Clinical and Laboratory Investigations

Dermatology

Dermatology 2005;211:98–102
DOI: 10.1159/000086436

Received: May 31, 2004
Accepted: November 12, 2004

Value of Direct Immunofluorescence for Differential Diagnosis of Cicatricial Alopecia

Stefan Trachsler Ralph M. Trüeb

Department of Dermatology, University Hospital of Zurich, Zurich, Switzerland

Key Words
Scarring alopecia · Histopathology · Direct immunofluorescence · Lupus erythematosus · Lichen planopilaris · Pseudopelade of Brocq

Abstract
Background: There are diverse causes of cicatricial alopecia characterized by lack of follicular ostia and irreversible loss of hair. While clinical differentiation between the causes may be difficult, particularly with regard to lichen planus (LP), lupus erythematosus (LE) and pseudopelade of Brocq (PB), it has been suggested that both histopathologic examination and direct immunofluorescence studies (DIF) are necessary for an accurate diagnosis. ***Objective:*** The aim of this study was to evaluate the diagnostic value of DIF studies in addition to histopathology in patients with cicatricial alopecia as a clinical feature. ***Methods:*** 136 scalp biopsy specimens received for histopathology and DIF during a 5-year period were reviewed. ***Results:*** Definitive diagnosis was achieved by careful evaluation of scalp biopsies. The most prevalent diagnoses in order of frequency were LP (26%), LE (21%) and folliculitis decalvans (20%). PB was diagnosed in 10%. In most cases, the diagnosis could be made on the basis of histopathology and independently of DIF. Characteristic DIF patterns showed high specificity, but low sensitivity for LP, and high specificity and sensitivity for LE. The DIF pattern in PB showed no difference to LP. ***Conclusions:*** Histopathology permits diagnosis in the majority of cicatricial alopecias. DIF is of value in histopathologically inconclusive cases, particularly when LE is in question.

Clinical differentiation between the major causes of cicatricial alopecia, which is recognized clinically by loss of hair and decrease in number or absence of follicular ostia, may be difficult. Particularly with regard to lichen planus (LP), chronic cutaneous lupus erythematosus (LE) and pseudopelade of Brocq (PB), it has been suggested that both histopathologic examination and immunofluorescence studies are necessary for accurate diagnosis [1–3].

Histologic features of LP include a perifollicular lymphocytic infiltrate, with interface alterations involving the follicular epithelium [4–6]. These changes tend to be top-heavy. Civatte bodies may be detected in proximity. End-stage lesions demonstrate reparative fibrosis with absence of elastic fibers and selective loss of hair follicles – changes similar to those seen in PB. There has been considerable debate as to whether PB represents a specific clinicopathologic entity or basically the same condition as LP [2, 3, 7–9], with which it shares a patchy lymphocytic infiltrate around the upper portion of the follicle in early lesions [10], and little or no inflammation together with selective loss of follicles in older lesions.

Histologic features of LE include liquefaction degeneration of the basal cell layer and a perivascular and periadnexal lymphocytic infiltration on all levels of the der-

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Prof. Dr. Ralph M. Trüeb
Department of Dermatology, University Hospital of Zurich
Gloriastrasse 31
CH–8091 Zurich (Switzerland)
Tel. +41 44 255 30 79, Fax +41 44 255 44 31, E-Mail Ralph.Trueeb@usz.ch

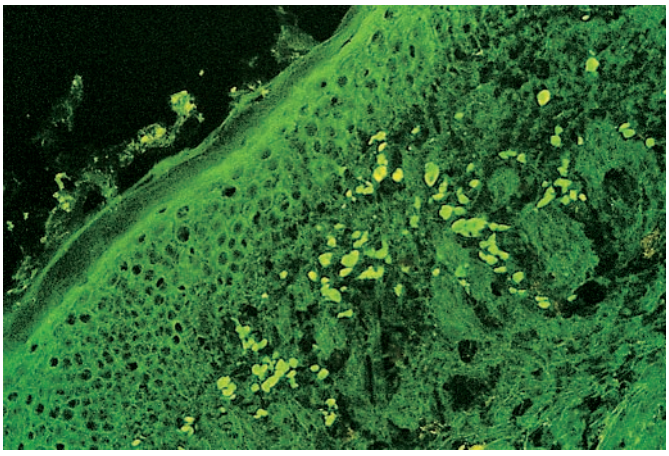

Fig. 1. DIF of LP. Grouped globular deposits of IgM.

