Using Smudge Cells on Routine Blood Smears to Predict Clinical Outcome in Chronic Lymphocytic Leukemia: A Universally Available Prognostic Test

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Recently developed prognostic tests in early Rai and Binet stage chronic lymphocytic leukemia (CLL) require considerable technological expertise and are not available worldwide. Smudge cells are CLL cells ruptured during smear preparation. We hypothesized that smudge cell formation is inversely correlated with expression of vimentin, a cytoskeletal protein and prognostic marker, and that the percentage of smudge cells would predict prognosis in CLL. We reviewed the blood smears of 75 patients with previously untreated early- and intermediate-stage CLL (Rai stage 0-II) who were seen at the Mayo Clinic in Rochester, Minn, between September 1989 and December 2000. A total of 200 lymphocytes and smudge cells were counted on each slide and the results expressed as a percentage of the total lymphocytes (intact and smudged). The median percentage of smudge cells was 27% (range, 4%-72%). The percentage of smudge cells inversely correlated with vimentin expression ($r = -0.57$; $P = 0.007$). The median percentage of smudge cells was higher in patients with the mutated immunoglobulin heavy chain gene than in those with the unmutated immunoglobulin heavy chain gene (31% vs 13%; $P = 0.02$). Patients with less than 30% smudge cells had a median time from diagnosis to initial treatment of 72.7 months, whereas the median time from diagnosis to initial treatment in patients with 30% or more smudge cells was not reached ($P = 0.001$). The percentage of smudge cells as a continuous variable correlated with overall survival ($P = 0.04$). The estimation of smudge cells on a blood smear could be a universally available prognostic test in early-stage CLL.


Patients and Methods

This study was approved by the Mayo Foundation Institutional Review Board in accordance with federal regulations and was supported in part by grants from the National Institutes of Health and National Cancer Institute (CA92541) and philanthropic support from Mr Edson Spencer and the Donner family.

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and the Declaration of Helsinki. Patients seen at the Mayo Clinic in Rochester, Minn, between September 1989 and December 2000 who were diagnosed as having early- and intermediate-stage (Rai stage 0-II) CLL, consented to be included in a prospective clinical database, and had peripheral blood smears archived before the initiation of treatment were included in this study. Criteria for diagnosis and indication of treatment adhered to the National Cancer Institute Working Group guidelines.11

**Peripheral Blood Smear Examination**

Archived Wright-Giemsa–stained blood smears originally obtained for clinical purposes were reviewed. The smear obtained closest to the date of diagnosis was used for each patient. In 15 patients, a second archived specimen was also evaluated to determine the variability of the percentage of smudge cells over time. The blood smears were prepared from either EDTA anticoagulated blood or a finger stick. All blood smears were prepared using a semiautomatic device used by our laboratory since the 1980s (Miniprep; Sedona Lab Products, Sedona, Ariz) in which a simple spring mechanism pulls a drop of blood along a slide. According to established criteria, smudge cells were defined as broken cells with no intact cytoplasm and a disrupted nuclear membrane (Figure 1).12 A total of 200 lymphocytes and smudge cells were counted on each slide and the results expressed as a percentage of the total lymphocytes (intact and smudged).

Because some other laboratories use the manual wedge method, in which a drop of blood is manually pulled by a glass cover, or the Autoprep (Sedona Lab Products) semiautomatic method (essentially the same procedure as the Miniprep), we also freshly prepared peripheral blood smears from 10 patients with CLL using each of these methods to allow comparison of smudging using these different techniques. In addition, to determine the reproducibility and...
consistency of smear smudge cell counts among different observers, peripheral smears were made and read in a blinded manner by 3 hematopathology technologists.

**Vimentin Expression by CLL Cells**

Vimentin expression in peripheral blood CLL cells was measured by flow cytometry. Briefly, mononuclear cells isolated by density gradient centrifugation were stained with monoclonal phycocerythrin–labeled anti-CD19 antibodies (BD Pharmingen, San Jose, Calif), permeabilized (Fix and Perm Solution; Caltag Laboratories, Burlingame, Calif), and stained with antivimentin fluorescein isothiocyanate–labeled monoclonal antibody RV202 (Santa Cruz Biotechnology, Santa Cruz, Calif). The vimentin content was assessed in CD19+ gated cells by flow cytometry (FACScan; BD Biosciences, San Jose, Calif). Mean fluorescence intensity (MFI) of vimentin was compensated for the isotype control MFI.

**Assessment of IgVH Gene Mutational Status**

The IgVH gene mutation status was assessed as previously described. Patients were classified as having unmutated status if there was 98% to 100% homology with the germline IgVH gene sequence and mutated status if there was less than 98% IgVH gene homology.

