Elastic staining versus fluorescent and polarized microscopy in the diagnosis of alopecia

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Background: Recently, polarized microscopy was reported as helpful in the evaluation of alopecia biopsy specimens.

Objective: We sought to determine the usefulness of polarized microscopy relative to elastic tissue staining and fluorescent microscopy.

Methods: Histologic sections from 60 alopecia specimens were evaluated to determine the pattern of elastic tissue in elastic van Gieson–stained sections. Comparable hematoxylin-eosin sections were examined under a fluorescent microscope to determine the elastic tissue pattern and examined under polarized microscopy to determine the pattern of birefringence.

Results: Elastic van Gieson staining demonstrated high sensitivity (1.0) and high specificity (1.0) for the identification of nonscarring alopecia. In 54 of 60 cases, fluorescent microscopy demonstrated an identical pattern of elastic tissue. High background eosin fluorescence made it impossible to interpret the elastic tissue pattern in the remaining 6 specimens. Strong birefringence in dermal collagen sparing fibrous tracts had high specificity (1.0) but lower sensitivity (0.59). Strong collagen birefringence within the dermis and broad fibrous tracts were present in all 6 cases of central centrifugal cicatricial alopecia.

Limitations: Elimination of the 6 uninterpretable specimens with high background fluorescence from our calculations may be a source of bias, as these cases could potentially all have been either negative or positive.

Conclusion: Elastic tissue staining is the most reliable means to determine the pattern of scarring in alopecia biopsy specimens. In most cases, fluorescent microscopy of hematoxylin-eosin sections shows an identical pattern. Although a pattern of collagen birefringence on polarized microscopy distinctly sparing fibrous tract is specific for nonscarring alopecia, not all cases of nonscarring alopecia demonstrate this pattern. Strong collagen birefringence within both the dermis and fibrous tracts suggests a diagnosis of central centrifugal cicatricial alopecia. (J Am Acad Dermatol 2013;69:288-93.)

Key words: alopecia; biopsy; cicatricial; elastic; fluorescent; histology; polarized; scar.

The histologic evaluation of alopecia biopsy specimens with dense or hyalinized collagen can be challenging, as it may be difficult to differentiate dense collagen from scarring in hematoxylin-eosin (H&E)-stained sections. Recently, the use of polarized microscopy was reported in this setting.[1] Specifically, the authors reported that polarized light microscopy allowed them to distinguish follicular scars from fibrous tracts

Abbreviations used:

CCCA:	central centrifugal cicatricial alopecia
EVG:	elastic van Gieson
FFA:	frontal fibrosing alopecia
H&E:	hematoxylin-eosin
LPP:	lichen planopilaris
NPV:	negative predictive value
PPV:	positive predictive value

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(fibrous streamers) because the fibrous tracts do not demonstrate birefringence in contrast to the birefringence of normal dermal collagen. We sought to validate their observation and evaluate the use of polarized microscopy compared with that of elastic tissue staining and florescent microscopy in differentiating nonscarring from scarring alopecia. We calculated sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with 95% confidence interval.

CAPSULE SUMMARY

- Elastic tissue patterns and the pattern of birefringence in polarized sections may be helpful in the evaluation of biopsy specimens from patients with alopecia.
- Elastic staining proved to be the most reliable means to differentiate scarring from nonscarring alopecia.
- Polarized microscopy and fluorescent microscopy have the advantage of immediacy and low cost, but must be interpreted carefully and may not be substitutes for elastic tissue staining.

METHODS

Histologic sections from 62 consecutive alopecia specimens that had elastic tissue stains performed at the time of diagnosis were pulled from the files of the Ackerman Academy of Dermatopathology, New York, NY. Two were excluded because of lack of comparable H&E- and elastic-stained sections, leaving a total of 60 evaluable cases. In each case, the pattern of elastic staining was determined in elastic van Gieson (EVG)-stained sections. Comparable H&E sections were examined under a fluorescent microscope to determine the elastic tissue pattern and examined separately under polarized microscopy to determine the pattern of birefringence of collagen bundles and fibrous tracts. Two investigators (C. A. E. and D. M. E.) reviewed all sections. Calculation of the Cohen kappa coefficient was used to assess agreement between the observers.[2] The Statistica 10.0 program package (StatSoft, Tulsa, OK) was used to perform the remaining statistical calculations.

The cases studied included 17 cases of lichen planopilaris (LPP) and 3 of frontal fibrosing alopecia (FFA), an entity considered by many to be a variant of LPP. The additional specimens included 14 cases of pattern alopecia, 10 of alopecia areata, 6 of central centrifugal cicatricial alopecia (CCCA), 2 of traction alopecia, 2 of trichotillomania (1 with hair fiber granulomas and focal scar), 2 of telogen effluvium, 1 of folliculitis decalvans, 1 of early lupus erythematosus without scar (follicular vacuolar interface dermatitis), 1 of spongiotic folliculitis, 1 of end-stage alopecia secondary to infection, 1 of folliculitis decalvans, 1 of alopecia with wedge-shaped scarring and a differential diagnosis of LPP versus folliculitis decalvans, and 1 of superficial suppurative folliculitis of uncertain origin. Two patients had more than 1 diagnosis. One had a background of pattern alopecia together with LPP and the other had a background of pattern alopecia together with CCCA.

RESULTS

EVG examination

There was concordance between both observers for all EVG-stained sections (Cohen kappa coefficient = 1). EVG staining confirmed preservation of elastic fibers in all 29 cases of nonscarring alopecia (Table I). Sensitivity, specificity, PPV, and NPV with 95% confidence interval for EVG-stained sections in the diagnosis of nonscarring alopecias are presented in Table II.

Elastic fiber abnormalities were detected in the 31 cases of scarring alopecia (Table I). The patterns of elastic fiber loss included:

1. **Superficial columnar (Fig 1) or wedge shaped.** This was seen in 15 cases, predominantly in LPP (11 cases), but also in FFA (2 cases), folliculitis decalvans (1 case), and in 1 specimen that had a differential diagnosis of LPP versus folliculitis decalvans.

2. **Superficial flat bottomed.** This was seen in 5 cases: 2 cases of LPP, 1 case of trichotillomania with granulomas and scarring, 1 case of superficial folliculitis, and 1 case of FFA.

3. **Perifollicular.** This was seen in 4 cases of LPP. In H&E-stained sections, this pattern of elastic fiber loss corresponded to perifollicular mucinous fibrosis.

4. **Widening of fibrous tracts with an intact elastic sheath.** This was seen in 6 cases of CCCA (Fig 2).

5. **Diffuse patchy loss.** There was 1 case of scarring alopecia secondary to infection.

Fluorescent microscopy

In the next phase of the study, H&E-stained sections were examined under a fluorescent microscope to determine the pattern of elastic fibers and compare the results with those obtained with an elastic tissue stain. In 6 specimens (3 nonscarring and 3 scarring), the intensity of eosin fluorescence made