Diagnostic and Prognostic Significance of Sézary Cells in Peripheral Blood Smears From Patients With Cutaneous T Cell Lymphoma

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Blood smears stained with Wright-Giemsa were obtained from 124 patients with pathologically confirmed cutaneous T cell lymphoma (CTCL), 70 patients with various other cutaneous disorders, and ten healthy adult volunteers. These were examined in a blinded fashion for atypical lymphocytes with cerebriform nuclei (CLs), which were characterized further according to cell diameter. CLs, comprising up to 15% of lymphocytes in smears, were observed in 20% of the patients with benign dermatitis. CLs, comprising up to 89% of lymphocytes in smears, were found in 22%, 30%, 50%, and 96% of patients with patch, plaque, tumor, and erythrodermic CTCL, respectively. Large-diameter CLs (15 to 20 μm) were observed only in smears from patients with CTCL. Total CL counts above 15 per 100 lymphocytes and/or the presence of large CLs occurred in 33 of 49 (67%) patients with erythrodermic disease and in only two patients with other skin manifestations. Blood smears obtained at the time of cytogenetic studies indicated that a total CL count above 15% was the smear criterion that correlated best with the demonstration of a chromosomally abnormal malignant clone in the blood. The presence of large CLs per se, although also predictive of a malignant clone, was less useful. Multivariate survival analysis showed that the duration of disease before the blood smear and the proportion of large CLs within the total CL population were the covariates that correlated most significantly with survival. We speculate that the reduced survival of patients with increased proportions of large CLs in smears reflects the presence of polyploid malignant lymphocytes in the blood.

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Hospital for review. An effort was made to obtain the earliest available smear from each patient. The majority of smears (81% of the total) were prepared at the time of initial evaluation, and the remainder were obtained later in the patients' course at times of restaging of the disease because of clinical evidence of disease progression (10% of total), a change in treatment (6% of total), and miscellaneous other reasons (3% of total). In addition, blood smears were prepared at the time of cytogenetic studies to allow for correlation with chromosome findings.

Smears from non-CTCL dermatologic conditions were obtained from patients with the following diagnoses (number of cases): extensive psoriasis vulgaris (four) and psoriatic erythroderma (four), pityriasis rubra pilaris (one), pemphigus vulgaris (two), lichenomatoid papulosis (one), cutaneous lymphoid hyperplasia (two), leukemid skin eruption (two), various forms of lupus erythematosus (nine), dermatomyositis (one), extensive atopic dermatitis (six), idiopathic exfoliative dermatitis (four), atypical mycobacterial infection (one), angioendothelial edema (one), nonneoplastic Kaposi's sarcoma (two), chronic digitate dermatitis (three), widespread nummular eczema (seven), contact allergic dermatitis (two), chronic hand dermatitis (one), extensive tinea corporis (one), lichen planus (four), lichenoid drug eruption (one), nodular exanthem (three), pityriasis rosea (three), sarcoidosis (four), and dermatitis herpetiformis (one). For the purpose of analysis, the dermatologic controls were combined into psoriasis, eczema, idiopathic erythroderma, collagen vascular, lichenoid, and miscellaneous groups. The possibility that some patients with apparent widespread eczema or erythroderma had CTCL in actuality was excluded since their eruptions either cleared or improved markedly, often spontaneously, during long-term follow-up.

Blood smears were stained with Wright-Giemsa and examined under the light microscope at ×100 by one of us (M.K.H.) in a random sequence without knowledge of the clinical diagnosis. At least 100 lymphocytes were identified and classified into three groups: atypical lymphocytes with cerebriform nuclei (CLs), atypical lymphocytes with a nuclear morphology different from CLs (usually "reactive" lymphocytes), and lymphocytes with a normal appearance.26 The CLs were categorized further according to cell diameter by means of an ocular micrometer as small- (8 to 11 μm), intermediate- (12 to 14 μm), and large- (15 to 20 μm) diameter CLs. These cellular dimensions were chosen to reflect reported size differences between normal lymphocytes and small and large Sézary cells in the blood.27,31,32,54