**Statistical Analyses**

The differences between percentages of smudge cells on peripheral blood smears generated by using different methods and by independent observers were compared using the tables for calculating the least significant differences for leukocyte differential counts with 200 cells and a 2-sided level of significance of 0.05 as developed by Rumke et al.

The nonparametric Spearman correlation test was used to assess correlations among continuous variables. The TTT was estimated using the Kaplan-Meier method, and differences between groups were assessed using the log-rank test. Univariate and multivariate analyses were performed using the Cox proportional hazard regression model. To discriminate between those who had a short TTT and those who had a longer TTT, a categorical variable for the percentage of smudge cells was defined using a minimum value approach to identify the optimal cutpoint.

**RESULTS**

The clinical characteristics of the 75 eligible patients with blood smears are given in Table 1. The median follow-up from diagnosis was 75 months (range, 9-202 months). Thirty-two patients (43%) had been treated as of last follow-up with a median TTT of 95.3 months. The IgVH gene mutational status was available for 45 patients (60%). The median TTT for patients with unmutated IgVH status was 64.2 months (95% confidence interval [CI], 40.6-100.3 months) and was not reached for patients with mutated IgVH status (95% CI, 95.3 months to not yet reached; P=.002). The median survival for the entire cohort was not reached; the predicted 10-year overall survival rate was 77.2% (95% CI, 64%–93%).

**Peripheral Blood Smear**

The median time from the date of diagnosis to the first available smear was 22 months (range, 0-162 months), and 39 patients (52%) had smears available for review within 24 months of diagnosis. In 15 patients, blood smear slides obtained at diagnosis were compared with slides made at later time points after diagnosis (median time between slides, 48 months; range, 9-72 months). The percentage of smudge cells had not changed significantly over time (P=.61).

The median ALC at the time the peripheral blood smear was made was 16.8×10^9/L (range, 5.3-165.7×10^9/L). The median percentage of smudge cells was 27% (range, 4%-72%). No correlation was found between the percentage of smudge cells and the ALC (r=0.04; P=.73).

Concordance was found between slides prepared using the Miniprep device and the Autoprep device in 29 (97%) of 30 readouts. A 93% (28/30 readouts) concordance rate was reached; the predicted 10-year overall survival rate was 77.2% (95% CI, 64%–93%).

**TABLE 1. Clinical Characteristics of the 75 Study Patients**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>46 (61)</td>
</tr>
<tr>
<td>Female</td>
<td>29 (39)</td>
</tr>
<tr>
<td>Median age (y) (range)</td>
<td>63.6 (33-86)</td>
</tr>
<tr>
<td>Rai stage</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>56 (75)</td>
</tr>
<tr>
<td>I</td>
<td>13 (17)</td>
</tr>
<tr>
<td>II</td>
<td>6 (8)</td>
</tr>
<tr>
<td>IgVH gene mutational status†</td>
<td></td>
</tr>
<tr>
<td>Mutated</td>
<td>29 (64)</td>
</tr>
<tr>
<td>Unmutated</td>
<td>16 (36)</td>
</tr>
<tr>
<td>Median ALC at the time of smear (×10^9/L) (range)</td>
<td>16.8 (5.3-165.7)</td>
</tr>
</tbody>
</table>

*ALC = absolute lymphocyte count; IgVH = immunoglobulin heavy chain.
†Available for 45 patients.


<table>
<thead>
<tr>
<th>Reader</th>
<th>Autoprep</th>
<th>Wedge method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10/10</td>
<td>5/10</td>
</tr>
<tr>
<td>2</td>
<td>9/10</td>
<td>6/10</td>
</tr>
<tr>
<td>3</td>
<td>10/10</td>
<td>6/10</td>
</tr>
</tbody>
</table>

*Data are presented as concordant/total readouts. The least significant differences (α=.05) were estimated for a 200-cell differential as described by Rumke et al.
found in the estimated percentage of smudge cells across the readers for the Miniprep and Autoprep methods, whereas concordance between readers when the manual wedge method was used was 57% (17/30 readouts).

**RELATIONSHIP AMONG THE PERCENTAGE OF SMUDGE CELLS, VIMENTIN EXPRESSION, AND IGVH GENE MUTATION STATUS**

We studied a subset of 21 patients with CLL who had pretreatment cryopreserved samples available for analysis. Cells from all 21 patients expressed vimentin with an MFI of 383 (range, 211-1666). Vimentin expression was inversely correlated with the percentage of smudge cells ($r = -0.57$; $P = .007$). The percentage of smudge cells was significantly higher in patients with mutated $\text{IgV}_H$ status (median, 31%) than in patients with unmutated $\text{IgV}_H$ status (median, 13%) ($P = .02$).

