit is replaced by scarring tissue.[14,30]

The classification of primary cicatricial alopecia, defined by the North American Hair Research Society (NAHRS) in 2001, remains a core of alopecia diagnostics.[1,2] The NAHRS classification groups diseases based upon the composition of the inflammatory infiltrate, as 'lymphocytic', 'neutrophilic' and 'mixed'. A more simplified classification scheme for primary cicatricial alopecia has been proposed by Sperling, with less emphasis on the nature of the inflammatory infiltrate, removing pseudopelade of Brocq (considered as a non-specific end-stage scarring alopecia) and merging folliculitis decalvans with central centrifugal cicatricial alopecia (CCCA).[14]

The new grossing techniques allowing examination of both vertical and horizontal sections enable not only more precise diagnostics but also revealed some limitations of the current classifications. Of note are the following:

1. Follicular scarring is the replacement of the hair follicle by connective tissue with no follicular epithelium left at all. Cicatricial alopecia implies that the follicular unit has been permanently replaced by scar tissue. However, when there is follicular drop out in long-standing alopecia, all that is left is an empty fibrous tract, which is histopathologically similar to the follicular scarring resulting from cicatricial alopecia. This process has been described as 'biphasic alopecia', since the early process has no scarring, but the later process results in total follicular loss. This follicular stem cell exhaustion and permanent hair loss, may be seen in pattern hair loss, particularly in males (Fig. 4), alopecia areata and traction alopecia.[14,30] In other words, follicular scarring should not only be seen only as inflammation selectively attacking the hair follicle, but also as follicular deletion in chronic alopecia, even in the absence of apparent inflammation. Thus, all alopecias, whether classified as cicatricial or non-cicatricial, may, in the end, be scarring, with complete loss of the follicular epithelium and permanent hair loss.
2. It is nearly impossible to distinguish the follicular scarring from the fibrous tracts seen

below miniaturized follicles (vellus hair) or below CT follicles. As the miniaturized follicles and the CT hairs are not long enough to extend down to the deep dermis and subcutis, the fibrous tracts in a deep section can be misdiagnosed as follicular scarring. Cases with marked decrease in the T : V ratio and/or increase in the CT count should therefore not be interpreted as cicatricial alopecia, particularly in deep horizontal dermal sections (Fig. 5).

3. We believe that classifying cicatricial alopecia in function of the nature of the infiltrate[2,39,40] may be misleading, because it lumps together clinically disparate disorders. The 'neutrophilic' primary cicatricial alopecias include folliculitis decalvans and dissecting cellulitis of the scalp. Both of these conditions have a mixed inflammatory infiltrate composed of lymphocytes, plasma cells, histiocytes and a variable number of neutrophils. In our experience, neutrophils may be sparse in folliculitis decalvans (Fig. 6). Acne keloidalis was classified as 'mixed', but it is histopathologically similar to folliculitis decalvans and distinction is based on clinical grounds. Sperling has proposed a new classification of primary cicatricial alopecia without taking into consideration the nature of the inflammatory infiltrate. In this classification, poorly defined entities such as Brock pseudopelade and CCCA have been withdrawn.[14] However, this classification joined diseases remaining controversial. Particularly, we are not convinced that folliculitis decalvans is the 'neutrophilic equivalent' of CCCA. Folliculitis decalvans has a mixed inflammatory infiltrate, arranged both around and between follicular units (Fig. 7) and it mostly affects Caucasian men. On the contrary, CCCA resembles LPP histologically (Fig. 8) with an infundibuloisthmic perifollicular lymphocytic infiltrate and it mostly affects the vertex of women of African descent.
4. Non-cicatricial alopecia cannot be considered as synonymous to reversible hair loss. Permanent alopecia after chemotherapy is both non-cicatricial and not reversible due to a toxicity of the drug to the follicular stem cells.[41–43]

Thus, we believe that the currently established classifications are not practical enough in our everyday alopecia diagnostics. We therefore wish to propose a new approach, which would be different than the conventional separation of alopecia into cicatricial and non-cicatricial.

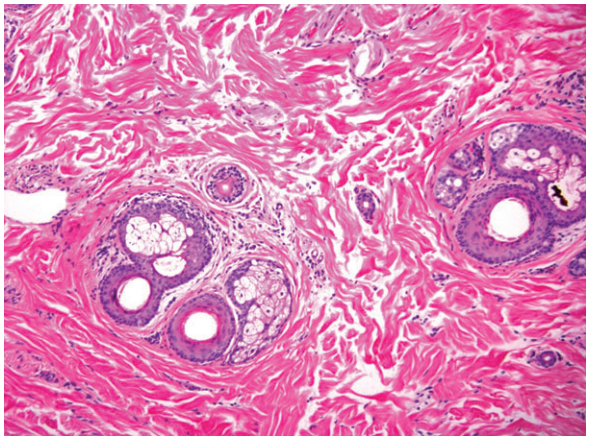

Fig. 4. Follicular dropout in male pattern hair loss.

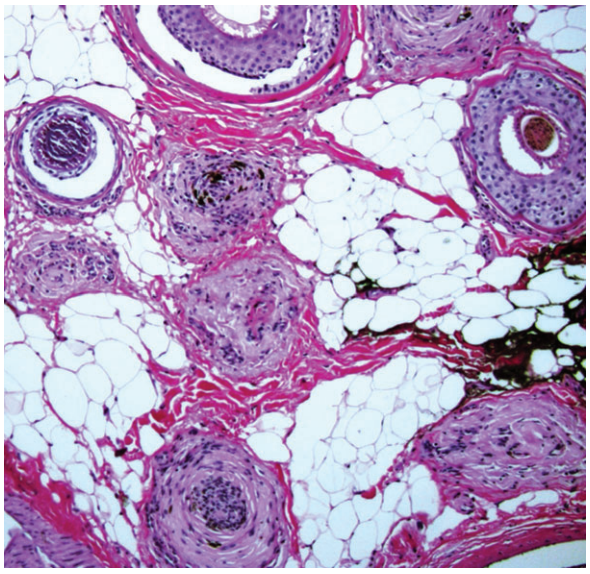

Fig. 5. Follicular fibrous tracts in horizontal deep dermal section.

