Immunophenotypic Correlation Between Skin Biopsy and Peripheral Blood Findings in Mycosis Fungoides

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Abstract

In mycosis fungoides (MF) with blood involvement, T-cell immunophenotypes in skin and blood have not been compared. Our aim was to evaluate T-cell immunophenotypes in skin by immunohistochemical analysis and compare results with flow cytometric (FC) findings in blood. Of 20 patients with MF with blood involvement, the immunophenotype was discrepant in 11 (55%). Compared with FC findings in blood, immunohistochemical analysis of skin samples failed to detect partial deletion of CD2 (5/11 [45%]), CD3 (3/11 [27%]), and CD5 (3/11 [27%]) and overrepresented deletion of CD7 in 2 (18%) of 11 patients. In addition, CD8+ MF was missed by immunohistochemical analysis in 2 (18%) of 11 patients. Identical T-cell populations were demonstrated by T-cell gene polymerase chain reaction in skin and blood in 8 of the 11 patients who had a discrepant immunophenotype. Awareness of the limitations of immunohistochemical analysis of skin samples is of practical value for pathologists interpreting skin biopsies in MF patients. In addition, our findings suggest CD8+ MF to be more common than previously reported.

Mycosis fungoides (MF) is the most common primary cutaneous lymphoma,[1] yet, the diagnosis of early MF represents one of the most difficult challenges of dermatopathology. Clinical features of early MF can resemble benign inflammatory skin conditions such as eczema and psoriasis, and the histologic features of early MF substantially overlap with inflammatory skin disorders.[2,3] Ancillary techniques that have been incorporated into the histologic evaluation of skin biopsy specimens to facilitate the diagnosis include immunohistochemical analysis to evaluate the CD4/CD8 ratio and loss of T-cell antigens and T-cell receptor (*TCR*) gene rearrangement assays to evaluate clonality. Just like clinical and histologic parameters, these ancillary techniques are not entirely specific or sensitive for MF, and findings may overlap with inflammatory skin conditions. In particular, the diagnostic usefulness of T-cell immunophenotyping in skin biopsy specimens has remained disappointing because sensitivity is low for the detection of aberrant T-cell antigen deletions. Since a direct comparison of the immunophenotype of the MF cells is not available, it is not clear whether the low detection rate of T-cell antigen deletions is a genuine feature of early cutaneous T-cell lymphoma or merely reflects technical limitations of immunohistochemical analysis in skin biopsy specimens.

Sézary syndrome, the leukemic variant of cutaneous T-cell lymphoma, is a rare and often late complication of MF; however, variable numbers of circulating cutaneous T-cell lymphoma cells can be detected in many patients with MF in earlier stages of disease by flow cytometry[4,5] and molecular methods.[6,7] Flow cytometry of the peripheral blood is a useful tool for the detection of circulating T-cell lymphoma cells in MF, yet a direct comparison between T-cell immunophenotypes detected in skin biopsy specimens and blood samples does not exist.

The aim of our study was to compare T-cell immunophenotypes detected in skin biopsy specimens by immunohistochemical analysis with flow cytometric immunophenotypes detected in peripheral blood. By correlating the results of these 2 techniques, we evaluated the performance and limitations of T-cell immunophenotyping by immunohistochemical analysis in skin biopsy specimens in MF.

Materials and Methods

Case Selection

This study was approved by the institutional review board of Oregon Health & Science University, Portland. The pathology database of Oregon Health & Science University was searched for cases of MF with histologic analysis of skin biopsy specimens and concomitant flow cytometric analysis of peripheral blood during an interval from January 1, 2000, to June 30, 2009. Morphologic, immunophenotypic, laboratory, and molecular findings were reviewed. T-cell immunophenotypes detected in skin biopsy specimens by immunohistochemical analysis were compared with the flow cytometric T-cell immunophenotypes detected in peripheral blood. Results of molecular studies for clonal T-cell gene rearrangement were compared between skin and blood samples to evaluate clonal identity of the T-cell lymphoma.

Histologic Studies and Evaluation of Skin Biopsy Specimens

Peripheral blood samples were stained with Wright-Giemsa for morphologic evaluation.

Skin biopsy specimens were fixed in 10% buffered formalin before embedding and were stained with H&E for histologic evaluation. H&E-stained slides were first reviewed by a dermatopathologist (C.R.W.); all H&E and immunohistochemical results were then reviewed by at least 2 hematopathologists (K.K. and K.G., R.M.B., or G.F.). For a diagnosis of MF, a combination of histologic features, immunohistochemical findings, and, when available, molecular results were considered. Morphologic diagnostic criteria for MF were based on those reported by Smoller et al[8] and included the following: (1) lymphocytic atypia, defined by nuclear enlargement, hyperchromasia, and irregular or cerebriform nuclear contours; (2) epitheliotropism of lymphocytes; (3) alignment of morphologically atypical lymphocytes along the epidermal side of the dermo-epidermal junction; and (4) papillary dermal fibrosis with coarsened collagen fibers that contain a dense infiltrate of lymphocytes.

Immunohistochemical features to consider included aberrant deletion of expression of 1 or more T-cell antigens in the epidermis, dermis, or both. A T-cell antigen (CD2, CD3, CD5, and CD7) was considered partially deleted when an estimated 30% or more of the T-cell population was negative for the marker. Deletion of T-cell antigens, however, was not required for a positive diagnosis. Molecular features to consider included the presence or absence of clonal T-cell gene rearrangement by polymerase chain reaction (PCR). Clonality was not required for a positive diagnosis.

