High Proliferative Activity Excludes Dermatofibroma
Report of the Utility of MIB-1 in the Differential Diagnosis of Selected Fibrohistiocytic Tumors

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Context.—Dermatofibroma is a benign fibrohistiocytic tumor composed of a mixture of fibroblastic and histiocytic cells. The diagnosis of this tumor is generally uncomplicated; however, rare variants may be difficult to distinguish from malignant fibrohistiocytic tumors. Deep penetrating dermatofibroma may be difficult to distinguish from dermatofibrosarcoma protuberans, and pseudosarcomatous dermatofibroma and dermatofibroma with monster giant cells share morphologic similarities with malignant fibrous histiocytoma and atypical fibroxanthoma.

Objective.—To find an immunohistochemical marker or markers that differentiate between fibrohistiocytic lesions of skin.

Design.—We evaluated the immunophenotypic characteristics of 83 fibrohistiocytic tumors (36 typical dermatofibromas, 16 cases of dermatofibrosarcoma protuberans, 16 malignant fibrous histiocytomas, and 15 atypical fibroxanthomas) using antibodies against MIB-1 (Ki-67), factor XIIIa, CD34 (HPCA-1), HHF35 (muscle-specific actin), S100 protein, and desmin.

Results.—A high proliferative index detected by MIB-1 staining excluded the possibility of dermatofibroma and was diagnostically useful in separating this entity from dermatofibrosarcoma protuberans, malignant fibrous histiocytoma, and atypical fibroxanthoma. A low proliferative index, however, could not differentiate dermatofibroma from dermatofibrosarcoma protuberans. Factor XIIIa reactivity was not helpful for the diagnosis of dermatofibroma, whereas CD34 reactivity was statistically significant in the diagnosis of dermatofibrosarcoma protuberans. The sensitivity of these 2 markers is low and therefore of questionable practical diagnostic value.

Conclusion.—Evaluation of the proliferative index may further assist in distinguishing dermatofibroma from dermatofibrosarcoma protuberans, atypical fibroxanthoma, and malignant fibrous histiocytoma.

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MFHs, and 15 AFXs. Hematoxylin-eosin–stained sections were reviewed to establish the diagnosis. Histologic criteria as detailed by Weiss and Enzinger7–9 were applied in the evaluation. Only classic examples of the entities under consideration were included in the study and, in particular, atypical variants of DF were excluded.

Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded tissue. Sections were cut at 4 μm, deparaffinized in xylene, and hydrated in graded series of ethanol. Endogenous peroxidase activity was blocked by preincubation with 1% hydrogen peroxide in phosphate-buffered saline. Heat-induced epitope retrieval are shown in Table 1. Staining was performed with the primary antibody followed by biotin-conjugated goat antirabbit or antimouse immunoglobulins followed by peroxidase-conjugated streptavidin and diamobenidine. Appropriate controls were included and evaluated.

Nuclear staining for MIB-1 was graded semiquantitatively as negative (no tumor cells staining), + (<10% of tumor cells positive; Figure 1), 2+ (10%–50% of tumor cells positive; Figure 2), and 3+ (>50% of cells positive; Figure 3). The MIB-1 reactivity was evaluated in lesional cells only. Reactivity with MIB-1 in more than 10% of tumor cells (2+ and 3+) was considered a high proliferative index. Staining was graded as either negative or positive for factor XIIIa, CD34, 1A4 (smooth muscle actin), HHF35 (muscle-specific actin), cytokeratins (AE1/AE3, CAM 5.2, and high molecular weight keratin), S100 protein, and desmin. These antibodies were evaluated in all 83 cases. The type of antibodies, dilutions, commercial sources, catalog numbers, and the use of heat-induced epitope retrieval are shown in Table 1. Staining was performed with the primary antibody followed by biotin-conjugated goat antirabbit or antimouse immunoglobulins followed by peroxidase-conjugated streptavidin and diamobenidine. Appropriate controls were included and evaluated.