A practical guide to diagnosis

A practical way to assess the scalp biopsies is (1) getting the clinical clues, (2) examining the biopsy specimen and (3) applying our two-step algorithm.

Getting the clinical clues

Getting the clinic notes is especially helpful because the patient's subjective history and the clinician's exam may provide useful clues. Expert alopecia clinicians can provide very exact differentials. On the contrary, clinicians who

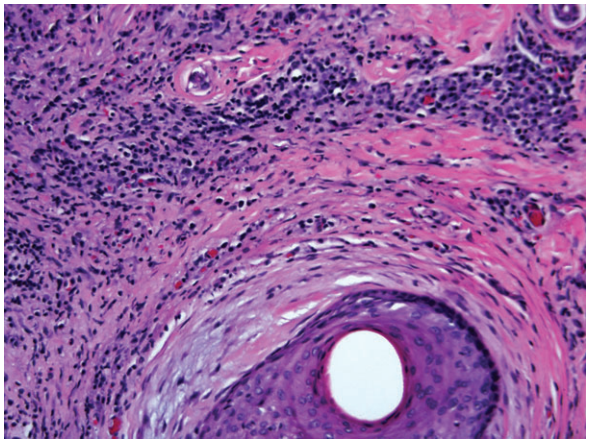

Fig. 6. Folliculitis decalvans with sparse neutrophils.

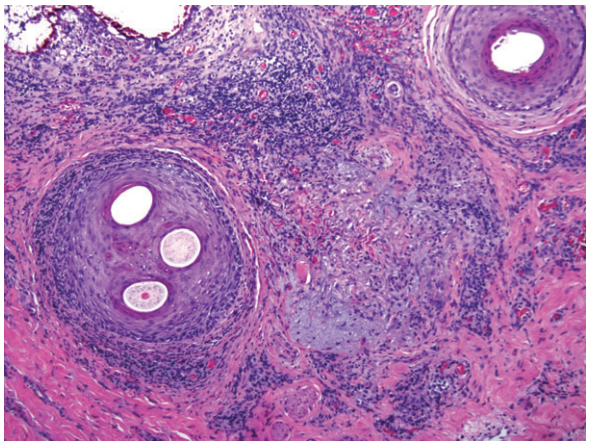

Fig. 7. Folliculitis decalvans with mixed inflammatory cell infiltrate arranged both around and between follicular units.

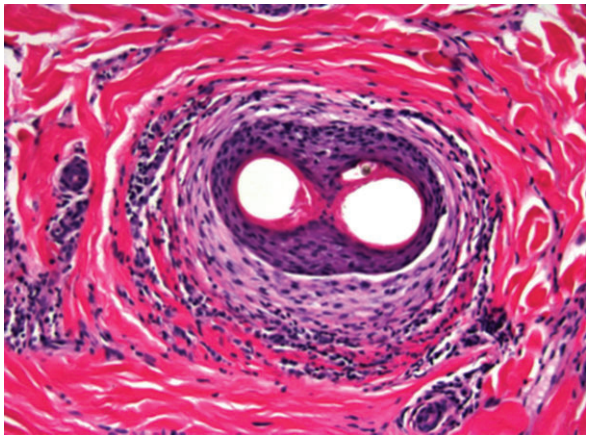

Fig. 8. Central centrifugal cicatricial alopecia with perifollicular fibrosis identical to lichen planopilaris.

see fewer alopecia patients often do not provide sufficient clinical information, often only indicating 'hair loss'. Additionally, most of dermatopathologists are geographically far from the clinics. Without accurate clinical information, however, it may be impossible to render an accurate histopathological diagnosis in alopecia. The most important clinical clue to obtain from the dermatologist is whether the alopecia is diffuse or patchy or whether it is present in a 'special' context.

Table 1 separates diagnoses, based upon 'Diffuse' or 'Patchy' loss and a 'Usual' or 'Special' clinical context. 'Special clinical context' entities may be diffuse or patchy, but they are listed separately, because the clinical history and presentation are unique and usually make the diagnosis obvious. It is important to note that alopecia areata and LPP may present with either diffuse or patchy alopecia, and systemic LE and syphilis may induce a telogen effluvium and present with diffuse alopecia.

A positive or negative hair pull (traction) test is a helpful clinical clue in the interpretation of diffuse or patchy alopecia. Clinicians routinely perform this test, in order to determine the severity and the location of the hair loss. Additionally, hair experts usually examine microscopically the pulled hair for diagnostic purpose. This test is useful for pathologists in order to indicate whether alopecia is active or non-active. Pulling more than 5–6 hairs after having grasped 50 to 60 hairs between the thumb, index and middle fingers is considered a positive hair pull test; a positive hair pull test indicates an active alopecia, and a negative test indicates a non-active alopecia.[44,45] Microscopic examination of the hair roots reveals terminal anagen hair (with a darkly pigmented and triangular-shaped bulb and preserved inner root sheath) and terminal telogen hair (with a lightly pigmented club-shaped bulb and without inner root sheath) (Fig. 9). Normal values for adult scalp are >80%–90% for anagen hair and <10%–20% for telogen hair. Increase in telogen hair in both fronto-parietal and occipital areas is observed in telogen effluvium and in subacute alopecia areata (both telogen effluvium and subacute alopecia areata show increase of the CT hair count). Pattern hair loss should be suspected if the increase of telogen hair is higher in the fronto-parietal area in combination with a variation of the hair shaft diameter. An increase in anagen hair can be found in anagen effluvium (indicating a toxic agent or chemotherapy) and in acute alopecia areata (the hair sheds in anagen phase because of the acute peribulbar infiltrate). Trichotillomania is

