Analysis of T-Cell Receptor Gene Rearrangement for Predicting Clinical Outcome in Patients With Cutaneous T-Cell Lymphoma

A Comparison of Southern Blot and Polymerase Chain Reaction Methods

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Objective: To extend previous observations regarding the prognostic value of analyzing lymph node DNA from patients with cutaneous T-cell lymphoma for the presence of a monoclonal T-cell population by Southern blot vs polymerase chain reaction (PCR) methods.


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

Patients: Fifty-five uniformly staged patients with the diagnosis of cutaneous T-cell lymphoma who underwent a lymph node biopsy, 21 with clinically abnormal nodes and 34 with normal nodes.

Interventions: Lymph nodes were evaluated for T-cell receptor (TCR) γ-chain gene rearrangement by 2 PCR methods: capillary electrophoresis and denaturing gradient gel electrophoresis. The same lymph nodes were evaluated by Southern blot analysis for TCR δ-chain gene rearrangement and examined histopathologically on the basis of the National Cancer Institute lymph node classification system. Patients were observed clinically for a mean of 9.5 years.

Main Outcome Measures: Skin stage, clinical lymph node examination, lymph node histologic examination, Southern blot analysis, and PCR analyses were evaluated as potential prognostic predictors by univariate and multivariate analyses. The statistical association of TCR analysis and clinical outcome was determined among all patients. Hazard ratios (HRs) by Cox proportional hazards regression analysis were used to estimate the risk of a poor clinical outcome. Cumulative survival rates were analyzed by the Kaplan-Meier method.

Results: A skin stage of T3 (tumors) or T4 (erythroderma) was the most powerful predictor of a poor clinical outcome (HR, 31.3 vs T1; P < .001). Patients with detectable TCR γ-chain gene rearrangement in lymph node DNA by PCR also were more likely to have a poor outcome (HR, 5.1; P < .001), but it was a less powerful predictor than skin stage. Even when the skin stage, presence or absence of lymphadenopathy, and histologic lymph node score were known for the patient, Southern blot analysis still added to prediction of a poor outcome (HR, 9.3; P = .007), whereas PCR provided no statistically significant additional information on outcome.

Conclusions: Detection of a monoclonal T-cell population by PCR in lymph nodes of patients with cutaneous T-cell lymphoma does not enhance prediction of clinical outcome and probability of survival beyond what can be determined from clinical examination and histologic lymph node scores. Skin stage and the presence or absence of lymphadenopathy remain the most important determinants of clinical outcome.

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Mycosis fungoides is the most common cutaneous T-cell lymphoma (CTCL) characterized by a highly variable clinical outcome. While most patients’ life expectancies are not altered by mycosis fungoides, a significant number of patients develop progressive disease characterized by large cutaneous tumors and extracutaneous spread that, despite aggressive treatment, results in death. Currently, the stage of disease, defined by standard clinical and histopathological criteria, is most often used to select therapy and determine prognosis. Advanced lymph node disease, based

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mediate lymph node classification have an aggressive course.\(^7\)

To address this issue, several investigators have used analysis of T-cell receptor (TCR) gene rearrangement to provide additional prognostic information in this patient population.\(^8\)-\(^1^2\) Conventional polymerase chain reaction (PCR) has emerged as the most commonly used tool in T-cell clonality detection. It has several advantages over Southern blot analysis (SBA), including technical ease, rapid analysis, and superior sensitivity in detecting a clonal population of T cells (<1% for PCR vs 2%-5% for SBA).\(^1^3\)-\(^1^5\) These qualities have led investigators to routinely use PCR to detect the presence of a cutaneous dominant T-cell clone. Although gene rearrangement studies appear to be promising in augmenting the diagnosis of CTCL,\(^8\)-\(^1^6\) few studies have addressed whether these molecular tests can more accurately predict the clinical outcome in patients with established disease.

