Analysis of T-Cell Receptor Gene Rearrangement in Lymph Nodes of Patients With Mycosis Fungoides

Prognostic Implications

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Objective: To determine the prognostic value of analyzing lymph node (LN) DNA from patients with mycosis fungoides for the presence of a monoclonal T-cell population.

Design: Inception cohort study.

Setting: A tertiary care referral center in Seattle, Wash.

Patients: Fifty-five uniformly staged patients with the diagnosis of mycosis fungoides and who had a lymph node biopsy, 21 with clinically abnormal nodes and 34 with normal nodes.

Main Outcome Measures: Lymph nodes were evaluated by Southern blot analysis for T-cell receptor β-chain (TCRB) gene rearrangement and by histopathologic examination for the LN classification using the National Cancer Institute system. Patients were observed clinically for a mean (±SD) of 4.7±3.4 years.

Results: Patients with detectable TCRB gene rearrangement in lymph node DNA had an increased likelihood of a poor clinical outcome and a decreased probability of survival (P<.001 for both) compared with patients with the TCRB germline. Although patients with clinically enlarged nodes were more likely to have the TCRB gene rearranged, those with normal nodes and the TCRB gene rearranged also had a poor clinical outcome and a decreased probability of survival. Similar to those with the TCRB gene rearranged, most patients with advanced histopathologic changes (LN3 and LN4) had a poor prognosis. The presence of a rearranged TCRB gene, however, correctly predicted some patients with intermediate LN scores (LN2) who had a poor clinical outcome.

Conclusions: Detection of a monoclonal T-cell population, as demonstrated by a rearranged TCRB gene on Southern blot analysis, in LNs of patients with mycosis fungoides is predictive of a poor clinical outcome and a reduced probability of survival. Lymph node TCRB gene analysis provides additional prognostic information for patients with mycosis fungoides with intermediate LN histopathology.

Mycosis fungoides (MF) is a lymphoproliferative disorder of T cells with a highly variable clinical outcome.1,4 It affects the skin primarily, and the life expectancy of many patients with limited patch/plaque disease is not notably affected.2,4 A substantial number of patients with MF, however, go on to have extensive extracutaneous involvement and die of their disease.2,4 Distinguishing patients who will have a more indolent course from those who will have a more aggressive course is important for prognosis and treatment. For example, patch/plaque MF often can be adequately treated with topical mechlorethamine hydrochloride, topical Carmustine, or phototherapy, without the risks of systemic chemotherapy.4,5 In fact, previous studies have demonstrated no improvement of the prognosis or survival for such patients with MF treated with systemic chemotherapy.3,4 The detection of lymph node (LN) involvement by MF has been considered an indicator of a more aggressive course, a worse prognosis, and the need for more aggressive treatment.

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Histopathologic assessment of lymph nodes is typically carried out using the National Cancer Institute classification.5,9 Advanced lymph node disease on histopathologic examination is usually associated with a poor clinical outcome. There are, however, a substantial number of patients with benign or intermediate node disease on histopathologic assessment who...
METHODS

PATIENTS AND SPECIMENS

Fifty-five patients with a diagnosis of MF were prospectively evaluated at the University of Washington Medical Center between 1982 and 1994. The diagnosis was based on clinical and histologic criteria, as previously described. A lymph node biopsy was performed as part of the staging workup. All patients with a diagnosis of MF and with sufficient DNA extracted from frozen lymph node tissue were included. Lymph node biopsy specimens were obtained from patients with clinically abnormal node(s) and from those with clinically normal nodes. A clinically abnormal lymph node was defined as one that was larger than 1.0 cm or hard. Multiple palpable nodes smaller than 1.0 cm were not considered abnormal unless hard. Lymph node biopsy specimens from patients with normal results on clinical examinations were obtained from the lymph node site draining the area of greatest skin involvement, whether or not nodes were palpable. Care was taken to avoid skin testing (eg, intradermal purified protein derivative [tuberculin], mumps, and Candida species) before the lymph node biopsy was done. For patients who had more than 1 lymph node biopsy during their clinical course, only the initial staging lymph node biopsy was included in this study.