Table 1. Diffuse and patchy alopecia in common and a special clinical context

Clinical context	Diffuse alopecia	Patchy alopecia
Common entities	Alopecia areata	Acne keloidalis
	Fibrosing alopecia in a pattern	Alopecia areata
	Distribution	Central centrifugal scarring alopecia
	Lichen planopilaris	Dissecting cellulitis
	Pattern hair loss (female and male)	Folliculitis decalvans
	Telogen effluvium (acute and chronic)	Lichen planopilaris
		Lupus erythematosus (discoid and non-scarring)
		Syphilis
		Traction alopecia (early and chronic)
		Trichotillomania
Special entities	Chemotherapy-related alopecia	Frontal fibrosing alopecia
	Anagen effluvium	Loose anagen syndrome
	Permanent alopecia after chemotherapy	Post-operative (pressure-induced) alopecia
	Loose anagen syndrome	Psoriatic alopecia and drug-induced psoriasiform alopecia
	Short anagen syndrome	Triangular alopecia

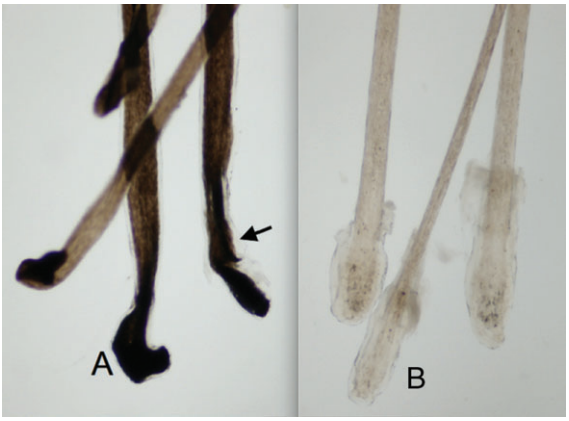

Fig. 9. Microscopic examination of the pulled hair. A) Anagen hair preserve their inner root sheath (arrow) and have a darkly pigmented and triangular-shaped bulb. B) Telogen hair are devoid of inner root sheath and have a lightly pigmented club-shaped bulb.

characterized by broken hair (anagen hair with a sharp horizontal proximal end).[14,46,47]

We should also always make the distinction between common and rare causes. While there are many causes of alopecia, a few entities provide the bulk of diagnoses, and a focus on the common causes will lead to more accurate diagnoses. Finally, the frequency of a particular diagnoses may vary widely depending upon the patient population being seen in a particular clinic.

Examine the biopsy specimen

The evaluation of the total number of hairs, of the overall size of hairs, of the total terminal telogen hairs and of the amount of the inflammation has already been integrated in a helpful four-step method by Sperling.[14] The following items should be assessed when examining the scalp biopsy.

Density

A normal 4-mm punch in a Caucasian patient has 25–30 terminal hairs in a transverse section through the deep dermis and five additional vellus hairs through the superficial dermis. Individual variations of the total hair count can range from 19 to 59 with an average of 38 follicles.[9,14] African-American patients have fewer follicles with an average of 21 hairs and Koreans (and presumably Asians) an even lower average of 16 hairs.[14,48,49] A 4 mm punch biopsy has 12.56 mm^2 so dividing a count of the follicles from one mid-dermal cross section by 12.56, produces the hair density result (example 36 total follicles/12.56 mm^2 = 2.86 follicles/mm^2).

Follicular size

Follicles with shafts less than or equal to the thickness of the adjacent inner root sheath, or less than 0.03 mm in diameter are vellus hairs, those with shafts between 0.03 mm and 0.06 mm in diameter are indeterminate hairs and those with shafts larger than 0.06 mm in diameter are terminal hairs.[5,14] A normal T : V ratio is over 3 : 1, and in patients older than 50 years old 2 : 1 (senescence).[14,50] A ratio 2 : 1 or less is diagnostic of miniaturization. When assessing the T : V ratio, the major difficulty lies in counting the indeterminate hairs.[14] In our experience,

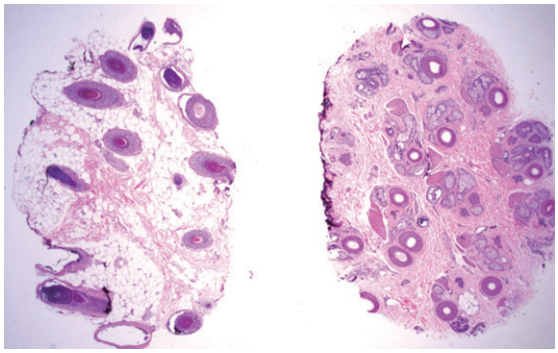

Fig. 10. The terminal hair count should be obtained in the superficial subcutis (left horizontal section) and the vellus hair count in the mid-dermis (right horizontal section).

assessing extension into the subcutis provides the easiest way to assess the T : V ratio. The terminal hair count should be obtained in the superficial subcutis, where adipocytes and collagen bundles are admixed. The vellus hair count should be obtained in the mid-dermis where either CT follicles or sebaceous lobules are present (Fig. 10). The number of hairs in the deep reticular dermis at the dermo-hypodermal junction reflects the number of terminal hairs and the difference between upper and lower reticular dermis reflects the number of vellus hairs. CT follicles also vary in size, so large CT follicles may be considered as terminal hair and small CT follicles considered as vellus hair. Of note, a biopsy near the frontal, lateral or posterior hair line will have many more vellus (miniaturized) follicles as a normal feature, as the scalp merges into adjacent skin, which is populated with vellus follicles.

Catagen and telogen percentage

For ease of diagnostics, CT phase follicles can be grouped, especially because they can be histologically similar depending on the level at which they are sectioned. It is important to search all sections for the dermal level with the most CT phase follicles. Of note, CT follicles are present in markedly different percentages. Catagen phase follicles should be <2.5% of the total, so seeing >1 per biopsy is diagnostics of a catagen shift. Telogen phase follicles may be seen normally up to 10%–15% and a count over 20% is abnormal.[9,14,51] Of note, as miniaturization progresses, the length of the follicular cycle shortens, and more and more CT follicles are present. Thus, a CT count of over 15%–20% may be seen in a miniaturizing process, such as pattern hair loss and subacute or chronic alopecia areata.