To evaluate predictors of clinical outcome, we have investigated whether the presence of a dominant T-cell clone in lymph node DNA improves prediction of the clinical outcome in patients with CTCL. Several studies have assessed clonal heterogeneity by means of PCR or SBA, but this is the first study, to our knowledge, to directly compare PCR, SBA, skin stage, clinical lymph node examination, and histologic lymph node score for their ability to predict clinical outcome from a large cohort of patients with CTCL with 9.5 years of follow-up. The prediction was that PCR, with its greater sensitivity in detecting T-cell clonality, would detect more cases or earlier cases with a poor outcome than SBA or clinical examination alone. The alternative prediction was that PCR would be too sensitive and identify more cases that displayed clonality but had a good clinical outcome. We also were interested in comparing 2 PCR methods with SBA on the same lymph node DNA.

### METHODS

**PATIENTS AND MATERIALS**

Fifty-five patients diagnosed as having CTCL were studied in the Dermatology Clinics of the University of Washington Medical Center, Seattle, from 1982 to 1998. Recruitment of new patients ended in 1994. The diagnosis of CTCL was based on a combination of clinical and histologic criteria and confirmed by a CTCL panel that consisted of pathologists and dermatopathologists as previously described.\(^1\)

A lymph node biopsy was performed as part of the staging workup. All patients with a diagnosis of CTCL and with sufficient DNA extracted from frozen lymph node tissue were included. Lymph node biopsy specimens were obtained from patients with clinically abnormal nodes as well as from those with clinically normal nodes. A clinically abnormal lymph node was defined as one that was 1.0 cm or larger 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, regardless of whether nodes were palpable. Care was taken to avoid skin testing before the lymph node biopsy was done, since that might result in additional lymphocytic proliferations in the lymph nodes. For patients who had more than one lymph node biopsy during their clinical course, only the initial staging lymph node biopsy was included in this study. All patients were classified according to the TNM classification of CTCL.\(^1^7\) The 55 patients with CTCL were prospectively studied and followed up for an average of 9.5 years from the time of diagnosis. They were classified into 5 categories according to their clinical status (outcome) during follow-up: complete remission, partial remission, progressive lymphoma, dead of disease, and dead of causes unrelated to CTCL. Complete remission was defined as no evidence of residual disease after a follow-up of at least 6 months. Partial remission was defined as the presence of residual disease but without progression to a more advanced stage of disease. Progressive lymphoma was defined as advanced disease (TNM stage IIB or greater) that was progressive despite therapy, including psoralen–UV-A and electron beam radiation. Dead of disease was defined as death related either to direct CTCL involvement of a vital organ or to secondary causes related to CTCL (eg, sepsis related to extensive cutaneous disease). Dead of other causes was defined as death from causes unrelated to CTCL during the follow-up period and without evidence of progressive lymphoma. Patients in complete remission or partial remission were classified as having a “good” outcome, whereas patients with progressive lymphoma or dead of disease were classified as having a “poor” outcome. Patients who died of other causes were considered to have survived without progression of their CTCL until the time of death, and for the purposes of this study were classified as having a “good” outcome.

**HISTOLOGIC LYMPH NODE ASSESSMENT**

All lymph node biopsy specimens were given a histologic diagnosis and a score based on the National Cancer Institute lymph node classification system.\(^1^6\) Briefly, a score of LN1 indicates occasional and isolated atypical lymphocytes, not arranged in clusters; LN2 indicates many atypical lymphocytes occurring singly or in 3- to 6-cell clusters; LN3 indicates atypical lymphocytes arranged in aggregates (nodal architecture essentially preserved); and LN4 indicates partial or complete replacement of nodal architecture by atypical lymphocytes or frankly neoplastic cells. Lymph node biopsy specimens were interpreted and scores assigned at the time of each biopsy by 1 of 2 hematopathologists during a 12-year period. This initial interpretation was used for all data analysis in this study.

**TCR β-CHAIN GENE REARRANGEMENT ANALYSIS USING SBA**

Lymph node DNA was analyzed by SBA for the TCR β-chain gene rearrangements in a study reported earlier by Kern et al\(^9\); the clinical outcomes for these patients were updated in the present study to reflect an additional 4 years of follow-up.

Briefly, a TCR β-chain gene rearrangement was determined to be present if a distinct nongermline band was present in the BamHI digestion and in either the EcoRI or HindIII digestion. The sensitivity of the SBA gene rearrangement studies was established by testing DNA extracted from mixtures of human promyelocytic leukemia cells (HL60) with human acute T-lymphocytic leukemia cells (MOLT3). The results of this method were consistently positive if the clonal cells made up 5% of the total population.