ASSESSING CLINICAL OUTCOME

The 55 patients with MF were subsequently classified according to the biologic behavior (outcome) of their disease: complete remission, partial remission, progressive lymphoma, dead of disease, and dead of causes unrelated to MF. Complete remission is defined as no evidence of disease after a follow-up of at least 6 months. Partial remission is defined as the presence of residual disease but without progression to a more advanced stage of disease. Progressive lymphoma is defined as advanced disease (TNM stage IIb or greater) uncontrolled by treatment. Dead of disease is defined as death related either to direct MF involvement of a vital organ or to secondary causes related to MF (such as sepsis related to extensive cutaneous disease).

go on to have an aggressive course. Cell surface marker analysis demonstrated that the proliferating cells of MF were of T-cell origin, but these markers lack prognostic sensitivity and specificity. The analysis of T-cell receptor (TCR) gene rearrangement has been applied to lymphoproliferative disorders, including MF. Although TCR analysis has been used to demonstrate monoclonal T-cell populations in the lymph nodes of some patients with MF, only limited data exist regarding the sensitivity, specificity, and positive predictive value of TCR analysis of lymph nodes with regard to the prognosis of MF.

In this study, we investigated the prognostic value of TCR analysis of lymph node DNA in patients with MF. Lymph node biopsy specimens from 55 patients with uniformly staged MF were evaluated for histo-pathologic assessment and TCR analysis by the Southern blot technique. The results were compared with the clinical outcome for each patient. The utility of lymph node TCR analysis in the management of patients with MF is discussed.

RESULTS

PATIENT INFORMATION

Fifty-five patients (35 men and 20 women) with a mean (±SD) age of 55.5±16.5 years were studied. At the time of lymph node biopsy, the skin stage distribution of the patients was as follows: T1, 25; T2, 19; T3, 4; and T4, 7. (T1 indicates that ≤10% of total body surface area has
patches, plaques, or papules; T2 indicates that >10% of total body surface area has patches, plaques, or papules; T3 indicates the presence of ≥2 tumors; and T4 indicates generalized erythroderma.) The TNM stage distribution of the patients at the time of lymph node biopsy was as follows: IA, 20; IB, 8; IIA, 8; IIB, 4; III, 2; and IV, 13. Thirty-four patients had normal nodes on clinical examination, whereas 21 patients had 1 or more clinically abnormal lymph nodes. The lymph node biopsy specimens were collected for 12 years. Clinical outcomes could be determined for 54 of the patients at the time of this analysis. One patient was unavailable for follow-up after 3 years. The length of clinical follow-up ranged from 3 months to 12 years; the shortest follow-ups were recorded for patients who died of disease or died of unrelated causes. The mean (±SD) length of follow-up was 4.7±3.4 years. The clinical outcome distribution of the patients was as follows: 9 patients had complete remission; 21 had partial remission; 6 died of causes unrelated to MF; 5 had progressive lymphoma; and 17 died of their disease. Complete and partial remissions were considered good clinical outcomes; progressive lymphoma or dying of disease were considered poor clinical outcomes; and dying of causes unrelated to MF was considered indeterminate.

REARRANGED TCRB GENE PREDICTS A POOR OUTCOME

Lymph node DNA from the 55 patients was evaluated by TCRB analysis. In 15 patients, TCRB gene rearrangement(s) (TCRB-R) was detected, demonstrating the presence of a monoclonal T-cell population. In the remaining 40 patients, only the germline bands were detected (TCRB-G).

For each patient, the results of TCRB analysis were compared with the clinical outcome (Table 1). The percentages given in all tables are rounded to the nearest whole number. Most patients (26 [67%]) with TCRB-G have had a good outcome (complete or partial remission) or died of causes unrelated to MF (5 [13%]). The TCRB-G gene, however, did not necessarily predict a good clinical outcome because 8 of 22 patients with a poor clinical outcome (progressive lymphoma or died of disease) had TCRB-G. Of the 15 patients with TCRB-R, 14 had a poor outcome (progressive lymphoma or died of disease). The only patient with TCRB-R who was not thus classified died of unrelated causes after 3 months of follow-up. The difference between the proportion of patients with TCRB-R and those with TCRB-G who had a poor outcome (14 of 15 vs 8 of 39) is significant (P<.001, Fisher exact test). Because no patient with TCRB-R had a good clinical outcome (complete or partial remission), the detection of TCRB-R appeared specific for a poor clinical outcome in our patients and, thus, predicted a poor clinical outcome.