Immunohistochemical Analysis

Immunohistochemical analysis was performed on formalin-fixed, paraffin-embedded skin biopsy specimens using the following primary antibodies: anti-CD2 mouse monoclonal and anti-CD4 mouse monoclonal (Novocastra Laboratories, Newcastle upon Tyne, England); anti-CD3 rabbit monoclonal, anti-CD20 mouse monoclonal, anti-CD30 mouse monoclonal, and anti–Ki-67 rabbit monoclonal (Ventana Medical Systems, Tucson, AZ); anti-CD5 rabbit monoclonal and anti-CD8 mouse monoclonal (Cell Marque, Hot Springs, AR); and anti-CD7 mouse monoclonal (Vector Laboratories, Burlingame, CA). After incubation with the primary antibody, immunodetection was performed with a biotin-conjugated secondary antibody formulation that recognizes rabbit and mouse immunoglobulins (Ventana), followed by peroxidase-labeled streptavidin and with diaminobenzidine chromogen as the substrate (Ventana UltraView Universal DAB Detection Kit). All immunostaining was performed using a BenchMark XT automated immunostaining device (Ventana).

Peripheral Blood Evaluation

Wright-Giemsa–stained peripheral blood smears were reviewed and evaluated in combination with CBC results and flow cytometric analysis. Absolute count of the circulating T-cell lymphoma cells was calculated based on the CBC and the percentage of aberrant T-cell population detected by flow cytometry. Criteria to diagnose peripheral blood involvement were as follows: (1) CD4/CD8 ratio of 10 or more by flow cytometry; (2) aberrant expression of pan–T-cell markers CD2, CD3, CD5, or CD7, defined as distinctively lower or higher intensity of expression in at least 30% or more of the gated T-lymphocyte population; and (3) evidence of a T-cell clone in the blood by TCRγ region PCR, with or without absolute lymphocytosis, identical to the T-cell clone identified in the skin biopsy specimen with MF.

Flow Cytometric Immunophenotyping

Cell suspensions of peripheral blood cells were prepared and stained for flow cytometry within 24 hours of sample collection. We incubated 50-μL aliquots of cell suspensions with 4- or 8-color combinations of fluorescent monoclonal antibodies including CD2, CD3, CD4, CD5, CD7, CD8, and CD45 (Becton Dickinson Biosciences, San Jose, CA). After 30 minutes, 2.5 mL of ammonium chloride was added for 10 minutes

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to lyse RBCs. The cells were washed with phosphate-buffered saline containing 0.1% bovine serum albumin and 0.1% sodium azide. The cell pellet was resuspended in 0.5 mL of phosphate-buffered saline containing 1% EM grade formaldehyde (Polysciences, Warrington, PA). Cells were analyzed on a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA). Data were analyzed using CellQuest (BD Biosciences) and WinList software (Verity Software House, Topsham, ME).

Molecular Studies

PCR for clonal *TCR* gene rearrangement was performed on paraffin shavings of skin biopsy specimens and on peripheral blood samples at Hematologics (Seattle, WA), or at the University of Washington Medical Centers Hematopathology Laboratory (Seattle). For a molecular comparison between skin and blood samples from the same patient, the same molecular laboratory was used in each case. Therefore, a direct molecular comparison was possible between the PCR amplified products.

To identify T-cell clonality, Hematologics used the *TCR*γ gene-rearrangement assay kit from InVivoScribe Technologies (San Diego, CA)[9] according to the instructions. Briefly, 0.5 to 1.0 μg of DNA was amplified in the presence of 0.15 μL of *Taq* polymerase and a mixture of fluorochrome-labeled primers specific for conserved regions within the variable (V) regions $V_\gamma 1$ through $V_\gamma 8$, $V_\gamma 10$, $V_\gamma 9$, and $V_\gamma 11$ and the J_γ exon of the joining region of the *TCR* gene. Monoclonal, polyclonal, and no-template control samples were analyzed by fluorescence detection using the ABI 310 capillary electrophoresis sequencer and ABI Prism GeneScan software (Applied Biosystems, Foster City, CA). Primer sequences, assay sensitivity, and assay specificity were published previously.[9]

At the University of Washington Medical Centers Hematopathology Laboratory, T-cell clonality was determined by PCR amplification of rearrangements at the *TCR*γ locus as previously described.[10] Briefly, 0.5 μg of DNA was amplified in the presence of a 400-nmol/L concentration of each of the upper and lower primers and 1.25 U of *Taq* DNA polymerase (Perkin Elmer, Norwalk, CT). After 40 cycles, PCR products were analyzed on an ABI 310 analyzer. The presence of a clonal T-cell population was determined by the presence of 1 or 2 peaks of fluorescence intensity at least 2-fold greater than background fluorescence among the 3 primer sets. Primer sequences, assay sensitivity, and assay specificity were published previously.[10]

Results

Subjects were 45 patients, including 29 men and 16 women who fulfilled inclusion criteria. The age of subjects ranged from 21 to 85 years, with a median age at time of the initial biopsy of 62.5 years.

Skin Biopsy Findings

The 45 patients underwent 53 skin biopsies. The diagnoses established on skin biopsy included the following: patch/plaque stage MF, 47 (89%) of 53; follicular mucinosis, 2 (4%) of 53; granulomatous MF, 2 (4%) of 53; tumor-stage MF, 1 (2%) of 53; and MF palmaris et plantaris, 1 (2%) of 53.

Peripheral Blood Findings

The 45 patients underwent a total of 57 peripheral blood analyses to rule out peripheral blood involvement by MF or Sézary syndrome. The median elapsed time between skin biopsy and peripheral blood sampling was 20 days (range, 0-724 days).