**TCR γ-CHAIN GENE REARRANGEMENT ANALYSIS USING PCR**

Two different PCR methods, capillary electrophoresis (PCR-Ce) and denaturing gradient gel electrophoresis (PCRDGGE), were used for detection of clonal TCR γ-chain gene rearrangements on the same lymph nodes studied by SBA and histologic methods.
The methods for PCR-DGGE and PCR-CE were performed as previously described, including primer sequences and PCR product separation methods. Positive controls included DNA from the Jurkat and MOLT3 cell lines, DNA from a reactive lymph node was used as a polyclonal control, and a no-template control was included as well to detect DNA contamination of PCR reagents. The principle of TCR γ-chain PCR is based on the fact that, at the time that T cells rearrange their antigen receptor genes, the enzyme terminal deoxynucleotidyl transferase adds random numbers of nucleotides at the V-J junction. Thus, each T-cell clone has a unique distance between the V and J segments. Consensus Vγ and Jγ primers are used to amplify across the V-J junction. The Vγ primers are specific for families I and II. Separate Jγ primers are used for Jγ and JγP. Either the PCR products are separated by DGGE and detected by staining with ethidium bromide, or fluorescently labeled primers are used and the PCR products are separated by CE with an automated DNA sequence analyzer. The results of these methods were expressed as positive when a dominant T-cell clone was present, as evidenced by a distinct band rated by CE with an automated DNA sequence analyzer. The principle of TCR template control was included as well to detect DNA contamination, which indicated the increased risk of a poor outcome at any given time for one category compared with another (eg, positive vs negative PCR). Thus, an HR estimate of 6.5 for a positive test vs a negative test indicates that people with a positive test face a 6.5-fold increased risk of a poor outcome at any given time.  

**STATISTICAL ANALYSIS**

To identify the factors that best predicted prognosis in our cohort, we used Kaplan-Meier plots and the Cox proportional hazards model for the time to progressive lymphoma or death from CTCL (poor outcome). We also used the Kaplan-Meier method to estimate the 10-year survival rate. We used the 10-year survival rate for each patient group as a summary statistic. The length of clinical follow-up ranged from 3 months to 21 years, with a mean of 9.5 years.  

**RESULTS**

**PATIENT INFORMATION**

Fifty-five patients were studied, 39 men and 16 women, with a mean ±SD age of 55.5 ± 16.5 years. Details of the patients are listed in Table 1. At the time of lymph node biopsy, 25 patients had T1 disease, 19 had T2 disease, 4 had T3 disease, and 7 had T4 disease. In 5 of the 7 patients with T4 disease, Sézary cell counts were greater than 1000/mm³, consistent with Sézary syndrome. By TNM staging, 36 patients (65%) presented with patch or plaque-type cutaneous lesions (stages IA, IB, and IIA), whereas 19 patients (35%) also had cutaneous tumors, erythroderma, and/or lymph node or visceral involvement (stages IIb, III, and IV, respectively), as previously reported. Palpable lymphadenopathy was detected in 21 patients (38%), whereas 34 patients (62%) had normal results of lymph node examination. The length of clinical follow-up ranged from 3 months to 21 years, with a mean of 9.5 years. The clinical outcome distribution of the patients at follow-up is shown in Table 2. Thirty-three patients had good outcomes (12 patients with complete remission, 13 with partial remission, and 8 who died without evidence of progressive lymphoma), while 22 patients had poor outcomes (1 patient with progressive lymphoma and 21 dead of disease).