The results from the TCRB analysis were also compared with those from the clinical node examination (Table 2) and the skin stage (Table 3) at the time of lymph node biopsy. Patients with abnormal nodes were more likely to have TCRB-R detected in their lymph nodes than patients with normal nodes. Thus, most (11 of 15) patients with TCRB-R LNsS had abnormal nodes on clinical examination. Conversely, about 50% (11/21) of patients with clinically abnormal nodes had TCRB-R (Table 2). The difference between the proportion of patients with clinically abnormal nodes and those with normal nodes who had TCRB-R (11 of 21 vs 4 of 34) is significant (P<.01, Fisher exact test). The 12% of patients (4 of 34) with clinically normal nodes and TCRB-R (Table 2), however, also had a poor clinical outcome (95% confidence interval, 3%-28%; binomial distribution). Patients with a more advanced skin stage were more likely to have TCRB-R than patients with a less advanced skin stage (Table 3). One patient with skin stage T1, however, had TCRB-R on lymph node DNA analysis. Furthermore,
Among the 19 patients with T2, 7 (37%) had TCRB-R (95% confidence interval, 16%-62%; binomial distribution). Based on this 95% confidence interval for these patients with T2 skin stage, patients with T2 skin stage may be equally likely to have TCRB-R or TCRB-G on analysis of lymph node DNA. Thus, neither clinical node examination findings nor skin stage was entirely predictive of the presence of TCRB-R, although patients with normal nodes were less likely, and patients with skin stage T1 are unlikely, to have TCRB-R on analysis of lymph node DNA.

The results of the TCRB evaluation were also compared with survival, using a Kaplan-Meier analysis (Figure 1). The mean (±SE) probability of survival for the patients with TCRB-R was 2.6±0.5 years. The mean (±SE) probability of survival for the patients with TCRB-G was 13±13 years. The difference between these 2 groups is significant (P<.001, log-rank test). Thus, the presence of TCRB-R predicts a poorer probability of survival.

ADVANCED LN DISEASE PREDICTS A POOR OUTCOME

The relationship between the LN histologic classification and clinical outcome was also evaluated (Table 4).

Among 18 patients with a histologic score of LN0 or LN1, 13 had a good outcome (complete or partial remission), and only 2 patients had a poor outcome (progressive lymphoma or died of disease). In contrast, among 13 patients with a histologic score of LN3 or LN4, 11 had a poor outcome (progressive lymphoma or died of disease), and only 1 had a good outcome. The 23 patients with LN2 scores were almost as likely to have a poor outcome as they were to have a good outcome. Thus, an LN0 or LN1 score predicts a good clinical outcome, an LN3 or LN4 score predicts a poor clinical outcome, and an LN2 score has the greatest predictive uncertainty.

Comparing lymph node histologic classification with the results of clinical node examinations, most (10 of 13) of our patients with a score of LN3 or LN4 had clinically abnormal lymph node scores (Table 5). Conversely, about 50% (10/21) of patients with clinically abnormal nodes had histologic scores of LN3 or LN4. Almost 10% (3/34) of patients with clinically normal nodes also had scores of LN3 or LN4, however, which are predictive of a poor outcome. These findings are similar to the data in Table 2 comparing TCR analysis with clinical node examinations. Furthermore, 56% of patients (19 of 34) with clinically normal nodes had intermediate (LN2) histologic scores. As noted earlier, in terms of clinical outcome, the LN2 group had the greatest prognostic uncertainty. Therefore, although patients with clinically abnormal nodes are more likely to have advanced node disease, the results of the clinical node examination are not necessarily predictive of LN histologic classification.