Of the 45 patients, 25 had no evidence of peripheral blood involvement. None of the 25 patients had an abnormal T-cell population detected in the peripheral blood by flow cytometry. In 20 patients (represented by 32 peripheral blood tests) there was involvement of peripheral blood. Of the 20 patients, 7 had several involved peripheral blood samples over time (range of number of involved blood samples, 2-5; median, 3). In the 7 patients, the flow cytometric immunophenotype of the aberrant T-cell population remained stable over time in all cases.

Diagnostic findings in 20 patients with involved peripheral blood included the following: (1) A flow cytometric finding of CD4/CD8 ratio of 10 or more was found in 12 patients (60%). (2) Aberrant expression of 1 or more T-cell antigens CD2, CD3, CD5, and CD7 (defined as distinctively lower or higher intensity of expression in at least 30% or more of the gated T-lymphocyte population) was found in 17 patients (85%). Of the 17, the individual T-cell antigen aberrancies in order of frequency were as follows: CD7 deletion, the most common in 7 (41%); a combination of CD2 and CD7 deletion, 5 (29%); CD5 deletion as a single aberrancy, 2 (12%); and combinations of CD3 with CD5 deletion; of CD2, CD3, and CD5; and of CD3, CD5, and CD7, 1 patient each (6%). An inverted CD4/CD8 ratio was detected in 4 (20%) of 20 patients. (3) Clonal T-cell gene rearrangement by PCR was tested in the blood in 15 of 20 patients: 13 were clonal and 2 were oligoclonal. Absolute lymphocytosis (defined as a lymphocyte count of ≥4,000/μL [4.0×10^9/L]) was present in 8 (40%) of 20.

Correlation Between T-Cell Immunophenotype Detected in Skin and in Peripheral Blood

In 20 patients with peripheral blood involvement, the T-cell immunophenotype in skin was compared with the flow cytometric immunophenotype detected in peripheral blood **Image 1** and **Image 2**. A discrepant immunophenotype was defined as 1 or more of the major T-cell antigens (CD2, CD3, CD5, or CD7) reported as deleted or partially deleted by one method but reported as normal by the other method.

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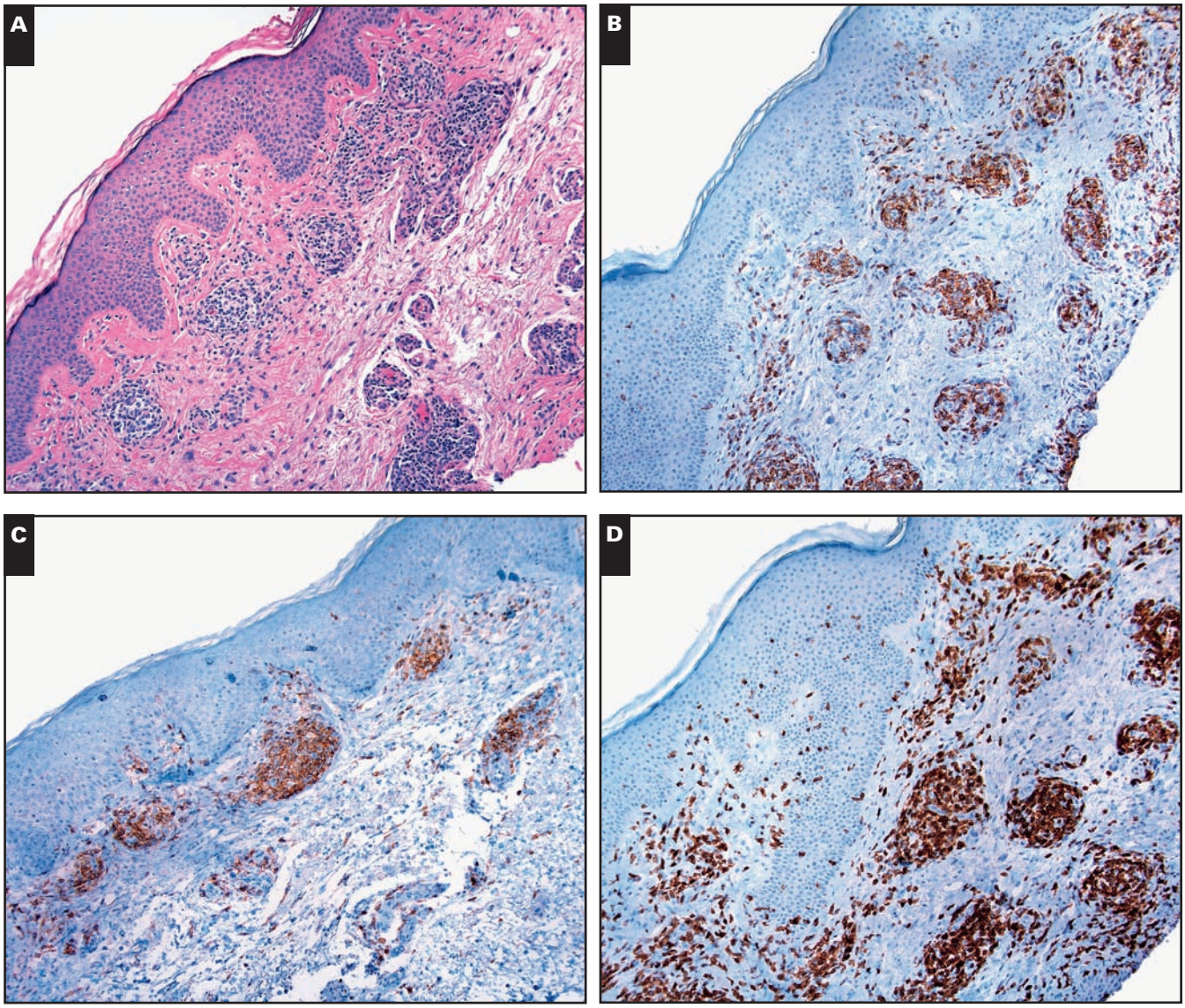

Image 1 (Case 9) Skin biopsy findings. **A**, H&E-stained section shows a superficial dermal perivascular and band-like infiltrate of small lymphocytes with minimal epidermotropism (×200). Immunohistochemical analysis shows a predominant CD4+ T-cell population (**C**, ×200) with deletion of CD7 (**H**, ×200) and retained expression of CD2 (**B**, ×200), CD3 (**D**, ×200), and CD5 (**F**, ×200).