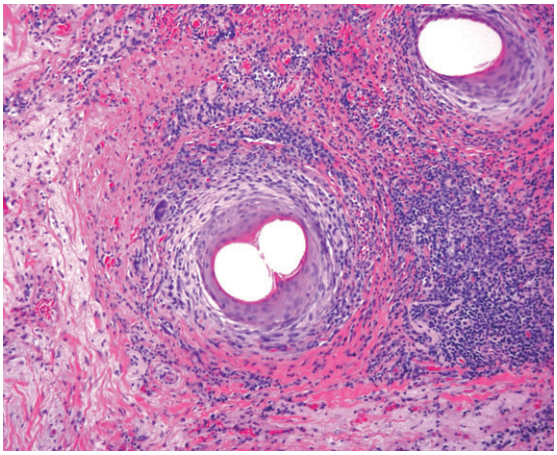

Fig. 11. Blue/gray-staining perifollicular fibrosis.

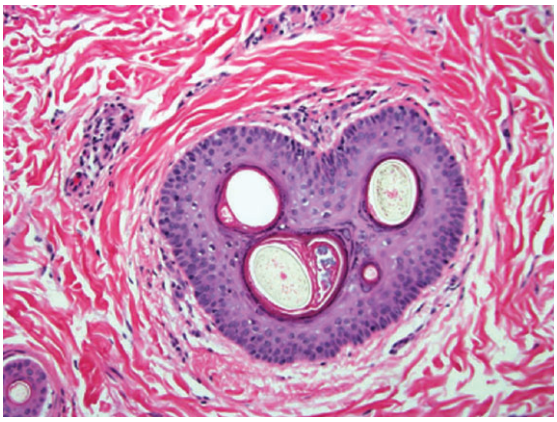

Fig. 12. Follicular ostium resembling compound follicle.

Perifollicular scarring

A diagnosis of true perifollicular scarring should be reserved for blue/gray-staining fibrosis (Fig. 11), since follicles normally have onion skin-like collagen around them (fibrous root sheath). If only a single follicle with perifollicular fibrosis is identified, this could be a resolving acneiform lesion rather than real cicatricial alopecia. Blue/gray-staining perifollicular fibrosis involving more than one follicle is usually seen in LPP, CCCA and folliculitis decalvans. In our experience, this process of perifollicular scarring is less prominent in LE. Of note, normal follicles often group as they exit the ostium, and a transverse section may appear to be a compound (tufted) follicle, when it is actually a normal finding (Fig. 12).

Inflammatory infiltrate

The composition of an inflammatory cell infiltrate should be noted. However, it should be correlated with the above-discussed features

rather than being used as the primary feature for a diagnosis. Sometimes, active inflammation will not be identified on a biopsy, though other features will still lead to a definitive diagnosis. Sampling variability produces a wide variety of features for a single disease. A granulomatous component may surround the naked hair shafts in all acute alopecias in which the follicular epithelium is destroyed.

Follicular fibrous tracts

As previously mentioned, fibrous tracts or stela may either be seen below viable follicles (vellus hair and CT follicles) or correspond to the follicular scarring replacing the dropped-out follicles. The fibrous tract below a viable follicle has, perhaps, more vascularity, but, in our experience, this feature is subtle and difficult to assess.

Apply the two-step algorithm

After obtaining the clinical information of diffuse or patchy hair loss and after the histopathological examination, the diagnosis can be obtained following the below simple two-step algorithm (Figs. 13 and 14):

1 Presence or absence of follicular miniaturization?
2 Increased or normal CT count?

Diffuse alopecia

Diffuse alopecia with follicular miniaturization

1. **Female/male pattern hair loss (androgenetic)**. Follicular miniaturization without any other findings is most certainly pattern hair loss. Female pattern hair loss seems to progress to a certain degree and then ceases, whereas male pattern hair loss will progress to complete follicular loss.[4,52] This is not a diagnostic problem, however, because male pattern hair loss is uncommonly biopsied and generally only in the early stages. Seborrheic dermatitis is often also present, characterized by superficial dermal, especially perifollicular lymphocytes. Of note, seborrheic dermatitis on the scalp does not always have perifollicular parakeratosis as seen on the face. Sebaceous lobules are preserved but reduced in sized in relation to the miniaturized follicles.[1,8,14]
2. **Female/male pattern hair loss (androgenetic) with superimposed chronic telogen effluvium**. Pattern hair loss is often unmasked by a chronic telogen effluvium. Follicular miniaturization with a CT shift over 20% in both involved and normal-appearing scalp is most consistent with the co-existence of these two entities.[19]
3. **Subacute alopecia areata**. Miniaturization with a dramatic CT shift is usually subacute alopecia areata. Profound miniaturization with a T : V ratio of <1 : 4 most likely is subacute alopecia areata, and <1 : 7 always is subacute alopecia areata. A CT shift of 50%–100% often secures a diagnosis, since only few diseases cause such a profound CT shift (see alopecia areata-like pattern below).[11,53] Lymphocytes may be quite limited, with the peribulbar 'hive-of-bees' being absent. As discussed above, CD3 IHC may identify deep dermal or subcutaneous lymphocytes in follicular tracts. This is particularly helpful in cases where the T : V ratio is between 1 : 1 and 1 : 7 and the CT count is between 20% and 50%, a range found in both pattern hair loss and subacute alopecia areata. Diagnosing so-called 'alopecia areata incognita' may be aided with CD3 IHC.[21] Though eosinophils are useful in diagnosing alopecia areata, in our experience, they are not commonly present in the subacute form. Another useful histologic feature is the so-called 'nanogen' follicles, which are abnormal, miniaturized follicles resulting from rapid cycling.[14] Rarely, there may be a granulomatous component.[54,55]
4. **Permanent alopecia after chemotherapy**. With a history of chemotherapy, this entity is not difficult to diagnose, though it is rarely biopsied. Histologic sections show miniaturization and a CT shift resembling pattern hair loss or alopecia areata. Basaloid structures resembling telogen phase follicles have been described as a unique feature, but these may simply be follicles arrested in telogen phase.[41,42] With the basaloid, telogen-like structures, it may not be possible to histologically distinguish this from alopecia areata on H&E sections alone. CD3 IHC studies could be helpful in making this distinction.[21]