When the LN score is compared with skin examination results, most (11 of 13) of our patients with LN3 or LN4 scores had a skin stage of T2 or greater. One of 4 patients with T3 skin disease, however, had an LN1 score, and 2 of 25 patients with T1 skin disease had advanced disease (LN3 or LN4). Furthermore, among our 19 patients with T2 skin disease, the probability of intermediate disease (LN2) (8 patients) was not significantly different from the probability of advanced disease (LN3 or LN4) (6 patients) (P>.05, Fisher exact test). Therefore, although the patients with an advanced skin stage are more likely to have advanced lymph node disease, the skin stage is not necessarily predictive of the LN histologic classification.

We also compared the LN score with survival, using a Kaplan-Meier analysis (Figure 2). The mean (±SE)

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Table 4. Lymph Node Histologic Classification vs Clinical Outcome

<table>
<thead>
<tr>
<th>Clinical Outcome†</th>
<th>LN0 (n=1)</th>
<th>LN1 (n=17)</th>
<th>LN2 (n=23)</th>
<th>LN3 (n=6)</th>
<th>LN4 (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>0 (0)</td>
<td>1 (6)</td>
<td>3 (13)</td>
<td>1 (17)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>PR</td>
<td>1 (100)</td>
<td>11 (65)</td>
<td>9 (39)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>D</td>
<td>0 (0)</td>
<td>3 (18)</td>
<td>2 (9)</td>
<td>0 (0)</td>
<td>1 (14)</td>
</tr>
<tr>
<td>PL</td>
<td>0 (0)</td>
<td>1 (6)</td>
<td>2 (9)</td>
<td>1 (17)</td>
<td>1 (14)</td>
</tr>
<tr>
<td>DOD</td>
<td>0 (0)</td>
<td>1 (6)</td>
<td>7 (30)</td>
<td>4 (67)</td>
<td>5 (71)</td>
</tr>
</tbody>
</table>

*The 1 patient who was unavailable for follow-up after 3 years is not included in this analysis.
†Clinical outcome is classified as follows: CR indicates complete remission; PR, partial remission; D, died of unrelated cause(s); PL, progressive lymphoma; and DOD, died of disease.
‡Lymph node score is as follows: LN0 indicates reactive, no atypical lymphocytes; LN1, dermatopathic, occasional atypical lymphocytes; LN2, dermatopathic, atypical lymphocytes singly or in small cluster (<6 cells); LN3, dermatopathic, numerous atypical lymphocytes singly or large cluster (>15 cells); and LN4, partial or complete effacement with lymphoma.

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The relationship between TCRB analysis and LN histologic classification was also evaluated for the entire study population and for each LN score subgroup. For the entire study group (Tables 1 and 4) regarding sensitivity for predicting a poor clinical outcome, the presence of TCRB-R identified 14 (64%) of 22 patients and scores of LN3 or LN4 predicted only 11 (50%) of 22 patients with a poor outcome (progressive lymphoma or died of disease). The difference was not statistically significant (P = .25, McNemar test). Regarding the positive predictive value for a poor outcome, the presence of TCRB-R predicted 14 (93%) of 15 patients, whereas a score of LN3 or LN4 predicted 11 (85%) of 13 (P = .5, resampling method). The specificity for predicting good outcomes (complete or partial remission) when the presence of TCRB-R and scores of LN3 or LN4 were not observed was 100% (26/26) for TCRB analysis and 96% (25/26) for scores of LN3 or LN4 (P = 1.0, McNemar test). Hence, for patients with LN histologic scores of LN3 or LN4 in our study group, TCRB analysis did not add statistically significant prognostic information.