A quantitative difference in the value of the CD4/CD8 ratio with a consistent CD4+ phenotype between skin and blood was not considered a discrepancy. However, if an aberrant T-lymphocyte population was reported as CD4+ in skin but it appeared as CD8+ in blood (or vice versa), the immunophenotype was interpreted as discrepant. In 9 of the 20 patients, the immunophenotypes detected in skin and peripheral blood were essentially identical, as follows: CD4+ with loss of CD7 antigen in 6 (67%), CD4+ with no major T-cell antigen loss in 1 (11%), CD4+ with loss of CD5 in 1 (11%), and CD4+ with combined loss of CD2 and CD7 in 1 (11%).

In the remaining 11 patients, the immunophenotype reported in skin and in peripheral blood was different. Immunophenotypic differences between skin and blood were observed in the following categories: (1) Flow cytometric analysis of blood detected 1 or more additional T-cell antigen aberrancy compared with the skin in 8 patients (73%). In order of frequency, additional CD2 deletion was detected in 5 patients (45%), additional CD3 in 3 (27%), additional CD5 in 3 (27%), and additional CD7 in 2 patients (18%). In 4 of the 8 patients, the blood analysis detected more than 1 additional aberrancy. (2) Immunohistochemical analysis of skin samples reported additional T-cell aberrancies compared with blood: This scenario was observed much less frequently, in 4 (36%) of 11 patients. Of note, 3 of these additionally detected aberrancies in skin were that of CD7 when a concomitant blood sample showed no evidence of CD7 deletion on the T cells. In 1 patient, a CD5 deletion was reported in the skin sample

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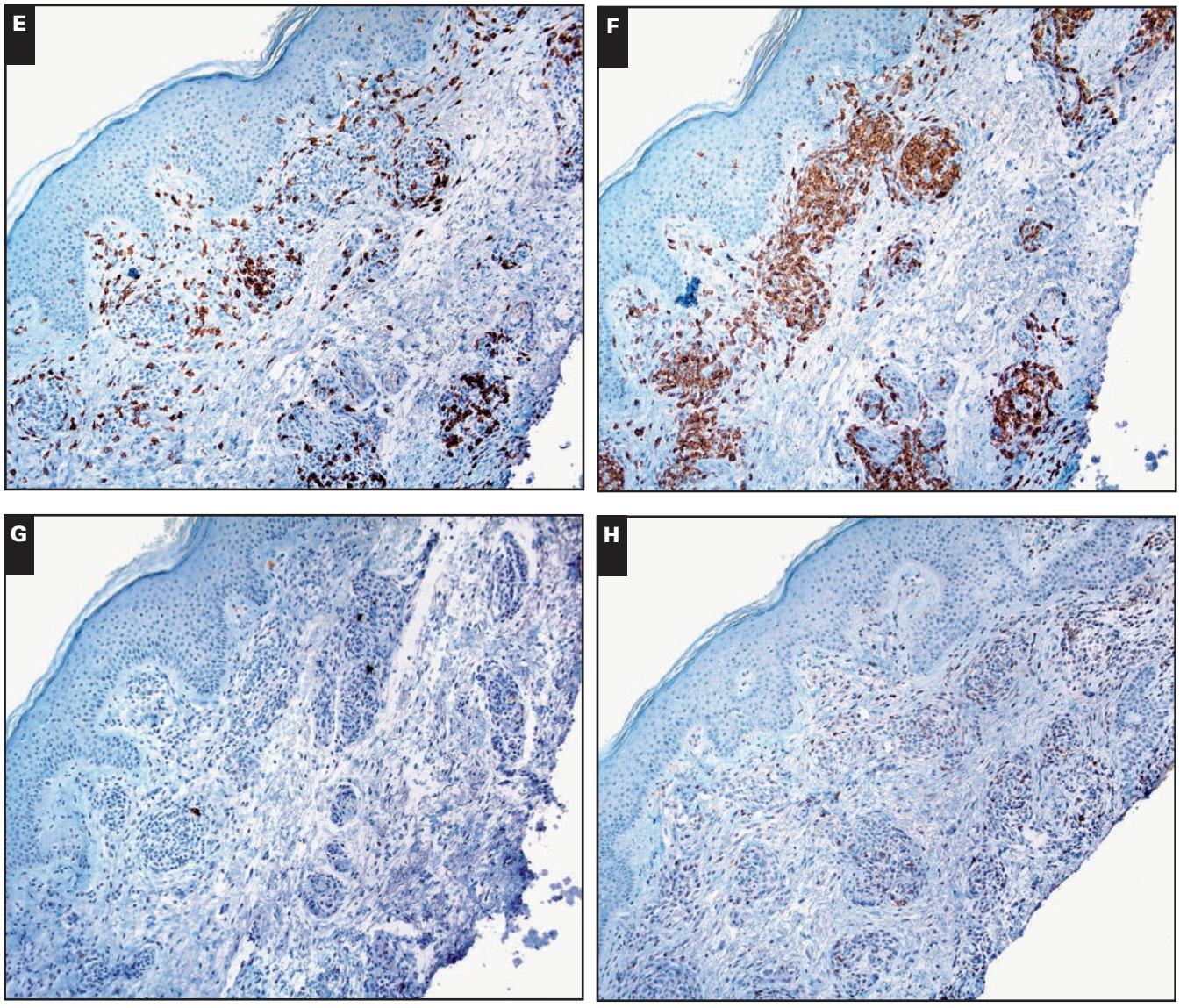

CD8+ T cells are rare (**E**, ×200), and CD20+ B cells are virtually absent (**G**, ×200).

that was not observed in the blood sample analysis. (3) Flow cytometric analysis of blood samples detected a CD8+ aberrant T-cell population, while the skin sample was reported as CD4+ in 3 patients.