Diffuse alopecia without follicular miniaturization

Diffuse alopecia without follicular miniaturization and with increased CT count

1. **Chronic telogen effluvium**. The term 'telogen effluvium' should always be preceded

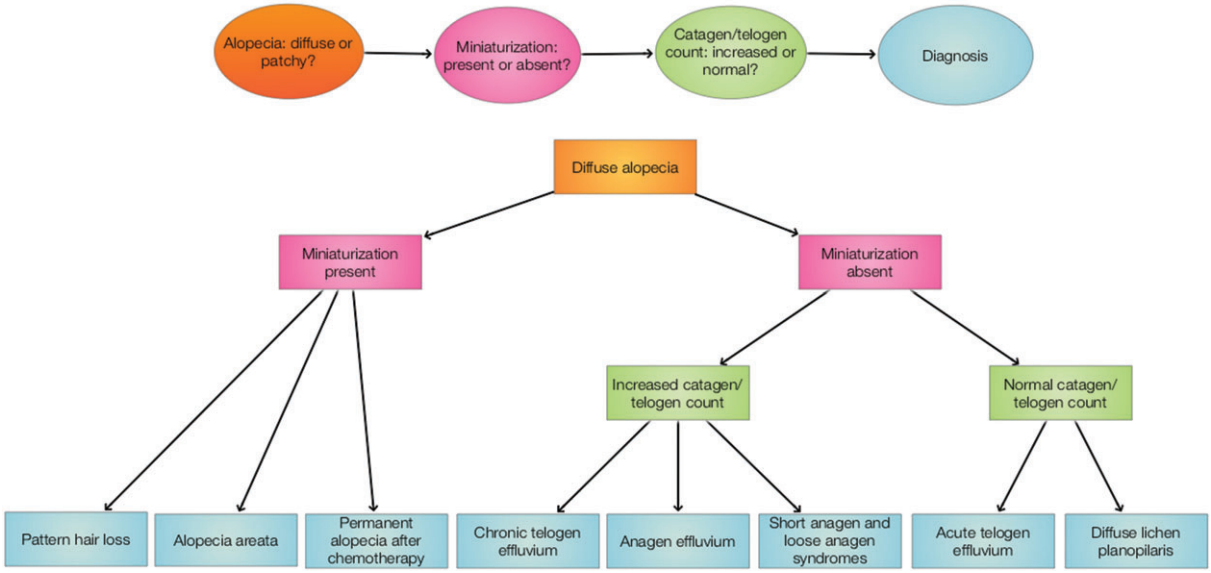

Fig. 13. Diagnostic algorithm for diffuse primary scalp alopecia.

by the word 'acute' or 'chronic', since acute telogen effluvium and chronic telogen effluvium are entirely different entities. Acute telogen effluvium is rarely biopsied, and the clinical history of a dramatic, acute shedding usually secures the diagnosis. The dramatic shedding typically occurs 2–3 months after an insult, such as high fever, operations, general anesthesia, crash diets, accidents, trauma, psychological stress, thyroid dysfunction and drugs, in which a majority of follicles cycle prematurely into catagen phase. Shedding, however, does not occur until the follicles re-enter anagen phase and the new hair shaft pushes out the old hair shaft. Thus, a biopsy of acute telogen effluvium may show nearly 100% anagen phase follicles. Chronic telogen effluvium, on the other hand, is quite common and is defined as an increased shedding of hair on the entire scalp for longer than 6 months.[51] A clinical evaluation may identify a nutritive deficiency of some sort, such as protein or iron deficiency, hypothyroidism, chronic infection, connective tissue disease, malignancy and drug intake.[46] A biopsy is often performed to distinguish it from pattern hair loss. Chronic telogen effluvium alone should not show follicular miniaturization and there is a catagen/telogen shift of >20%. This percentage however rarely exceeds 50% and it can be variable because of the waxing and waning course.[14,31,56,57]

2. **Anagen effluvium**. Following an exposure to a toxicity agent (chemotherapy, high dosage of vitamin A, radiation), the anagen phase is abruptly interrupted and the hair falls out 2–4 weeks after the initiating event without entering catagen and telogen phases.[46,58,59] Since the anagen hair will shed, in skin biopsy we will observe the remaining CT hair, which will appear to be increased over 15%–20%. The clinical history and positive pull test with anagen hair make the diagnosis obvious.
3. **Short anagen and loose anagen syndromes**. In short anagen syndrome there is a persistent hair shedding and an impression that the hair does not grow due to the abnormal short anagen phase duration.[60–62] Loose anagen hair syndrome is due to a defective anchorage of the hair shaft to the follicle, resulting in easily pluckable hair.[63,64] In both these disorders, anagen hairs are shed and the remaining CT hairs in the skin biopsy would appear to be increased.

Diffuse alopecia without follicular miniaturization and with normal CT count

1. **Acute telogen effluvium**. As previously mentioned, massive shedding in acute telogen effluvium occurs when the new anagen hair shafts push out the prior shafts (2–3 months after the initiating trigger). Thus, despite the name, a biopsy will show a very low CT count of 0%–5%.
2. **Lichen planopilaris**. LPP is usually patchy, but occasional cases present with diffuse loss. LPP is discussed further below.