**TCRB ANALYSIS PROVIDES ADDITIONAL PROGNOSTIC INFORMATION IN A SUBSET OF PATIENTS**

The results from TCRB analysis and histologic classification are compared directly for each lymph node subgroup in Table 6. A monoclonal T-cell population was not detected (TCRB-G) in the LN's of any patients with LN0 or LN1 scores. Although most patients (n=24) with a score of LN2 also had TCRB-G, 3 (13%; 95% confidence interval, 3%-32%; binomial distribution) did have a detectable monoclonal T-cell population (TCRB-R), predictive of a poor outcome. All 3 of those patients died of disease. Conversely, although most patients with a score of LN3 or LN4 had TCRB-R, 1 patient with a score of LN3 had TCRB-G, predictive of a good outcome. This patient with a score of LN3 and TCRB-G is in complete remission (132 months' follow-up). Thus, TCRB analysis may provide additional information clinically useful for predicting a poor prognosis for patients with LN2 disease or a good prognosis for patients with a score of LN3 or LN4.

**RELIABILITY OF HISTOLOGIC INTERPRETATION AND TCRB ANALYSIS**

The original histologic interpretations were done during a 12-year period by 2 different hematopathologists (including P.G.K.). To determine the reproducibility of the LN classification, biopsy slides of all available lymph node biopsy specimens (n=32) were coded and reread by a single hematopathologist (P.G.K.) at a single point in time. They were again scored LN0 through LN4, as previously described.7,17 In 20 of the cases, the LN grade was identical. In 12 cases, there was discordance between the initial and the reread interpretations. Seven changes were from LN1

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**Table 5. Clinical Node Examination Results vs Lymph Node Histologic Classification**

<table>
<thead>
<tr>
<th>Lymph Node Score*</th>
<th>Normal (n=34)</th>
<th>Abnormal (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LN0</td>
<td>1 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>LN1</td>
<td>11 (32)</td>
<td>6 (29)</td>
</tr>
<tr>
<td>LN2</td>
<td>19 (56)</td>
<td>5 (24)</td>
</tr>
<tr>
<td>LN3</td>
<td>2 (6)</td>
<td>4 (19)</td>
</tr>
<tr>
<td>LN4</td>
<td>1 (3)</td>
<td>6 (29)</td>
</tr>
</tbody>
</table>

*See the third footnote in Table 4 for an explanation of the lymph node score.

**Table 6. Lymph Node Histologic Classification vs Lymph Node T-Cell Receptor β-Chain (TCRB) Analysis**

<table>
<thead>
<tr>
<th>TCRB Status</th>
<th>LN0 (n=1)</th>
<th>LN1 (n=17)</th>
<th>LN2 (n=24)</th>
<th>LN3 (n=6)</th>
<th>LN4 (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germine</td>
<td>1 (100)</td>
<td>17 (100)</td>
<td>21 (87)</td>
<td>1 (17)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Rearranged</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (13)</td>
<td>5 (83)</td>
<td>7 (100)</td>
</tr>
</tbody>
</table>

*See the third footnote in Table 4 for an explanation of the lymph node score.
to LN2 or LN2 to LN1, and all 7 patients had TCRB-G. One change was from LN4 to LN3, and that patient had TCRB-R. There were, however, 3 changes from LN3 to LN2 or LN2 to LN3 and 1 change from LN4 to LN2, and all 4 had TCRB-R. These last 4 patients all had a poor outcome (died of disease), which was correctly predicted by TCRB analysis but which would not have been correctly predicted by histologic classification alone, if the lower reading (LN2) was accepted and interpreted as predicting a good outcome. Of the 12 cases with discordant histologic interpretations, 11 involved an LN2 score in 1 of the 2 readings. If these 11 LN2 cases were analyzed for TCRB, 4 with TCRB-R (all dying of the disease) would have been identified, and none with TCRB-R (predictive of a poor clinical outcome) would have avoided detection. These data suggest that there is some variability in the histologic interpretation and that the addition of TCRB analysis is more reliable than histologic interpretation alone, particularly for the LN2 subset of patients.