The specificity of factor XIIIa for the diagnosis of DF was 100% and the specificity of CD34 for the diagnosis of DFSP was 94%. Factor XIIIa and CD34 both showed a low diagnostic sensitivity of 14% and 56% for the diagnosis of DF and DFSP, respectively. Factor XIIIa failed to demonstrate statistical significance for the diagnosis of DF, whereas CD34 reactivity was statistically significant for the diagnosis of DFSP. Smooth muscle actin (1A4) and muscle-specific actin (HHF35) were reactive in 25% (21/83) and in 11% (9/83), respectively, of the tumors studied; they failed to demonstrate significant specificity. Cytokeratins, S100 protein and desmin were nonreactive in all lesions.

**COMMENT**

Immunohistochemistry is extremely useful in the classification of spindle cell and pleomorphic cutaneous tumors.10,11 The lack of specific markers for fibrohistiocytic lesions, however, has diminished the usefulness of this method when applied to these tumors. Previous results using proliferative index with MIB-1 failed to allow the distinction of DF from DFSP: up to 10% of cells were reactive with DF, whereas DFSP showed a range of reactivity from 2% to 15%.12 The proliferative index of MFH is typically high and, on average, is above that of AFX.13 We showed that DF never demonstrates a high proliferative index (2+ or 3+; >10% of cells positive), whereas a high percentage of DFSP (44%), MFH (94%), and AFX (93%) demonstrated a high proliferation rate. A high proliferative index is statistically significant in excluding DF from all of these tumors. Use of MIB-1 may be useful when faced with this differential diagnostic problem only if a high proliferative index is detected because a low proliferative index does not differentiate DF from DFSP.

Deep penetrating DF may be difficult to distinguish from DFSP! Both lesions show a bland spindle cell proliferation extending into the subcutaneous fat. The utility of factor XIIIa and CD34 to differentiate DF from DFSP is well documented and is generally considered helpful.14–18 The literature strongly supports the role of CD34 in the diagnosis of DFSP: between 80% and 100% of cases have been reported to stain positively with this antibody.10,17–21 Rare reports, however, show a lower sensitivity of CD34 in the diagnosis of DFSP because the reactivity for this antibody may be lost in areas with myxoid or fibrosarcomatous changes.22–25 We were able to confirm the utility of CD34 in supporting the diagnosis of DFSP; however, reactivity with factor XIIIa was not statistically significant in the diagnosis of DF. We found that the sensitivity of these markers was below that which has been previously reported in the literature. Factor XIIIa and CD34 staining may be seen at the periphery of nonfibrohistiocytic tumors that induce a reactive stromal response. Consequently, we...

### Table 1. Primary Antibodies With Dilution, Source, Catalog Number, and Use of Heat-Induced Epitope Retrieval (HIER)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Dilution</th>
<th>Source*</th>
<th>Catalog No</th>
<th>HIER</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIB-1 (Ki-67)</td>
<td>1:50</td>
<td>Immunotech</td>
<td>0505</td>
<td>Yes</td>
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<tr>
<td>Factor XIIIa</td>
<td>1:1200</td>
<td>Calbiochem</td>
<td>233498</td>
<td>No</td>
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<tr>
<td>CD34 (HPCA-1)</td>
<td>1:200</td>
<td>Becton Dickinson</td>
<td>34766</td>
<td>No</td>
</tr>
<tr>
<td>Smooth muscle actin (1A4)</td>
<td>1:250</td>
<td>DAKO</td>
<td>M851</td>
<td>No</td>
</tr>
<tr>
<td>Muscle-specific actin (HHF35)</td>
<td>1:200</td>
<td>DAKO</td>
<td>M635</td>
<td>No</td>
</tr>
<tr>
<td>Cytokeratin (AE1/AE3)</td>
<td>1:200</td>
<td>DAKO</td>
<td>M3515</td>
<td>Yes</td>
</tr>
<tr>
<td>Cytokeratin (CAM 5.2)</td>
<td>1:1500</td>
<td>Becton Dickinson</td>
<td>92-0005</td>
<td>Yes</td>
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<tr>
<td>Cytokeratin 34BE12</td>
<td>1:50</td>
<td>DAKO</td>
<td>Z311</td>
<td>No</td>
</tr>
<tr>
<td>S100 protein</td>
<td>1:6000</td>
<td>DAKO</td>
<td>M760</td>
<td>Yes</td>
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<tr>
<td>Desmin</td>
<td>1:500</td>
<td>DAKO</td>
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* Immunotech, Fullerton, Calif; Calbiochem, San Diego, Calif; DAKO, Carpinteria, Calif; and Becton Dickinson, Franklin Lakes, NJ.
interpret peripheral nontumoral staining of factor XIIIa and CD34 as nonspecific when intact tumor cells in the center of the lesion fail to stain with these antibodies.