Demographic, immunophenotypic, and molecular findings for the 11 patients with immunophenotypic discrepancies between skin and blood are summarized in **Table 1**.

Correlation of Molecular Findings Between Skin and Peripheral Blood

A possible explanation for a different T-cell immunophenotype between skin and blood in patients with MF could be that the immunophenotypically abnormal T-cell populations detected in skin and blood samples are not the same. This question is especially important in patients with a CD4+ T-cell population detected in the skin and CD8+ T cells identified in the blood. A comparison between the results of molecular studies for T-cell gene rearrangement might be helpful to address this issue. Results of PCR for clonal T-cell gene rearrangement performed on skin biopsy and peripheral blood were available for comparison in 9 of the 11 patients with discrepant immunophenotypes **Image 3**. In 7 of the 9 patients, there was evidence of identical clonal T-cell populations in the skin and peripheral blood samples, providing strong support that the same aberrant T-cell population is present in skin and blood. Another patient had a clonal T-cell population in the skin, and oligoclonal peaks were detected in peripheral blood (case 5 in Table 1), one of which was identical to the clonal peak detected in skin, also supporting the presence of the same T-cell population in the skin and blood.

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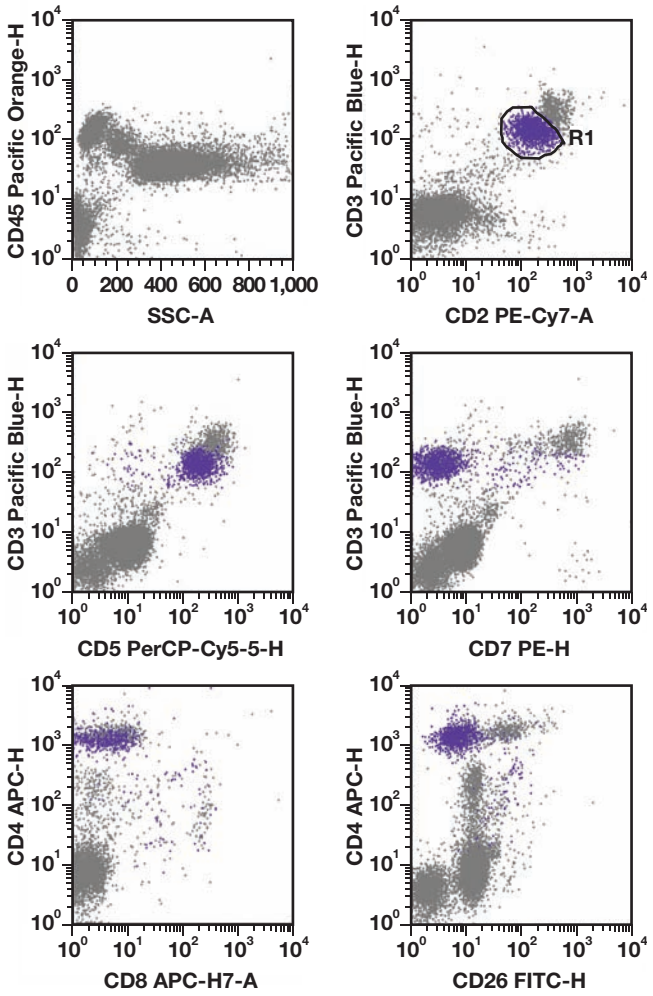

Image 2 (Case 9) Peripheral blood, flow cytometric findings. The absolute lymphocyte count of the peripheral blood is within normal range (3.4 × 10^3/μL). Of the lymphocytes, 90% are CD3+ T cells, 85% of which show an aberrant immunophenotype with the following immunophenotype: dim CD3+, dim CD2+, CD4+, dim CD5+, CD7–, and CD26– (purple gate). The CD4/CD8 ratio within the aberrant T-cell population is 90:1. APC, allophycocyanin; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin chlorophyll protein; SSC, side scatter.

Finally, 1 patient had a clonal T-cell population present in the skin, and oligoclonal peaks were detected in the peripheral blood; however, none of the molecular peaks were identical to the peak detected in the skin. Of note, this patient had a CD8+ T-cell population in the blood and a CD4+ T-cell population in the skin. In this patient, the molecular results suggest that the 2 T-cell populations are likely not the same.

Of the 9 patients who had overlapping immunophenotypes between skin and peripheral blood samples, 6 had clonality studies performed by PCR on skin and blood samples. All 6 patients had identical clones present in the skin in blood, with a specific observation that one of them had the same identical 3-peak oligoclonal product present in blood and multiple skin biopsy specimens.

In the group of 25 patients who had no evidence of peripheral blood involvement and had unremarkable CBC and flow cytometric results, 7 underwent PCR for T-cell gene rearrangement. In 5 of the 7 patients, the results were polyclonal; in 2 patients, however, there was evidence of clonal T-cell gene rearrangement in the blood. In 1 of the 2 patients, there was also a clonal T-cell population identified in a skin biopsy specimen; however, the PCR product in the blood sample was different; therefore, the clone in the blood was likely not the same as in the skin. The other patient with a clonal result in the blood did not have molecular comparison available for the skin biopsy specimen. Since the morphologic, CBC, and flow cytometric findings are entirely unremarkable in these 2 patients, these clonal T cells are likely present in very low levels and their significance is not clear. Possibilities include very low-level involvement by MF cells (although not the same clone as the skin) and T-cell clones of undetermined significance.