**COMMENT**

The detection of a TCRB gene rearrangement (TCRB-R) by Southern blot analysis in lymph node DNA of patients with MF was a marker for a poor prognosis and a reduced probability of survival in the patients studied. Although TCRB-R is more likely to be detected in the lymph nodes of patients with MF with clinically abnormal nodes and with more advanced skin stage, it can be detected in patients without palpable adenopathy and/or with early-stage skin disease. The data suggest that detecting TCRB-R has useful predictive value in patients with both early and more advanced skin stage and also with either clinically normal or abnormal lymph nodes. The study, however, lacks the statistical power to determine whether TCRB-R is a variable independent of the skin and LN stages. The results are in agreement with those of studies of fewer patients. The detection of TCRB-R by this technique may also indicate patients who would be appropriate for either more aggressive or more experimental (or both) treatments. Although no treatment to date has altered the natural history of advanced disease, several treatments currently in clinical trials show promise.

The predictive value of determining lymph node involvement of MF using the histopathologic classification has been well documented, and our results are consistent with this information (Table 4 and Figure 2). A discordance between histologic interpretations of the same node biopsy specimen was observed in 12 of 32 cases. Similar variability in the histologic interpretation of skin biopsy specimens of patients with MF, both among different pathologists and by the same pathologist, also has been reported. T-cell receptor analysis may be more objective, although we did not specifically examine this issue.

The data in this article, taken together with those of previous reports, suggest the following use of lymph node TCRB analysis in the management of patients with a diagnosis of MF. Patients with histopathologic grade LN0 or LN1 do not need Southern blot analysis of their lymph node DNA because there seems to be little likelihood for eliciting additional information. Patients with an intermediate LN grade (LN2 in our series and that of Bakels et al and LN3 for the series of Lynch et al) warrant doing Southern blot analysis of their LN DNA because there is the potential to gain additional information regarding the prognosis. Patients identified as having a poor prognosis could be considered for more aggressive treatment options. The utility of Southern blot analysis on node DNA for patients with MF with an advanced histopathologic classification is less clear. For our patients, those with an LN3 or LN4 classification generally had a poor clinical outcome (85% with progressive lymphoma or died of their disease). The TCRB analysis would have predicted a good outcome for 1 patient with histologic grade LN3 at the time of the initial staging. More data are necessary to determine whether TCRB analysis for patients with an advanced node classification is sufficiently instructive to be cost-effective. As with any test, a positive result (the presence of TCRB-R) with Southern blot analysis must be interpreted in the context of each patient. Furthermore, a negative result with TCRB analysis shares with the histologic examination the problem of sampling error, as well as the possibilities of γ- or δ-MF and of a lymphoma that has lost the TCRB gene.

Recently, the detection of a monoclonal T-cell population by polymerase chain reaction analysis of TCR in patients with MF has been developed. It has been shown to be more sensitive than detection by Southern blot analysis. The use of a more sensitive technique such as polymerase chain reaction will probably detect a clonal T-cell population in lymph nodes with an increased frequency compared with that detected by Southern blot analysis. The value of lymph node analysis with the polymerase chain reaction for predicting the clinical outcome in patients with MF, however, will need to be determined in studies with sufficient follow-up to permit the assessment of the clinical outcome.

Regarding the utility of lymph node biopsies in patients with normal findings on a clinical examination, 4 (12%) of 34 patients with normal nodes had TCRB-R, predictive of a poor clinical outcome. This is consistent with previous reports evaluating the histopathologic stage of clinically normal nodes in patients with MF. Because there is morbidity associated with lymph node biopsies and currently no effective early treatment of MF with lymph node involvement, we do not perform biopsies on patients with clinically normal lymph nodes. When effective treatment is available for patients with affected lymph nodes, obtaining a biopsy of clinically normal lymph nodes may be indicated.

Regarding which patients with MF with clinically normal lymph nodes would be most appropriate to do a biopsy on, none of our 24 patients with T1 skin stage and normal nodes had TCRB-R. The only patient with stage T1 who had TCRB-R had clinically abnormal lymph nodes. These observations are consistent with those of Sausville et al, who recommended that patients with stage T1 with clinically normal lymph nodes (and without involvement of the peripheral blood) do not need further tests. In contrast, 3 of our 4 patients with clinically normal LNs and TCRB-R had a skin stage of T2, and the fourth had skin stage T3. This information suggests that the biopsy of normal nodes in patients with MF might be reserved for those with more advanced (skin stage T2 or greater) disease.
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