For cytogenetic studies, peripheral blood lymphocytes were cultured for 24 hours without mitogens or for 72 to 96 hours with various mitogens using standard techniques for whole blood as previously reported.44,45 Chromosome preparations were prepared in routine fashion from all cultures and examined with standard Giemsa staining and with G banding. At least 30 chromosome counts and five banded karyotype analyses were done on each specimen, with additional counts and analyses done as required to characterize specific cytogenetic abnormalities and their clonal nature.

For statistical analysis of data, the Student's t test was used to compare mean values for quantitative variables, eg, percentage of large CLs.44 Equal standard deviations were assumed only if the P value for the test of equality of standard deviations was greater than .2. All correlations presented are Kendall rank order correlations since it was often the case that some observations for one of the variables were zero. Fisher's exact test for comparing proportions was used instead of the approximate χ² test because of the small sample sizes involved.44 Finally, the Cox proportional hazards survival analysis method was used to test for variables, eg, proportion of large CLs, which have prognostic significance.45 Both SPSS and BMDP statistical software computer packages were used.44,77

RESULTS

Correlation of blood smear findings with disease status. Some CLs were identified in the circulating lymphocyte population of 14 of 70 (20%) patients with various disorders involving the skin other than CTCL (Table 1). These Sézary-like cells were not found randomly within the dermatologic control group, but were associated primarily with eczematous conditions (number of cases): atopic dermatitis (two), numbular dermatitis (two), idiopathic erythroderma (three), and chronic hand dermatitis (one). Higher proportions of CLs in the lymphocyte population (six to 15 total CLs per 100 lymphocytes) occurred only in those patients with extensive or generalized skin involvement. In addition, a small number of CLs (one to two total CLs per 100 lymphocytes) were observed infrequently in blood smears from patients with a variety of other conditions, including psoriasis (one), cutaneous lymphoid hyperplasia (one), leukemid skin eruption (one), lupus erythematosus (one), sarcomatoide (two), and even on smears from healthy volunteers (two). The CLs in patients with the foregoing conditions were mostly small and only occasionally intermediate in size. In no instance were CLs with large diameters (>14 μm) seen. By contrast, atypical "reactive" lymphocytes were identified in only three patients with eczematous disorders and ten patients with other diseases, particularly viral exanthems.

In the confirmed CTCL group, CLs were identified in five of 23 (22%) patients with patch disease, nine of 30 (30%) patients with plaque disease, 11 of 22 (50%) patients with tumor disease, and 46 of 49 (94%) patients with generalized erythrodermic disease (Table 1). The only statistically significant differences in the relative frequency of finding CLs was between the erythrodermic group and the patch or plaque groups (P < .0005) and the tumor group (P < .0001). However, there was a statistically significant trend (P < .0001) for increased likelihood of the presence of CLs with advanced disease. When patients with pretumorous CTCL were grouped according to the extent of cutaneous involvement, the difference between patients with patches or plaques covering less than 10% of the skin surface (T1 rating) and patients with cutaneous tumors (T3 rating) was significant (P < .05). Thus the probability of identifying some CLs in the circulating lymphocyte population from patients with CTCL relates in part to the type and magnitude of skin involvement.