Table 1
Demographic, Immunophenotypic, and Molecular Findings in 11 Patients With Immunophenotypic Discrepancies Between Skin and Blood

	Skin Biopsy Immunohistochemical Results		
Case No./ Sex/Age (y)	Location	CD4/CD8	T-Cell Immunophenotype
1/M/56	Trunk	10/1	CD2+, CD3+, CD4+, partial drop of CD5, CD7–
2/M/59	Thigh	Increased	No antigen loss; CD2+, CD3+, CD4+, CD5+, CD7+
3/M/79	Palmar and plantar skin	Increased	CD2+, CD3+, CD4+, CD5+, partial drop of CD7
4/F/84	Shoulder	10/1	CD2+, CD3+, CD4+, CD5+, partial drop of CD7
5/M/79	Hand	2/1	CD2+, CD3+, CD4+, CD5+, partial drop of CD7
6/M/70	Leg	Increased	CD2+, CD3+, CD4+, CD5+, partial drop of CD7
7/F/80	Abdomen	Increased	CD2+, CD3+, CD4+, CD5+, minimal drop of CD7
8/M/50	Arm	1/1	CD2+, CD3+, CD4+, CD5+, partial drop of CD7
9/F/74	Arm	5/1	CD2+, CD3+, CD4+, CD5+, CD7–
10/M/63	Arm	Increased	No antigen loss; CD2+, CD3+, CD4+, CD5+, CD7+
11/F/88	Back, thigh	10/1	CD2+, CD3+, partial drop of CD5 and CD7

Discussion

Histologic diagnosis of early MF is challenging because of the substantial overlap between the clinical and histopathologic features of MF and numerous inflammatory skin conditions.[2,3]

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Peripheral Blood Flow Cytometric Results		
CD4/CD8	**T-Cell Immunophenotype**	**Molecular Results**
129/1	Dim CD2+, CD3+, CD4+, CD5+, CD7–, CD26–	Identical T-cell clone in skin and peripheral blood
Increased	Dim CD2+, CD3+, CD4+, CD5+, CD7–, CD26–	Identical T-cell clone in skin and peripheral blood
8/1	CD2+, CD3+, CD4+, CD5+, CD7+, CD26–	Identical T-cell clone in skin and peripheral blood
10/1	CD2+, dim CD3+, dim CD5+, CD7+, CD26–	T-cell clone in skin; blood not tested
0.5/1	CD2+, CD3+, CD5+, CD7–, CD8+	T-cell clone in skin; oligoclonal peripheral blood (identical peak)
14/1	Dim CD2+, CD3+, CD4+, CD5+, dim CD7+	Identical T-cell clone in skin and peripheral blood
40/1	Dim CD2+, CD3+, CD4+, CD5+, CD7–	Not done
0.3/1	CD2+, CD3+, CD5+, dim CD7+, CD8+	Identical T-cell clone in skin and peripheral blood
90/1	Dim CD2+, dim CD3+, CD4+, dim CD5+, CD7–, CD26–	Identical T-cell clone in skin and peripheral blood
10/1	CD2+, dim CD3+, CD4+, dim CD5+, dim CD7+, CD26–	Identical T-cell clone in skin and peripheral blood
0.5/1	CD2+, CD3+, dim CD5+, CD7+, CD8+	T-cell clone in skin; blood oligoclonal without overlapping peak with skin clone

Without ancillary techniques, histologic and cytologic interpretation of skin biopsy specimens is characterized by low accuracy and reliability.[11-14] Ancillary studies such as T-cell immunophenotyping and *TCR* gene rearrangement studies are often applied to facilitate the diagnosis.

Immunohistochemical analysis typically evaluates the CD4/CD8 ratio and the loss of expression of the T-cell antigens CD2, CD3, CD5, and CD7 in epidermal or dermal lymphocytes as a finding associated with MF. CD7 is by far the most commonly reported T-cell antigen to be lost in MF; however, it is also the least specific because CD7 is observed to be lost in many inflammatory skin conditions.[15-17] Decreased expression of CD2 or CD5 has been described as very specific but relatively insensitive for MF; for example, a recent study by Furmanczyk et al[18] observed sensitivity and specificity of 22% and 100%, respectively, for decreased expression of CD2 or CD5 in epidermal T cells.[18] Other investigators observed similarly low sensitivity for decreased expression of CD2, CD3, and CD5.[15,19] In another recent study, Florell et al[15] found that immunohistochemical stains were of limited usefulness in establishing a diagnosis in difficult cases in which the routine pathologic features were insufficient for the diagnosis of MF. In their hands, the majority of skin biopsies, including benign and normal skin, showed infiltrates of CD7– T cells. Epidermal or dermal deletion of CD3, CD5, or CD7 was not associated significantly with a final diagnosis of MF. In fact, the additional