**LYMPH NODE TCR GENE REARRANGEMENT STUDIES**

The SBA showed clonal rearrangements in 4 (12%) of 34 clinically normal lymph nodes, whereas 11 (32%) of 21...
clinically abnormal nodes showed clonal rearrangement. The PCR-CE showed clonal rearrangements in 7 (21%) of 34 normal nodes and 11 (52%) of 21 abnormal nodes, while PCR-DGGE showed clonal rearrangement in 4 (12%) of 34 normal nodes and 9 (43%) of 21 clinically abnormal nodes. Thus, the positive clonality yield for “blind” (clinically normal) lymph node biopsy was 12% for SBA, 21% for PCR-CE, and 12% for PCR-DGGE. The summary results of the lymph node TCR studies for patients are presented in Table 3. Clonality was detected in 15 patients (27%) by SBA, 18 patients (33%) by PCR-CE, and 13 patients (24%) by PCR-DGGE. For the 15 patients with lymph nodes positive for clonality by SBA, 8 showed rearrangement by all 3 methods, 4 were rearranged by PCR-DGGE but not PCR-CE, 1 was rearranged by PCR-CE but not PCR-DGGE, and 2 were rearranged by neither PCR method. Ten lymph nodes were determined to be clonal by 1 of the 2 PCR methods but not by SBA.

According to our clinical outcome measures, 33 patients experienced a good clinical outcome and 22 patients experienced a poor clinical outcome. Each gene rearrangement study is compared with clinical outcome in Table 2. For the 33 patients with good outcomes, clonality was detected in 7 unique patients. Clonality was detected by PCR-CE in 5 patients, by PCR-DGGE in 3 patients, and by SBA in 1 patient. These were designated “clinically benign positive” cases (ie, positive for clonality with a good outcome). However, it was difficult to determine the significance of this finding in 5 patients who died of unrelated causes, as there was no way to determine with certainty their disease-specific survival. There was short follow-up on 3 of the 5 patients who died of causes other than CTCL within 7 months of their initial diagnosis. The most compelling data for a clinically benign positive test result were from the 2 patients in partial remission with positive PCR-CE results.

In the 22 patients with a poor outcome, clonality was detected in 14 patients (64%) by SBA, in 13 patients (59%) by PCR-CE, and in 10 patients (45%) by PCR-DGGE. If we use “clinically aggressive negative” to denote the failure of the gene rearrangement method to predict a poor outcome, then SBA yielded 8 clinically aggressive negative findings (36%), while PCR-CE had 9 (41%) and PCR-DGGE had 12 (55%).

The use of PCR-CE had the highest frequency of clinically benign positive results (15%), with PCR-DGGE at 9% and SBA at 3%. On the other hand, PCR-DGGE had the highest frequency of clinically aggressive negative results (55%), followed by PCR-CE (41%) and SBA (36%).

PREDICTION OF SURVIVAL AND CLINICAL OUTCOME

The estimated 10-year survival rate and HRs were determined. Survival rates in relation to the clinical variables are given in the Figure and Table 4, while survival rates and HRs for gene rearrangement methods are given in Table 3. The data in Table 4 indicate that 25 patients (45%) with T1 disease had an estimated 10-year survival rate of 92%.

The estimated 10-year survival rate for T2 was 27%. The estimated HR

<table>
<thead>
<tr>
<th>Method and Clonality Result</th>
<th>No. (%)</th>
<th>Estimated 10-y Survival, %</th>
<th>Estimated HR</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBA Negative</td>
<td>40 (73)</td>
<td>80</td>
<td>1.0</td>
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<tr>
<td>SBA Positive</td>
<td>15 (27)</td>
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<tr>
<td>PCR-CE Negative</td>
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<tr>
<td>PCR-CE Positive</td>
<td>18 (33)</td>
<td>24</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>PCR-DGGE Negative</td>
<td>42 (76)</td>
<td>70</td>
<td>1.0</td>
<td>&lt;.001</td>
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<tr>
<td>PCR-DGGE Positive</td>
<td>13 (24)</td>
<td>17</td>
<td>6.5</td>
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</tr>
</tbody>
</table>

Abbreviations: CE, capillary electrophoresis; DGGE, denaturing gradient gel electrophoresis; HR, hazard ratio; PCR, polymerase chain reaction; SBA, Southern blot analysis; TCR, T-cell receptor.
of identifying patients who are candidates for more aggressive therapy. Most TCR studies have focused on diagnosis of CTCL or response to therapy,10–12 and relatively few have addressed whether these molecular methods are a useful staging tool for predicting prognosis.8,11 To address the value of lymph node DNA for prognosis, we compared 3 TCR methods with several readily discernible clinical signs in a cohort of 55 patients with CTCL who were followed up for an average of 9.5 years to determine outcome. Another unique aspect of this study was the comparison of SBA with 2 separate PCR methods (PCR-DGGE and PCR-CE) for analysis of TCR.