**PERCENTAGE OF SMUDGE CELLS AND CLINICAL OUTCOME**

The percentage of smudge cells as a continuous variable was associated with both a prolonged TTT ($P = .002$) and longer overall survival ($P = .04$). Using a minimum $P$ value approach, we established an optimal cutpoint of 30% to define a categorical variable for the percentage of smudge cells. Patients with less than 30% smudge cells had a significantly shorter median TTT (72.7 months; 95% CI, 48.5-92.2 months) compared with patients with 30% or more smudge cells (median TTT, not yet reached; $P = .001$) (Figure 2, middle). The difference between the groups remained statistically significant for a subset of patients with Rai stage 0 disease only (Figure 2, bottom). The estimated percentage of all patients with less than 30% smudge cells who required treatment within 10 years of diagnosis was 77% compared with 37% of patients with 30% or more smudge cells ($P = .001$). In a multivariate Cox model adjusted for Rai stage, the percentage of smudge cells (<30% vs $\geq 30\%$) and $\text{IgV}_H$ gene mutation status remained significant prognostic factors (Table 3).

**DISCUSSION**

In 1896, Gumprecht$^{17}$ described the presence of smudge cells (also known as shadows of Gumprecht or basket cells) on the blood smears of patients with “lymphocytic leukemia.” The formation of smudge cells was initially considered an unimportant artifact of slide preparation, reflecting the “fragility” of CLL cells.$^{18}$ In 1959 Heinivaara$^8$ made 2 intriguing observations: first, that the percentage of smudge cells was not dependent simply on the degree of lymphocy-

**TABLE 3. Multivariate Analysis of the Impact of IGVH Gene Mutational Status and Percentage of Smudge Cells on Time From Diagnosis to Initial Treatment in Early-Stage Chronic Lymphocytic Leukemia Adjusted for Rai Stage**$^*$

<table>
<thead>
<tr>
<th>Risk group</th>
<th>HR (95% CI)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rai stage of I or II at diagnosis</td>
<td>1.98 (0.82-4.79)</td>
<td>.13</td>
</tr>
<tr>
<td>Smudge cells $\geq 30%$</td>
<td>0.17 (0.05-0.56)</td>
<td>.003</td>
</tr>
<tr>
<td>Mutated $\text{IgV}_H$ gene status</td>
<td>0.21 (0.07-0.60)</td>
<td>.004</td>
</tr>
</tbody>
</table>

$^*$CI = confidence interval; HR = hazard ratio; $\text{IgV}_H = $ immunoglobulin heavy chain.
nosis or the slide stain method, and second, that “smudging” appeared to be patient specific. In 1993, Binet et al described a correlation between the presence of dense chromatin and the number of smudge cells and postulated that smudge cells are from lymphocytes on the “verge of apoptosis.”

We recently reported that a high expression of the cytoskeleton protein vimentin is associated with the presence of an unmutated \( IgV_H \) gene and shortened TTT. Because vimentin is critical for lymphocyte rigidity, low vimentin expression could render cells more “fragile” and susceptible to smudging when making peripheral blood smears. We have now shown that low vimentin expression correlates with a high percentage of smudge cells on the peripheral blood smear. In addition to its mechanical role, vimentin is involved in signal transduction and cell activation of normal and malignant cells. Vimentin expression is associated with adverse prognosis in patients with solid tumors.

In the current study, we demonstrate that the percentage of smudge cells on a peripheral blood smear is lower in patients with unmutated \( IgV_H \) genes and that the percentage of smudge cells as a continuous variable correlates with both TTT and overall survival. The cutoff of 30% smudge cells was identified on the basis of the minimal \( P \) value approach and stratified patients into 2 groups with significantly different TTT. Importantly, similar to other investigators, we found no correlation between ALC and the percentage of smudge cells, and the percentage of smudge cells in patients did not change significantly over time. These findings indicate that the percentage of smudge cells is not simply a surrogate marker of disease burden but reflects differences in the biologic characteristics of the leukemic cells.

Simple semiautomatic devices (Miniprep and Auto-prep) and the manual wedge method are routinely used to prepare slides in hematopathology laboratories. In our study, generation of blood smears with semiautomatic devices yielded consistent smudge cell percentages; however, significant differences in intraobserver and interobserver counts were seen when the manual wedge method was used. This finding is consistent with previous findings, likely reflecting the nonuniform spread with the manual method. The reproducibility of a white cell differential count can be improved by counting more cells per slide. Whether counting more than 200 cells to estimate the percentage of smudge cells would improve reproducibility of all the methods used remains to be established. Currently, we recommend using slides prepared by semiautomatic methods and a minimum of a 200-cell count for future studies.

CONCLUSION

Our finding that the high percentage of smudge cells on a peripheral blood smear is associated with mutated \( IgV_H \) gene status, a longer TTT, and better overall survival in patients with early-stage CLL could provide a simple and inexpensive prognostic test available to nearly all patients worldwide with a diagnosis of CLL.

REFERENCES