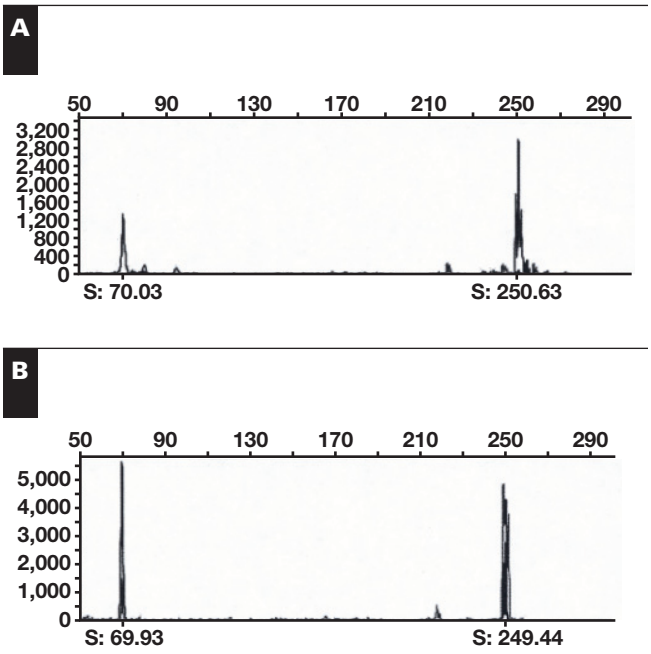

Image 3 (Case 9) Molecular comparison between T-cell populations present in skin biopsy (**A**) and peripheral blood (**B**). Polymerase chain reaction for the T-cell receptor γ region identifies monoclonal amplicons in both specimens, with distinct peaks of identical size at 70 base pairs (bp) and 249-251 bp, respectively. This result suggests that the clonal T-cell populations present in skin and blood are the same.

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information provided by T-cell immunophenotyping most often resulted in no change in diagnosis or downgrading the reaction pattern.[15]

Overall, the performance of T-cell immunophenotyping by immunohistochemical analysis has been disappointing as an aid in diagnosis of MF. It is not clear from these studies whether the lack of detectable CD2, CD3, or CD5 T-cell antigen loss is a genuine feature of early MF or simply reflects a limitation of the immunohistochemical technique performed on skin biopsy specimens. To answer this question, we took advantage of flow cytometric immunophenotyping of the circulating T cells in patients with MF with peripheral blood involvement to perform a direct comparison of the T-cell immunophenotype detected in skin and blood samples.

Although leukemic involvement of peripheral blood is infrequent in MF, circulating tumor cells are often detectable by flow cytometry. The reported frequency of peripheral blood involvement varies considerably in different studies, from 3% to 27%.[20,21] With erythrodermic MF, peripheral blood involvement is more frequent (42%).[7] This frequency becomes even higher, up to 73% when a V_β antibody repertoire is used to establish clonality by flow cytometry[22] or with the highly sensitive technique of PCR.[6,7] In our study, of 45 patients who had parallel testing of skin and peripheral blood for MF, 20 (44%) had evidence of peripheral blood involvement based on a combination of flow cytometric and molecular diagnostic criteria. This high percentage of 44% blood involvement clearly reflects a sampling bias toward more advanced disease because peripheral blood flow cytometry is preferentially ordered for patients who already have advanced features, such as erythroderma. In our patient group, 7 of 45 had erythroderma, and they were all positive for blood involvement. Nevertheless, a direct comparison of the skin and blood immunophenotypes was possible in 20 patients. Overall, the frequency and spectrum of deletion of different T-cell antigens detected in the blood was comparable to previously published results.[4] Of important note, the aberrant flow cytometric T-cell immunophenotype in the peripheral blood was stable and reproducible over time in patients who had several involved blood samples.

By comparing the immunophenotype between skin and blood samples, we found that in 9 of 20 patients, the immunophenotypes reported for skin and peripheral blood were essentially identical. In the remaining 11 patients, however, immunophenotypic differences were observed between skin and blood. The most frequently observed type of discrepancy was the detection of 1 or more additional T-cell antigen aberrancies in blood compared with the skin, and this was observed in 8 patients (73%). In order of frequency, an additional CD2 deletion was detected in 5 patients (45%), an additional CD3 in 3 (27%), an additional CD5 in 3 (27%), and an additional CD7 in 2 patients (18%). In 4 of the 8 patients, the blood analysis revealed more than 1 additional aberrancy.

A discrepancy of the opposite nature—immunohistochemical analysis of a skin biopsy specimen detected additional T-cell aberrancies compared with blood—was observed much less frequently, in 4 (36%) of 11 patients. Of note, in almost all of these cases (3/4), the additionally detected aberrancy in skin was that of CD7 when a concomitant blood sample showed no evidence of CD7 deletion in the T cells. In 1 patient, a CD5 deletion was reported in the skin sample that was not observed in the blood analysis. These data suggest that CD2, CD5, and even CD3 deletions are common features of the aberrant T cells in MF, and the low sensitivity observed in skin immunophenotyping is likely a consequence of the limitations of the technique.

In addition to higher sensitivity of the technique itself, flow cytometry has the power of gating on aberrant subpopulations of T cells and, as such, is able to distinguish between an aberrant T-lymphocyte population and a background reactive T-cell population with normal immunophenotypic features. In this regard, the frequent finding of additional abnormalities by flow cytometry is somewhat predictable. Furthermore, flow cytometry also detects differences in fluorescence intensity ("dim," "moderate," or "bright" intensity), a feature that would be unlikely to be reproduced by immunohistochemical analysis. As a consequence, immunohistochemical analysis of lymphocytic infiltrates of the skin is more prone to be skewed by an admixed reactive T-cell population that is often present in the superficial dermis of early MF.

In 3 of our patients, loss of CD7 antigen was reported in skin, yet flow cytometry showed CD7 expression within the normal range. This result suggests that loss of CD7 in skin biopsy specimens is probably overreported, and, in some cases, it reflects CD7 loss in the admixed reactive T cells rather than in MF cells. Another possible source of immunophenotypic discrepancy between skin and blood could be variable access of aberrant T cells from different layers of the skin to the peripheral blood. Discordant expression of antigens between intraepidermal and intradermal T cells has been described in MF.[23] In our 20 cases with peripheral blood involvement, however, we found only 2 cases in which we observed a localization of T-cell antigen aberrancy (CD5 and CD7, respectively) exclusively to the epidermis in the skin biopsy sample. Therefore, the low number of cases does not allow for a conclusion about this question.