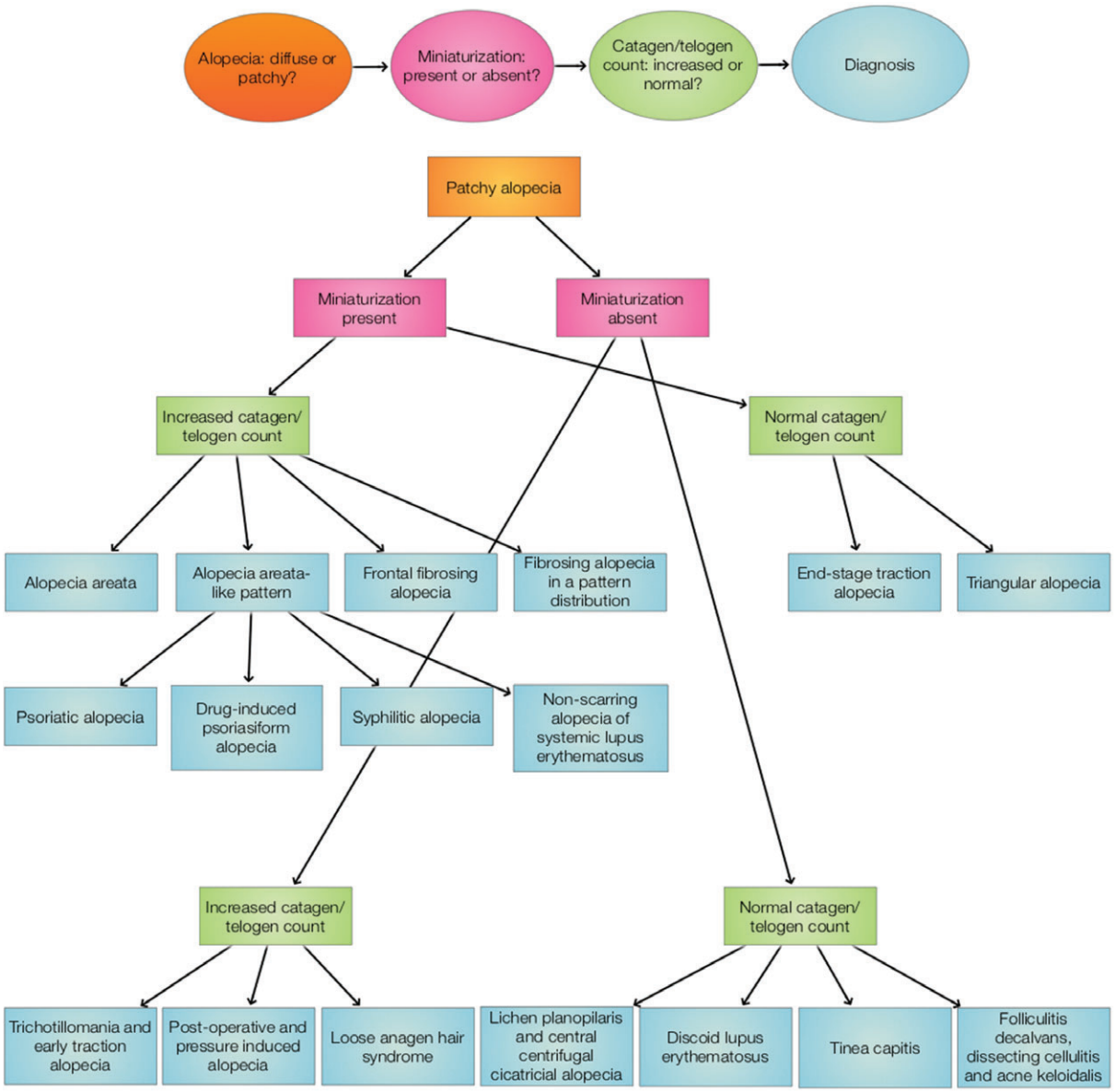

Fig. 14. Diagnostic algorithm for patchy primary scalp alopecia.

Patchy alopecia

Patchy alopecia with follicular miniaturization

Patchy alopecia with follicular miniaturization and with increased CT count

1. **Alopecia areata**. As the patients are often young and the diagnosis of patchy alopecia areata is obvious clinically, a biopsy is often not obtained initially. As discussed above in the diffuse presentation of alopecia areata, a biopsy of early, acute alopecia areata is generally not problematic, and the peribulbar 'hive-of-bees' infiltrate of lymphocytes is often identified. Eosinophils are helpful but often not present. Subacute alopecia areata, in which there has been significant miniaturization may be more challenging because of the absence of the peribulbar infiltrate and requires CD3 immunostaining as described above.
2. **Frontal fibrosing alopecia**. Frontal fibrosing alopecia, which may be a form of LPP, has a typical clinical presentation with loss of the frontal hairline and/or eyebrows. Smaller follicles, such as vellus and eyebrow follicles appear to be targeted, which may account for the loss of the frontal hairline, a location where the T : V ratio is decreased normally as the scalp transitions into the

forehead. Biopsy findings are discrete with minimal perifollicular inflammation at the level of the infundibulum/isthmus, subtle vacuolar change and limited blue/gray-staining perifollicular fibrosis.[65–68] Since the eyebrow hairs cycle so frequently, they may disappear without developing any perifollicular fibrosis, and the biopsy may only show lymphocytes with no remaining follicles. A definitive diagnosis relies on careful histologic analysis in the correct clinical setting. Even with HoVert sections, the inflammatory foci are often difficult to find, with level sections through the tissue block being necessary.