From a quantitative standpoint, more than 15 CLs per 100 lymphocytes (the maximum proportion in non-CTCL controls) occurred in one patient with disseminated plaque disease, one patient with tumor disease, and 33 patients with erythrodermic disease. Typically, cell diameters of CLs in smears from these patients varied considerably, a finding that has been reported by others.11,13,22,26 Small, intermediate, and large CLs were present in the same smear from 24 (69%) of these patients; small and intermediate CLs in seven (20%); and intermediate and large CLs in four (11%). By cell size, the mean percentages of CLs ± 1 SE were 7.3% ± 7.9% for small CLs, 30.0% ± 16.6% for intermediate CLs, and 9.0% ± 11.7% for large CLs in smears from patients with more than 15 total CLs in the lymphocyte population.
Large CLs (absent in controls) were found in 33 of 49 (67%) patients with erythrodermic disease and in only three of 75 (4%) patients with other skin manifestations of CTCL (Table 1). The correlation between the proportions of large CL and total CL within the lymphocyte population (large CLs vs total CLs per 100 lymphocytes) was high ($r = .71, P < .001$) for patients with erythrodermic CTCL. This finding indicates that large CLs are more apt to be observed in such patients when larger proportions of CLs are present on smears.

**Correlation of blood smear and cytogenetic results.** Chromosomal analysis of peripheral blood lymphocytes was performed on 34 patients with suspected or confirmed CTCL. A malignant clone of lymphocytes (three or more cells with the same karyotypic alteration) was demonstrated on initial blood samples from 15 patients and on a second sample from one patient (Table 2). Detailed cytogenetic data on a number of these patients has been published previously.16

At least one CL was identified on concurrent blood smears from 28 of the 34 (82%) patients studied cytogenetically. The likelihood of observing at least one CL was not significantly different on smears from patients with a malignant clone compared with patients without a clone ($P > .2$). However, using cell size, the difference in observed frequencies between the two groups became progressively more significant (small CLs, $P < .05$; intermediate CLs, $P < .01$; large CLs, $P < .005$).

Blood smear findings can be related to the results of cytogenetic analysis. Figure 1 shows the relationship between various proportions of total CLs in smears and the probability of demonstrating a chromosomally abnormal clone of malignant lymphocytes on the first cytogenetic study. The presence of at least one CL (total CLs per 100 lymphocytes $> 0$) was associated with an abnormal clone in 14 of 28 (50%) studies compared with one of six (17%) studies in which CLs were not found, a difference that was not significant ($P > .2$). However, as increasing proportions of CLs are used to define the cutoffs for positive and negative smear criteria, the probability of finding malignant clones in patients with a positive smear increases progressively, and the difference in the frequency of an abnormal cytogenetic study between positive and negative groups quickly becomes significant. Indeed, the lowest cutoff providing optimal discrimination occurred at total CLs per 100 lymphocytes $> 15$, which identified a malignant clone in 11 of 13 (85%) cases with a positive smear criterion vs four of 21 (19%) cases with a negative smear criterion, a difference that was highly significant ($P < .0005$). Likewise, this cutpoint was the best smear criterion for predicting the absence of a malignant clone, ie, 17 of 21 (81%) cytogenetic studies were negative for patients with 15 or fewer CLs per 100 lymphocytes, while only two of 13 (15%) cytogenetic studies were negative for patients with more than 15 CLs per 100 lymphocytes.

The presence of large CLs also appears to have substantial
predictive value in terms of cytogenetic results. For 28 patients with at least one CL in the smear, a malignant clone was identified in 11 of 15 (73%) patients with large CLs present compared with three of 13 (23%) without large CLs (P < .05). In addition, a significantly higher mean percent of chromosomally abnormal cells in culture were found in samples with large CLs than without (respectively, 38.5% and 9.5%; P < .05). The apparent predictive value of large CLs per se may reflect the fact that a high correlation exists between the proportions of total CLs and large CLs per 100 lymphocytes within this data set.

Diagnostic and prognostic implications from blood smears. The foregoing observations suggest that two smear findings, ie, more than 15 total CLs per 100 lymphocytes (total CLs > 15) and the presence of large CLs (large CLs > 0), are useful in distinguishing erythrodermic CTCL from benign erythrodermas and in identifying patients with chromosomally abnormal clones of lymphocytes in the blood. These tentative criteria for leukemic involvement (Sézary syndrome) are shown on a scattergram of large total CLs per 100 lymphocytes in smears from 49 patients with erythrodermic CTCL (Fig 2). It is apparent that 32 of 49 (65%) patients have smears with both criteria present; this group contains patients on whom cytogenetic studies invariably were abnormal (11 positive/11 patients). On the other hand, leukemic involvement was confirmed by chromosome analyses in four of ten patients from the remaining group without both smear criteria. Thus a definition of Sézary syndrome using both total CLs > 15 and large CLs > 0 results in a low frequency of false positives but a high frequency of false negatives.