We directly compared skin stage, clinical lymph node examination, histologic lymph node examination, SBA, and 2 PCR methods for predicting clinical outcome. In each method of analysis (univariate or multivariate Cox proportional hazards regression, Kaplan-Meier), skin stage was clearly the best method for predicting the clinical outcome. Two patients (8%) with T1 disease progressed to a poor outcome, whereas 58% of patients with T2 disease and 73% of patients with T3/T4 disease progressed to a poor outcome (progressive lymphoma or dead of disease). Patients with T3/T4 disease had an estimated HR of 31.3 compared with patients with T1 disease and an estimated 10-year survival of 27% or less compared with 92% for T1. Because our T3/T4 group is heavily weighted with patients with T4 disease, our survival data are comparable to those reported by Kim et al8 for the very large database at Stanford University, Stanford, Calif. Although the cumulative overall survival for our patients with T2 disease is similar to that reported by Kim et al at 5 years (approximately 67% vs 72%), our projected 10-year survival for the T2 group is considerably lower (approximately 27% vs 58%). We have no clear explanation for this observation except that the number of patients in our study is considerably smaller.

One of the main goals of this research was to evaluate whether TCR analysis was able to improve prediction of survival. Southern blot analysis was first used to assess T-cell clonality in patients with CTCL nearly 20 years ago.23,24 Most studies have found that SBA is able to detect a clonal T-cell population only if 5% to 10% of the cells are monoclonal. Polymerase chain reaction, on the other hand, has superior sensitivity (<1% clonality detected), technical ease, and rapid analysis, which explains why it is the method of choice for detecting clonality in T-cell lymphocytic populations.25–27

The PCR for TCR γ-chain gene rearrangement was more effective than SBA (TCR β-chain) in predicting out-
come in this study. In fact, PCR methods generally were somewhat less effective than SBA for predicting outcome. Clearly, the technical advantages of PCR make it the method of choice for clonality detection, and it will continue to be the method of choice unless newer methods, such as those discussed by Jones and Duvic,14 are shown to have advantages (eg, PCR methods for TCR α/β-chain clonality or analysis of TCR α/β-chain proteins present on the surface of tumor cells). Our study used 2 different PCR methods for TCR γ-chain gene rearrangement. With the PCR-CE method we used 3 primer sets, whereas with PCR-DGGE 2 primer sets were used. The latter do not detect TCR γ-chain gene rearrangements involving Vγ10 or 11. The addition of the third primer set in PCR-CE resulted in the detection of 3 additional patients who ultimately died of CTCL. However, whereas the additional primer set may have added sensitivity to the method, it may also be less specific: 5 patients with a positive PCR-CE had a good clinical outcome (ie, clinically benign positive cases). Optimal PCR primer sets will be important in predicting outcome. However, in our study, when skin stage, results of clinical lymph node examination, and the histologic lymph node score were known, neither PCR method was significant for predicting outcome.

Our study reports results of PCR for TCR γ-chain gene rearrangement in lymph node DNA; however, many studies have evaluated clonality in skin or blood. Clonal TCR gene rearrangements in lesion skin or blood and lymph nodes were not directly compared in the present study. It is possible that some nodal T-cell clones did not match the CTCL clones in skin lesions.

The specificity of PCR to detect a dominant T-cell clone for blood in CTCL has been questioned. A dominant clone in the peripheral blood not present in the skin was a frequent finding in a series of 363 patients presenting for clinical suspicion of cutaneous lymphoma. It occurred in 30% of patients with CTCL, 41% with non-CTCL malignant infiltrates, and 34% with benign cutaneous infiltrates. This peripheral-blood phenomenon was particularly common in patients older than 60 years.28 Clonality in the blood has been observed in conditions considered benign, such as small-patch parapsoriasis (9 of 14 cases),29 and dominant T-cell clones have been observed in the blood of elderly patients without evidence of disease.30,31