3. **Fibrosing alopecia in a pattern distribution**. This entity is reported to have miniaturization similarly to pattern hair loss, with additional focal vacuolar interface dermatitis of the upper portion of the follicular epithelium and a variably dense perifollicular lymphocytic infiltrate and perifollicular fibrosis. Reports of this entity are sparse and it is still debated whether it consists of diffuse LPP, or of a diffuse presentation of frontal fibrosing alopecia, or of LPP with superimposed pattern hair loss, or of a particular manifestation of advanced pattern hair loss. Similarly to LPP, both vellus and terminal hairs may be inflamed, whereas in frontal fibrosing alopecia only vellus hairs are affected.[14,69,70] We suspect that fibrosing alopecia in a pattern distribution may really be diffuse LPP and that miniaturization results from dropout of the terminal follicles.
4. **Psoriatic alopecia**. In the presence of psoriasis, there may be subjacent follicular miniaturization with an increased CT shift approaching 100%, simulating alopecia areata. The differential diagnosis between psoriatic alopecia and alopecia areata may be impossible. Hair regrowth may or may not occur after treatment of the psoriasis. Epidermal changes are not requisite for diagnosis of psoriatic alopecia, as the patients may have received treatment, but it is usually present.[14,71]
5. **Tumor necrosis factor (TNF)-alpha inhibitor associated (drug-induced) psoriasiform alopecia**. Closely related to psoriatic alopecia is TNF-alpha inhibitor associated alopecia. For diagnostic purposes, it can be considered to be the same as psoriatic alopecia. Subtle histologic differences have been reported with increased superficial and deep lymphocytic inflammation, presence of plasma cells and eosinophils, as well as focal follicular destruction.[72–74] This diagnosis should not be confused with TNF inhibitor-induced alopecia areata.[75,76]
6. **Syphilitic alopecia**. Syphilis is another simulator of alopecia areata showing pronounced follicular miniaturization and CT increase over 50%, even approaching 100%. Syphilis may produce different patterns of alopecia, and the histologic findings are diverse. Usual features of secondary syphilis may be present, or the syphilis may also induce a chronic telogen effluvium. Though plasma cells may normally be present in the scalp, the presence of easily found plasma cells in the infiltrate of an alopecia biopsy should raise the possibility.[14,31,77]
7. **Non-scarring alopecia of systemic lupus erythematosus**. LE may also simulate alopecia areata with marked follicular miniaturization and CT shift superior to 50%. Vacuolar interface changes, perivascular, periadnexal and perieccrine lymphocytes and plasma cells, increased mucin and positive direct immunofluorescence are clues in favor of non-scarring alopecia of systemic LE. The presence of eosinophils could also be considered as finding in favor of alopecia areata, however occasional eosinophils may also be found in LE.
8. **Comment on 'alopecia areata-like' pattern**. Of note, in addition to psoriasis, syphilis and systemic LE, findings of follicular miniaturization and increase of CT hair over 50% simulating alopecia areata have also been reported in tick bite alopecia.[78] We suspect that this alopecia areata-like process may be non-specific and associated with acute inflammation, and we believe that the pathologists should be wary of diagnosing these cases as alopecia areata.

Patchy alopecia with follicular miniaturization and with normal CT count

1. **End-stage traction alopecia**. End-stage traction alopecia also shows decrease T : V ratio, but this is mostly due to a selective loss of terminal hairs rather than follicular miniaturization. Typically, in end-stage traction alopecia most of the follicular units still have associated sebaceous glands.[31]
2. **Triangular alopecia**. This hamartomatous lesion is easily diagnosed because of the typical clinical presentation of a lancet-shaped

bald spot. Histology shows almost exclusively vellus hairs because of the hamartomatous origin and not because of miniaturization. Fibrous tracts are thus absent helping the distinction from end-stage traction alopecia, in which they are preserved.[79,80]

Patchy alopecia without follicular miniaturization

Patchy alopecia without follicular miniaturization and with increased CT count

1. **Trichotillomania/early (acute) traction alopecia**. Clinical information often points toward this diagnosis from the start. The presence of CT phase follicles is the most sensitive finding in trichotillomania and traction, also known collectively as 'trichotillosis'. The changes follow a spectrum from acute to chronic. A dramatic catagen shift involving the terminal follicles is the hallmark, and there are variable hair shaft changes (trichomalacia). Pigment casts, which occur from release of melanin from the hair shaft, may be present, but they may also be seen in other processes, particularly alopecia areata, especially when the hair is very dark. The popular 'Hamburger' sign, with a fracture in the hair shaft and pink-staining material in the middle, is only rarely found.[81] Histologic knife cutting chatter may also produce fractures in the hair shaft, simulating external trauma. Hemorrhage may be present, but punch biopsies also have biopsy-associated hemorrhage.[81,82] There may be superimposed lichen simplex chronicus. As previously mentioned, there may be a decreased T : V ratio in older lesions associated with a low follicular count.[31]
2. **Post-operative (pressure-induced) alopecia**. Post-operative alopecia is usually obvious because of the clinical history. A biopsy shows CT shift, which can sometimes exceed 50%. Alopecia areata is in the differential, and the absence of miniaturization, presence of vascular thrombosis and fat necrosis help this distinction.[14,83,84] Once again, a CD3 immunostaining study could be helpful in ruling out alopecia areata.
3. **Loose anagen hair syndrome**. Loose anagen hair syndrome may be both diffuse or patchy (on parietal areas).[63,64] This disorder has been previously mentioned.
4. **Dissecting cellulitis of the scalp**. CT hairs may be increased in dissecting cellulitis of the scalp because the intense inflammation triggers the conversion of anagen into CT hairs. This disorder will be discussed further.

Patchy alopecia without follicular miniaturization and with normal CT count

1. **Lichen planopilaris**. LPP is a very common entity, and the diagnostic features are usually easily found. Early cases, however, may have only focal findings, and level sections through the tissue block may be necessary. There is almost always blue/gray-staining perifollicular fibrosis confined to the level of the infundibulum and superficial isthmus with subtle interface change and squamatization of the basalis. Peripheral to the fibrosis, there is a perifollicular lymphocytic cell infiltrate, which varies in density, and sometimes the lymphocytes scatter into the follicle. The interfollicular epidermis is almost never affected in LPP, and the presence of such should raise a strong suspicious of lupus alopecia. Of great aid in the diagnosis is the reduction or absence of sebaceous glands.[85–87] A useful diagnostic feature is the near absence of CT phase follicles in lichen planopilaris, which has been credited to early destruction of the cytokeratin15+ follicular stem cells in the bulge.[28] Of note, often a resolving acneiform lesion, which may be incidentally biopsied in a variety of alopecic processes, may show changes identical to lichen planopilaris. For this reason, we believe that the changes should be seen in two separate foci in a biopsy before a diagnosis of LPP is rendered.
2. **Central centrifugal cicatricial alopecia**. In our experience, CCCA is nearly histologically identical to LPP. In CCCA, similarly to LPP, there is almost always blue/gray-staining perifollicular fibrosis at the infundibuloisthmic portion of the hair follicle. Interface changes are subtle or absent and confined to the follicular epithelium.[14] As with LPP, CCCA has more pronounced squamatization of the basal layer than lupus alopecia. Both CCCA and LPP have a perifollicular lymphocytic infiltrate, which is closely associated with the infundibuloisthmic area of fibrosis, with no