For this reason, we looked more closely at the clinical features of those patients with only one smear criterion, ie,
patients with more than 15 total CLs but without large CLs (group I), and those with 15 or fewer total CLs but with large CLs (group II). Patients in group I were found to have a somewhat higher frequency of lymph node or visceral involvement and death compared with patients in group II (Table 3). This finding suggests that total CLs >15 per 100 lymphocytes is a better criterion than the presence of large CLs per se for the diagnosis of leukemic CTCL, perhaps because it encompasses patients with the small-cell variant of Sézary syndrome.

The fact that increased proportions of CLs and large-diameter CLs are associated with abnormal cytogenetic studies suggests that smear findings may have important prognostic implications. This question was addressed further using multivariate survival analysis for the 49 patients with erythrodermic CTCL. As shown in Table 1, at least one CL was present on all but three smears from these patients. Large CLs were observed in 33 of the remaining 46 smears and the percentage of large CLs per total CLs ranged considerably from 0% to 75% with a median of 11%.

When the survival analysis was performed using prognostic variables restricted to age at smear, duration of symptoms, and results of routine hematologic tests (total leukocyte count, relative and absolute lymphocyte count), the patient’s duration of symptoms was the only significant covariate. Duration of disease continued to be the only significant variable related to survival as laboratory test covariates were expanded to include the proportion of total CLs per 100 lymphocytes and the total CL count per cubic millimeter of blood. However, a significant correlation to survival of patients with erythrodermic CTCL was identified when large CLs were included as a covariate in the analysis. Specifically, the proportion of large CLs among all CLs and absolute large CL count per cubic millimeter of blood were found to be almost equal as prognostic factors, even when the analysis was limited to patients with total CLs >15, the putative Sézary syndrome subgroup.

The probability of surviving to at least y years after the smear, S(y), can be estimated as a function of age at smear, duration of disease, and proportion of large CLs among all CLs in the smear. The formula is as follows:

\[ S(y) = S_0(y)^b \]

where \( S_0(y) \) is the “null” survival probability as estimated from the data and \( b = (0.0250 \cdot \text{age at smear}) - (0.3063 \cdot \text{duration of disease}) + (2.4551 \cdot \text{ratio of large CLs-total CLs} \).

Table 3. Clinical Features of Patients With Erythrodermic CTCL Subgrouped According to Inconclusive Blood Smear Findings

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Patients</th>
<th>Histopathologic Involvement</th>
<th>Palpable Death</th>
<th>Palpable Spleen</th>
<th>Palpable Lymph Node</th>
<th>Palpable Visceral</th>
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<td>I</td>
<td>7</td>
<td>5/7</td>
<td>3/7</td>
<td>3/7</td>
<td>6/7</td>
<td>0/5</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>1/5</td>
<td>0/5</td>
<td>1/5</td>
<td>2/6</td>
<td>0/5</td>
</tr>
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Group I: total CLs >15, large CLs = 0 per 100 lymphocytes; group II: total CLs ≤15, large CLs >0 per 100 lymphocytes.

*Number positive/number available for study at any time in patient’s course.
and never became positive in ten (20%) patients. Four patients in this series whose earliest available blood smears were classified as negative later developed positive smears in their course.

Treatment was found to have an important effect on smear findings of 37 evaluable patients in which additional smears were obtained during follow-up. The total CL count decreased substantially as a result of therapy in 27 (73%) patients, often with loss of the large cell component. In those patients with more than 15 total CLs per 100 lymphocytes, abnormal lymphocytes usually continued to be observed in small numbers or occasionally became undetectable.