A third group of discrepancies between skin and blood immunophenotype occurred in the category of CD4+ vs CD8+ immunophenotype. MF tumor cells are typically of the CD4 T-helper phenotype,[24] and only a minority of cases are CD8+. In a large study by Massone et al,[25] CD8+ cases represented 15% of total MF cases, and they concluded that a CD8+ immunophenotype has no influence on the prognosis of the disease. Immunohistochemical assessment of the CD4/CD8 ratio has been used in the evaluation of MF in skin biopsy

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specimens; however, the cutoff value varies between studies.[15,17,26] Therefore, a quantitative difference in the value of the CD4/CD8 ratio with a consistent CD4+ phenotype in skin and blood was not considered as a discrepancy in our study. However, if an aberrant T-lymphocyte population was reported CD4+ in skin but it appeared as CD8+ in blood (or vice versa), the immunophenotype was interpreted as discrepant.

Our study suggests that a CD8+ immunophenotype might be more common than reported in skin biopsy results. In 3 of our 11 patients with discrepant immunophenotypes, a CD8+ aberrant T-cell population was detected by flow cytometric analysis of the blood, while the MF in the skin biopsy sample was reported CD4+. In careful retrospective analysis, 2 of the 3 cases had an unusually low CD4/CD8 ratio reported for the skin biopsy sample (CD4/CD8 ratios of 2:1 and 1:1, respectively; cases 5 and 8 in Table 1). In their blood samples, flow cytometry identified a distinct subpopulation of T cells with partially deleted CD7 expression and a CD8+ immunophenotype. PCR for T-cell clonality revealed identical clones in skin and blood in both of these cases, providing evidence that the aberrant T cells in skin and blood are in fact, the same.

The third case was somewhat different. Retrospective review of the skin biopsy showed a high CD4/CD8 ratio of 10:1 in skin, highly suggestive of a true CD4+ phenotype, with additional drop of CD5 and CD7. In the blood, an aberrant T-cell population was revealed; however, it showed a CD8+ phenotype and only CD5 was dim and CD7 was retained. The molecular study showed a clonal T-cell population in the skin and oligoclonal T-cell populations in the blood. Of note, no molecular peak overlapped with the clonal peak in the skin. Based on the molecular results, the T-cell populations, although both appear aberrant, are likely different. This might be an example of MF with a reactive oligoclonal T-cell population in the blood rather than a true CD8+ example of early MF.

In general, the finding of a different immunophenotype between skin and blood samples in patients with MF should raise the question of identity of the 2 T-cell populations. Clonal heterogeneity between skin biopsy specimens of different anatomic sites has been described in MF, with the frequency varying in different studies from 10% to 23%.[27,28] Patients who had the same clone detected in multiple concurrent skin biopsy specimens at diagnosis were more likely to have progressive disease than patients who had clonal heterogeneity.[27] With the sensitive molecular method of PCR, circulating clonal T cells have been described in a high percentage of patients with early MF,[6] and a peripheral T-cell clone related to the cutaneous clone appeared to carry prognostic significance, while unrelated clonal T-cell populations were also common and appeared increasingly with age.[7,29,30]

In our study, we were able to compare the molecular profiles of T-cell clonality studies in 9 of 11 patients who had discrepant immunophenotypes between skin and blood samples. Except for the previously discussed patient who had oligoclonal T-cell gene rearrangement in the peripheral blood and no overlap with skin, all other patients had evidence of an identical clonal T-cell population present in skin biopsy and peripheral blood samples. This validates the comparison of the immunophenotypes between skin and blood in these patients. It is interesting that although the time between skin biopsy and blood analysis varied from 0 to 724 days (median, 20 days), clonal heterogeneity was not observed in any case in our study. This might be related to the limited number of patients. In 2 patients, molecular tests were not done; therefore, the identity between the T-cell populations in the skin and blood has not been proven. Both of these patients showed highly increased CD4/CD8 ratios in the blood with aberrant immunophenotypes, making the presence of a reactive T-cell population in the blood unlikely; however, clonal heterogeneity has not been excluded.

It is interesting that in the group of 25 patients with no peripheral blood involvement by morphologic, CBC, or flow cytometric results, 2 patients had clonal *TCR* gene results in peripheral blood. One of them had a skin biopsy sample with a clonal T-cell population but not the same clone. The other patient had no comparison available in a skin sample. The significance of these T-cell clones in the peripheral blood is not clear. Low-level trafficking of clonal T cells through blood vessels has been demonstrated even when a disease seems confined to the skin clinically.[31] Another possibility is T-cell clones of undetermined significance.

Immunophenotypic discrepancies are observed between T-cell immunophenotypes in skin and peripheral blood samples in patients with MF. Immunohistochemical analysis performed in skin samples failed to detect partial deletion of CD2 (45% of patients), CD3 (27%), and CD5 (27%) and falsely overrepresented deletion of CD7 in 18% of patients. In addition, CD8+ MF was missed in 18% of patients, likely owing to the inability of immunohistochemical analysis of skin samples to distinguish from an admixed reactive T-cell population rich in CD4+ T cells. This finding suggests that CD8+ MF might be more common than previously reported in the literature. To date, this is the first study to provide a quantitative evaluation of the limitations of immunohistochemical analysis to detect deletion of individual T-cell antigens in skin biopsy specimens. Awareness of the extent of these limitations is important and is of practical value for pathologists interpreting skin biopsies in patients with MF.

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