Prior studies also have shown the complicated interpretation of factor XIIIa staining when it is sometimes difficult to separate reactive dermal dendrocytes from tumor cells.15 Strict interpretation of factor XIIIa and CD34 in this manner may partially explain the rates of expression noted with these markers in our study. These findings are in contrast to rates of factor XIIIa and CD34 reactivity reported in the initial studies of DF and DFSP. However, subsequent authors have confirmed wide variability in expression of these markers in DF and DFSP, and have demonstrated rates of expression similar to those noted in this present investigation. Consequently, we believe the preponderance of information currently available cautions against overreliance of factor XIIIa and CD34 in the separation of DF from DFSP. Furthermore, Horenstein et al26 reported a group of tumors that demonstrate overlapping clinical, histologic, and immunohistochemical features of both DF and DFSP in which diffuse immunoreactivity for factor XIIIa and CD34 was noted in all 10 tumors studied. We believe that these findings confirm the lack of utility of factor XIIIa and CD34 in cases of diagnostically challenging fibrohistiocytic lesions. Interestingly, all of the tumors they studied were described as having low mitotic counts.

In conclusion, evaluation of proliferative activity using MIB-1 staining is a helpful adjunct in the diagnosis of fibrohistiocytic lesions when the pathologic differential diagnosis includes DF because a high proliferative index excludes this diagnosis. However, a low proliferative index using MIB-1 cannot differentiate DF from DFSP. Factor XIIIa, although specific for DF, was not statistically significant in this study to establish the diagnosis of DF. When used in conjunction with appropriate morphologic analysis, CD34 is a confirmatory marker for the diagnosis of DFSP. The interpretation of immunohistochemical stains for factor XIIIa and CD34 may be complicated by nonspecific peritumoral reactivity, which could explain the differences in the diagnostic sensitivity of these markers reported in the literature. Sole reliance on factor XIIIa or CD34 in differentiating between DF and DFSP should be avoided.

References
5. Zelger B, Sidoroff A, Stanzl U, et al. Deep penetrating dermatofibroma ver-

Table 2. Immunohistochemical Results of Proliferative Activity, Factor XIIIa, and CD34*

<table>
<thead>
<tr>
<th>Tumor</th>
<th>MIB-1</th>
<th>Factor XIIIa</th>
<th>CD34</th>
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<tr>
<td>DF</td>
<td>36</td>
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<td>10</td>
</tr>
<tr>
<td>DFSP</td>
<td>16</td>
<td>3</td>
<td>6</td>
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<tr>
<td>MFH</td>
<td>16</td>
<td>0</td>
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<td>AFX</td>
<td>15</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>29</td>
<td>18</td>
</tr>
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</table>

*MIB-1 staining was graded semiquantitatively as negative (no tumor cells staining), 1+ (<10% of tumor cells positive), 2+ (10%–50% of tumor cells positive), and 3+ (>50% of cells positive). Factor XIIIa indicates DF, dermatofibroma; DFSP, dermatofibrosarcoma protuberans; MFH, malignant fibrous histiocytoma; and AFX, atypical fibroxanthoma.