Because leukemic cell numbers usually decreased with treatment, it was difficult to determine whether the proportion of large CLs relative to total CLs changed over time. However, in two patients (PB and GB) on whom four cytogenetic studies were performed serially, the proportion of polyoid sublines related to the initial abnormal clone progressively increased from 0% to 36% and 16% to 100% over a two-year and 3 1/2-year interval, respectively, until the patients’ deaths. Concurrent blood smears showed a simultaneous increase in the proportion of large to total CLs in one of these patients, but definite conclusions are impossible because total CLs decreased substantially in both instances. These observations, although admittedly quite limited, suggest that the clonal evolution toward more marked aneuploidy underlies the clinical course of some patients with Sézary syndrome, perhaps prompted by the effect of mutagenic treatment.

Finally, blood smears were obtained at the same time as lymph node biopsies on 36 patients with erythrodermic CTCL (Table 3). No statistically significant correlation was found between the histopathologic diagnosis and the presence or absence of a total CL count >15 ($P = .16$), a large CL count >0 ($P = .48$), or both smear parameters occurring together ($P = .09$). This finding indicates that a positive blood smear is a useful parameter in staging since it provides prognostic information independent of lymph node biopsy results.

**DISCUSSION**

Various studies including this one have shown that atypical lymphocytes with cerebriform nuclei (CLs) often are found in the peripheral blood of patients with CTCL, including mycosis fungoides as well as Sézary syndrome, various inflammatory dermatoses, and even in healthy subjects. The frequency of observing CLs in such situations and the exact proportion of CLs within the lymphocyte population for a given case varies considerably in reports from the literature, partly because the criteria used to define CLs and the methods used to examine lymphocyte populations range from qualitative assessments of whole cells in routine blood smears to computer-assisted nuclear morphometry on thin sections of plastic-embedded lymphocyte preparations. Although measurement of CL numbers on routine smears has the advantage of being generally available, it is less accurate than more sophisticated methods because some CLs, particularly those with small compact nuclei, are difficult to recognize by visual examination. Myrie et al compared CL counts in blood smears with counts on semithin and thin sections and concluded that smear findings were quite inaccurate although our calculations from their data indicate a high correlation between methods ($r = .82, P < .00001$).

In our blinded study, CLs were identified in blood smears from almost all patients with erythrodermic CTCL and one third of patients with other skin manifestations (mycosis fungoides). The frequent association of CLs and erythrodermic CTCL has been noted by other investigators as well. In addition, our observations agree with those of Bunn et al in that the frequency of finding circulating CLs in other clinical expressions of CTCL relates to the magnitude of skin involvement (T rating) and hence stage of disease. Such data have been presented as evidence that malignant T cells readily enter the peripheral circulation and provide a rationale for systemic therapy of patients with early CTCL apparently confined to the skin.

Quantitation of CLs in the peripheral blood of patients with CTCL is important for two reasons. First, a certain CL count has diagnostic usefulness since it can be used to distinguish CTCL from benign conditions and to identify patients with leukemic involvement (Sézary syndrome). Second, a certain CL count probably has prognostic usefulness since the presence of CLs in the blood has been associated with an unfavorable clinical course. Considering the differences in methodology, it is not surprising that the criteria for a “clinically significant CL count” differs from center to center. Using routine smears, Duncan and Winkelmann concluded that an absolute Sézary count greater than 1,000/μL would distinguish most cases of Sézary syndrome from benign dermatoses. Bunn et al considers a smear with
20 or more CLs per 100 lymphocytes to be positive for leukemic involvement. Using quantitative morphometry to define CLs, Willemze et al\(^9\) also found greater than 20 CLs per 100 lymphocytes to be a diagnostically useful cutpoint, but Stolz,\(^9\) using comparable techniques, presented data to suggest that lower percentages may be more appropriate. Myrie et al,\(^50\) using a more qualitative definition of CLs on thin sections, suggested that a cutpoint as low as five CLs per 100 lymphocytes may be diagnostically important.