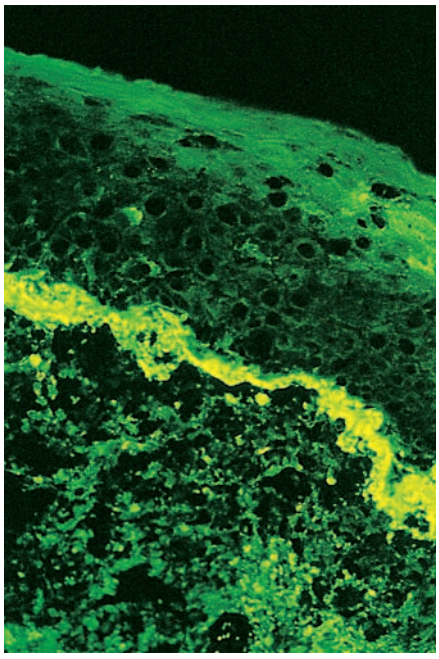

Fig. 2. DIF of LE. Granular deposits of IgG at the dermoepidermal junction.

mis [6]. Civatte bodies may also be seen, although in smaller numbers than in LP [11, 12]. Again, end-stage lesions demonstrate loss of hair follicles and reparative fibrosis with an absence of elastic fibers, often in a more diffuse pattern than in LP. The Alcian blue-PAS stain may be helpful in demonstrating an increase in dermal mucin and thickening of the basement membrane zone of chronic lesions [6].

Specific immunopathologic patterns are discernible by direct immunofluorescence (DIF) in both LP and LE [11, 13]. The most characteristic DIF findings in LP consist of grouped globular deposits of IgM (cytoid bodies), adjacent to the follicular epithelium or at the dermoepidermal junction (fig. 1), and heavy deposits of fibrin at the dermal-epidermal junction [1, 2, 5, 11, 14–17]. In LE, DIF studies most commonly demonstrate granular deposits of immunoglobulin (fig. 2) and C3 at the dermoepidermal junction [11, 18–24]. In PB, DIF is negative or occasionally demonstrates IgM [8, 25].

To assess the diagnostic value of histopathology and DIF in the diagnosis of cicatricial alopecias, we performed a retrospective clinicopathologic and immunopathologic review of 136 cases of cicatricial alopecia.

Material and Methods

The pathologic material reviewed in this study came from scalp biopsy specimens received for histopathology and DIF studies from 136 patients with cicatricial alopecia as a clinical feature. This material was collected in the Department of Dermatology, University Hospital of Zurich, during the period from 1995 to 2000. Tissue stored at –80°C for DIF studies had been cut at 5 μm, and standard direct DIF procedures were used as previously described [26]. Commercially prepared fluorescein-labeled antisera to human fibrinogen, complement factor C3 and IgG, IgA and IgM were used.

DIF patterns considered typical of LP, as previously described [1, 2, 5, 11, 14–17], are globular deposits of immunoglobulins, principally IgM, and deposits of C3 and fibrinogen localized in the papillary dermis adjacent to the dermoepidermal junction. In some cases, globular deposits may be present in the external root sheath of the hair follicles [20]. Additionally, a diffuse band of fibrin stains along the dermoepidermal junction and around follicles [15, 16]. Typically the globular deposits in LP are numerous, tend to cluster and usually contain various immunoglobulins and C3 [14, 16, 17].

DIF patterns considered typical of LE, as previously described [11, 18–24], are granular immune deposits, principally of IgG and IgM, and C3 found along the dermoepidermal junction. Deposits of fibrinogen in the same distribution are often found as well [27, 28]. In most cases, the deposits are diffuse, although they may also be focal. It must be taken into account that weak fluorescent deposits at the dermoepidermal junction, composed primarily of IgM, also occur in normal sun-exposed skin.

In all cases, material from the same excisional specimen was available for routine histopathologic examination. Either spindle or paired punch biopsies (4 mm) had been obtained from the active edge or borders of scarred lesions. Of the paired biopsies, one was used for vertical histologic examination and DIF studies, the other for transversal histologic examination [29]. For histopathology, 1-μm sections were stained with hematoxylin and eosin, orcein for elastic tissue and Alcian blue-PAS. Each biopsy was re-examined

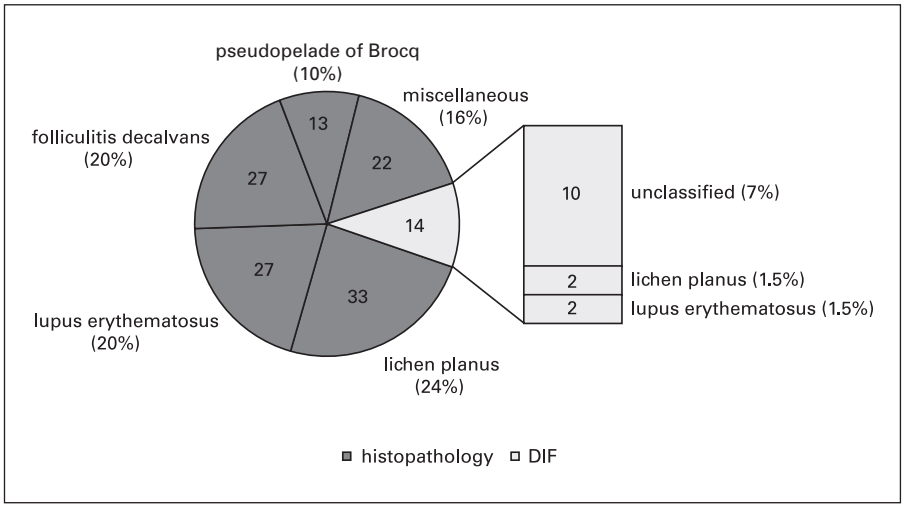

Fig. 3. Cicatricial alopecia: diagnosis on the basis of histopathology and DIF.

by one of the authors (R.M.T.) who was only aware that the biopsies were obtained from patients with cicatricial alopecia, without any information with respect to DIF results. After examination of the specimens, a histologic diagnosis was given to each case.