Polymerase chain reaction for TCR γ-chain gene rearrangement on skin biopsy specimens has been used to assess response to therapy in a study by Delfau-Larue et al.10 The authors reported that absence of a detectable T-cell clone was an independent predictor of a complete response in their study population with a median follow-up period of 4 years. It should also be noted that the result of TCR γ-chain clonality analysis was positive in skin biopsy specimens from 11 of 19 patients reported with T1 disease and that 2 long-term outcome studies have shown that patients with T1 disease have the same life expectancy as the general population.3,32 Only 1 of 11 patients with T1 disease who had a positive TCR γ-chain result was dead of disease at the end of the 4-year study.15 Thus, for the 11 TCR γ-chain–positive skin biopsy specimens from patients with T1 disease, it is possible that 10 of 11 patients would meet our criteria of having clinically benign positive findings for a poor outcome if clonality is being considered. The clonality analysis of lesion skin in the study by Delfau-Larue et al10 was intended principally for diagnostic rather than prognostic purposes. The skin lesions generally are expected to be positive in all T stages as long as the tumor cell population exceeds the clonal detection threshold of the assay being used.

In contrast, we used PCR analysis on lymph node biopsy specimens in 55 patients. Although a good outcome at last follow-up may eventually change to a poor outcome, the nearly 10 years of average follow-up suggest that the clinically benign positive rate from these data would be similar to that from follow-up to the end of life. Only 7 of 33 patients with a good outcome had a positive TCR result, and 5 of those died without a disease-specific outcome. The high rate of clinically benign positive results in TCR γ-chain analysis of skin10 (10 of 11) compared with lymph node tissue (7 of 33) may be due to the relatively larger number of total lymphocytes present in lymph nodes compared with skin. Polymerase chain reaction is able to detect clonality if 1% of the T cells under analysis are clonal, determined as the number of clonal T cells divided by the number of total T cells (clonal + reactive + naive). Because patients with CTCL have a concentration of epidermotropic and clonal T cells in the skin, there exists a disproportionate number of clonal T cells in the numerator compared with total T cells in the denominator (clonal and nonspecific [ie, reactive + naive]). Thus, the absolute number of clonal T cells required is much larger in the lymph nodes than in the skin to represent greater than 1% of the total T-cell population. The higher threshold for clonality for SBA of 5% may also help to explain why SBA was somewhat better than PCR in predicting a poor outcome, since the absolute number of clonal T cells in the lymph nodes required to have a positive TCR test is approximately 5 times higher for SBA than for PCR.

Another important issue with TCR studies is that a dominant T-cell clone does not equate to malignancy. Several benign lymphocyte-mediated dermatoses exist, including lymphomatoid papulosis, Mucha-Habermann disease, lymphomatoid drug eruptions, actinic reticuloid, and lichen planus, all of which demonstrate a dominant T-cell clone in some cases.31,32-36 Thus, although PCR may have a greater sensitivity in detecting T-cell clonality, the development of more predictive primer sets or entirely different methods31 may be needed to help distinguish between benign and malignant T-cell clones. Delfau-Larue et al10 suggested “gating” the PCR-DGGE method to diminish sensitivity so that approximately 5% clonality would be required for the test to be positive. They noted that this gating approach decreased the likelihood of having positive tests with benign conditions.10

Further long-term outcome studies are needed to more fully determine the prognostic utility of PCR analysis in patients with CTCL. Although PCR is a more sensitive assessment of clonality than SBA, it did not improve our ability to predict clinical outcome from lymph node DNA in this patient population. This study demonstrated the independent predictive value of dominant T-cell clone detection in lymph node biopsy specimens of patients with CTCL in univariate analysis; however, the predictive value of TCR by any method was inferior to that of clinical skin stage. Furthermore, with the exception of a subgroup of

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patients with sufficient sample size for analysis and intermediate disease (LN2). PCR was unable to provide additional prognostic information beyond that available from the clinical and histologic examination alone. In fact, the HR for a poor outcome was 7.2 for palpable abnormal lymph nodes vs clinically normal lymph nodes, while the HRs for clonality measured by PCR-DGGE and PCR-CE were 6.5 and 5.1, respectively (Table 3 and Table 4). Thus, the clinical examination of the lymph nodes was as useful as the PCR methods for predicting a poor clinical outcome.

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