Our findings likewise show that substantial proportions of circulating lymphocytes (up to 15\%) from patients with extensive benign dermatitis have a nuclear appearance indistinguishable from small Sézary cells. Moreover, reports from the literature suggest that actinic reticuloid, an unusual disorder characterized by severe photosensitivity, may have even higher proportions of CLs in the blood at times.\(^73-75\)

Most likely, this phenomenon occurs from increased numbers of activated normal T lymphocytes in the blood that are involved in inflammatory reactions in the skin.\(^31-32\) Thus it is often uncertain as to what portion of CLs in the blood of patients with extensive CTCL (particularly erythrodermic disease) represent nonmalignant T cells present because of cutaneous inflammation.

Because malignant T cells tend to be larger than normal T cells,\(^25,43,68\) we wondered whether measurement of the cell diameters of CLs would provide a simple means of identifying malignant T cells on blood smears. In fact, atypical lymphocytes with large hyperchromatic nuclei (the so-called mycosis cell) have long been recognized to be a cellular marker of mycosis fungoides\(^80\) and more recently, the presence of CLs with nuclei measuring more than 7.5 \(\mu\)m in diameter has been advocated to distinguish dermatopathic lymphadenitis from early lymph node involvement with mycosis fungoides.\(^77\) Litovitz and Lutzner\(^43\) found no relationship between the degree of nuclear convolutions and cell diameter of circulating Sézary cells. Furthermore, there is some evidence from quantitative morphometry that cell area is inversely related to the diameter of circulating Sézary cells. The association between increasing proportions of large CLs in the blood and decreasing survival may reflect the fact that large CLs encompass malignant T cells with polyploid DNA content\(^7,8,12,15,17\) and that a significant number of such aneuploid cells in the blood has dire connotations for the patient.\(^14,17\) This idea could not be substantiated by comparing blood smear results in samples with or without demonstrable polyploid sublines in the malignant clone, perhaps because of the small number of studies. An alternative explanation for the association may be that the reactive small CL component becomes less evident as the result of warning immunologic responses in advanced disease. These two mechanisms are not necessarily mutually exclusive. Thus we believe that the total CL count per se is less useful as a prognostic indicator than the proportion of large CLs per total CLs because some CLs, presumably small CLs, are not malignant.

Finally, we assessed the usefulness of the two potential smear criteria (total CLs >15 and large CLs >0) for establishing a diagnosis of Sézary syndrome. A definition of Sézary syndrome using both criteria simultaneously resulted in a low frequency of false positives as judged by chromosome findings but was too restrictive, since four of ten (40\%) patients lacking both smear criteria had abnormal clones in the blood (high false-negative rate). Subsequent comparison of subgroups with only one criterion present on smears indicated that a total CL count above 15 was more clinically relevant than the presence of large CLs per se, probably because the total CL count is less likely to exclude patients with the small cell variant of Sézary syndrome. For this reason, we have adopted at our center the criterion of more than 15 CLs per 100 lymphocytes as presumptive evidence of leukemic involvement in patients with CTCL, and use the proportion of large CLs among total CLs as a supportive diagnostic criterion and for prognostication. It also must be recognized that the above smear criteria will fail to detect leukemic involvement in some patients because the malignant T cells are difficult or impossible to recognize on routine blood smears. Using cytogenetic analysis, we have uncovered three cases of leukemic involvement in which careful examination of smears consistently showed few total CLs and no large CLs among the lymphocytes (Fig 2). Electron microscopy, particularly if combined with quantitative morphometry of nuclear images, is a more reliable screening procedure than smears, although it is more time-consuming.\(^80\)
SÉZARY CELLS IN CUTANEOUS T CELL LYMPHOMA

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