deep dermal component. In our experience, compound follicles (the 'google' follicles typically described in CCCA) are seen in both disorders, and the eccentric epithelial atrophy described as a feature of CCCA may also be seen in LPP. Traction alopecia and trauma, which may be components of CCCA, may also produce this feature.[88] In addition, we have observed the premature desquamation of the internal root sheath not only in CCCA but also in LPP. Premature desquamation may also be artifactually created with knife chatter during tissue sectioning.[14,89] Sperling has published that the premature desquamation of the inner root sheath is specific for CCCA only in early disease.[90] Of note, less inflammatory CCCA has been called 'follicular degeneration syndrome'.[91,92] We hypothesize that the reported diffuse and non-inflammatory manifestations of CCCA may be a manifestation of diffuse LPP.[93] Clinically, CCCA is identical to diffuse LPP and the additional crusting and pustule formation may be due to bacterial superinfection. LPP is commonly seen in women of African descent, and we suspect that CCCA may be a particular manifestation of diffuse LPP seen in the vertex of black African women, induced and intensified by chemical relaxers for styling purposes, caustic cosmetics, hot combs and chronic traction.

3. **Lupus erythematosus**. Lupus alopecia shows the usual histologic features similar to discoid lupus elsewhere on the body. Lupus alopecia almost always has vacuolar change in the surface epidermis and a superficial and deep lymphoplasmacytic infiltrate. Thus, most cases of lupus alopecia are easily distinguished from LPP, because of the surface interface change and the deep infiltrate. As with other sites on the body, a diagnosis of LE may be more challenging if the interfollicular interface change is absent.[94,95] Occasionally, a distinction between LE and LPP is difficult. As described above, we have reported the utility of CD123 immunostaining.[13] In contrast to LPP, lupus alopecia usually has minimal perifollicular fibrosis. The absence of CT follicles is a usual feature of LPP but not of lupus alopecia.[28] As discussed above, non-cicatricial alopecia of systemic LE may have a markedly increased CT count, simulating alopecia areata. Increased interstitial mucin is helpful, but the normal scalp has more interstitial mucin than other sites. Plasma cells, a hallmark of lupus, are less helpful in the scalp, because they may be present in any scalp dermatitis. Syphilitic alopecia may be excluded with immunohistochemistry or serologic studies. Occasionally, direct immunofluorescence studies may be helpful in making a distinction between LPP and lupus alopecia. Other histopathological clues in favor of LPP and against lupus alopecia are mucinous perifollicular fibroplasia and superficial wedge shaped scar destroying only the upper portion of the elastic sheath.[44,96,97]
4. **Folliculitis decalvans/dissecting cellulitis/acne keloidalis**. Formerly known as the 'pustulofollicular'-type of scarring alopecia, the entities in this group of diseases must be distinguished based upon clinical grounds. It is the function of the pathologist to make a diagnosis of this group, and the onus is on the clinician to make a specific diagnosis. Thus, for a histopathological diagnosis, we prefer to group folliculitis decalvans with dissecting cellulitis (perifolliculitis capitis abscedens et suffodiens) and acne keloidalis. Folliculitis decalvans represents the variant with more superficial and follicular centered inflammation, whereas dissecting cellulitis has deeper and widespread inflammation. Acne keloidalis is closely related to folliculitis decalvans but the clinical presentation is very distinctive. Generally, all of the entities involve the presence of clinical pustules, though dissecting cellulitis is typically deeper with the formation of suppurative tracts. It would be better with the NAHRS classification to group these under 'mixed cell type' because the infiltrate varies and neutrophils, which are essentially always present, may be quite few in number. The driving feature of these diagnoses is not only perifollicular inflammation and fibrosis but also interstitial involvement between the follicular units. All eventuate in compound follicles, which are often tufted (having multiple follicles). Of note, in dissecting cellulitis, in contrast to most other cicatricial alopecias, sebaceous glands are preserved because of the deep follicular involvement.[14,98,99]
5. **Tinea capitis**. In all the aforementioned diseases, in which neutrophils are present among the inflammatory cells, a PAS stain should always be performed to rule out tinea capitis. Of note, a biopsy from a child with

a scarring process should be examined very carefully for tinea, including multiple PAS stains, as the fungal forms may be rare and difficult to find.

6. **Pseudopelade of Brocq**. Pseudopelade of Brocq is not a distinct clinical entity and causes ongoing debate among experts. Adding further confusion, the term 'pseudopelade' has also been used to describe patients with CCCA.[14] Pseudopelade of Brocq may be considered to be end-stage, primary cicatricial alopecia, for which there is no established specific diagnosis.[14,100,101] The classic description as 'footprints in the snow' refers to cutaneous hypopigmentation and atrophy. With the use of horizontal sections, this entity has progressively disappeared, likely because horizontal sectioning finds foci of peri-infundibular inflammation and a diagnosis of LPP or CCCA is rendered.[100,102,103] In support of this is the observation that when mild inflammation is identified, there is also perifollicular erythema and hyperkeratosis, as seen in LPP. Pseudopelade of Brocq limited to the crown and vertex may correspond to burned out CCCA, which is possibly a variant of LPP. Thus, we recommend abandoning this diagnosis.

Conclusion

With this review we have attempted to move away from the established cicatricial (scarring) vs. non-cicatricial (non-scarring) classification to a more simplified pathway, which we hope will allow a more exact diagnosis of alopecia; by starting with a diffuse versus patchy clinical presentation, we rely on follicular size (miniaturization) and phase (catagen/telogen count). In addition, a number of alopecic entities can be diagnosed by correlating the histopathological findings with very obvious clinical presentations. In our experience, the new HoVert grossing technique, offering both vertical and horizontal studies in one biopsy, greatly simplifies diagnostics, mostly by reducing analysis time and limiting the need for step level sections. Finally, we have described the utility of immunohistochemistry in a few diagnostic situations.

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