Results

136 scalp biopsies were reviewed. In 126 of 136 biopsies (93%), a definitive diagnosis was made on the basis of histopathology and DIF.

LP was diagnosed in 35/136 (26%), LE in 29/136 (21%), folliculitis decalvans in 27/136 (20%), PB according to the criteria of Braun-Falco et al. [8] in 13/136 (10%) and miscellaneous in 22/136 (16%), i.e. postmenopausal frontal fibrosing alopecia [30] in 7/136 (5%), circumscribed scleroderma in 4/136 (3%), cutaneous lymphoma in 4/136 (3%), traumatic alopecia in 2/136 (1%), follicular degeneration syndrome [31] in 2/136 (1%), acne keloidalis nuchae in 1/136 (1%), alopecia parvimaculata in 1/136 (1%) and stem cell folliculitis [32] in 1/136 (1%). 10/136 (7%) were judged as unclassified cicatricial alopecia (fig. 3).

On the basis of histopathology alone and independently of DIF, an accurate diagnosis could be made in 97% of diagnosed cases, specifically in 94% of LP cases and in 93% of LE cases.

The typical DIF pattern, as described above, showed for LP a sensitivity of 34% and a specificity of 95%, and for LE a sensitivity of 83% and a specificity of 93%.

Discussion

Since clinical differentiation between the various causes of cicatricial alopecia may be difficult, scalp biopsy is mandatory for diagnosis. As recently outlined by other authors [33], this study confirms that scalp biopsy enables an accurate diagnosis in the majority of cases of cicatricial alopecia, especially if the correct biopsy technique is used, and the pathologist is familiar with the histopathology of the scalp.

Of 136 scalp spindle or paired punch (4 mm) biopsy specimens from patients with cicatricial alopecia received for histopathology and DIF studies, a definitive diagnosis was made in 126 cases (93%). Histopathologic examination of lesional scalp biopsy enabled a specific diagnosis in 97% of cases irrespective of DIF findings, the remaining 3% of diagnosed cases were resolved by DIF.

In order of frequency, the following diagnoses represented the most common causes of cicatricial alopecia: LP in 26%, LE in 21% and folliculitis decalvans in 20%. Using the criteria of Braun-Falco et al. [8], PB was diagnosed in 10%. PB should not be confused with the pseudopelade state of Degos, a common end stage of inflammatory chronic disease such as LP, LE and others, in which the cause defines the genus [34]. A minority of cases (7%) were judged as unclassified cicatricial alopecia, since a specific diagnosis on the basis of histopathology and DIF was not possible, and the diagnosis criteria for PB were not fulfilled.

With respect to the most frequent causes of cicatricial alopecia, histopathologic examination enabled the diagnosis in 94% of LP and in 93% of LE cases, irrespective

Table 1. DIF-positive cases of LE and LP in the literature

	Present study	Tan et al. [33] 2004	Whiting [13] 2001	Annessi et al. [25] 1999	Mehregan et al. [1] 1992	Abell [12] 1977
LE	24/29 (83%)	20/32 (62.5%)	16/21 (76%)	6/6 (100%)	–	8/9 (89%)
LP	12/35 (34%)	6/25 (24%)	9/23 (39%)	15/21 (71%)	18/30 (60%)	3/3 (100%)

of DIF. The remaining 6% of LP and 7% of LE cases were resolved by DIF. Characteristic DIF patterns for LP showed a sensitivity of 34% and a specificity of 95%. Characteristic DIF patterns for LE showed a sensitivity of 83% and a specificity of 93%. These findings reflect the percentage numbers for DIF-positive cases of LP and LE described in the more recent literature with higher numbers of biopsy specimens (table 1).

In PB, DIF showed negative results or an unspecific pattern with predominantly IgM deposits at the dermoepidermal junction. DIF was not helpful in differentiating PB from LP. The delineation of PB from LP is traditionally done on the basis of clinical and histopathologic criteria, though this remains subject of an ongoing debate [2, 3, 7–9].

Of the total of 136 scalp biopsies, DIF led to a diagnosis in 4 of 14 (26%) histopathologically doubtful cases.

Conclusion

Under the prerequisites that a correct biopsy technique is used [29] and the pathologist is familiar with the histopathology of the scalp, histopathologic examination of scalp biopsies enables a precise diagnosis in the majority of cicatricial alopecias. Due to a low sensitivity, DIF is of limited help in diagnosing LP. DIF does not help differentiate PB from LP. Due to a high sensitivity and specificity, DIF can be of value in the diagnosis of LE. We suggest not to perform DIF on a routine basis on all scalp biopsies. Preferably, frozen tissue samples are stored, and DIF is performed in histopathologically inconclusive cases.

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