Original Study

Prognostic and Predictive Significance of Smudge Cell Percentage on Routine Blood Smear in Chronic Lymphocytic Leukemia

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Abstract

We highlight, the use of smudge cell percentage on peripheral smear of chronic lymphocytic leukemia (CLL) patients at centers with limited resources.

Introduction/Background: Smudge cells are ruptured lymphocytes present on routine blood smears of chronic lymphocytic leukemia (CLL) patients. We evaluated prognostic and predictive significance of smudge cell percentage on a blood smear in CLL patients. **Materials and Methods:** We calculated smudge cell percentages (ratio of smudged to intact cells plus smudged lymphocytes) on archived blood smears of 222 untreated CLL patients registered at Institute Rotary Cancer Hospital, All India Institute of Medical Sciences, New Delhi over the past 12 years. **Results:** The male:female ratio was 3:1, and median age 60 (range, 28-90) years. Median absolute lymphocyte count was 42×10^9/L. The median smudge cell percentage was 29.6% (range, 4%-79%). We found no correlation of proportion of smudge cells with age, sex, lymphocyte count, organomegaly, or response to therapy, although there was a significant correlation with the Rai stage at diagnosis. Median smudge cell percentage in stage 0 and I was 33% (range, 12%-79%), in stage II 31% (range, 12%-61%), and stage III and IV 21% (range, 4%-51%) ($P < .001$). Patients with $\leq 30\%$ smudge cells had a shorter median progression-free period (PFP) of 30 months compared with patients who had more than 30% smudge cells (PFP, 45 months; $P = .01$). The 5-year survival rate was 51% for patients with 30% or fewer smudge cells, and it was 81% for patients with more than 30% smudge cells ($P < .001$) at a median follow-up of 3.5 years. **Conclusion:** Simple and inexpensive detection of smudge cells on routine blood smears seems useful in predicting progression-free and overall survival in CLL patients and might be beneficial in countries with limited resources.

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Introduction

Chronic lymphocytic leukemia (CLL) is a common leukemic disorder in the West, with an estimated incidence of 5.17 per 100,000 in the United States, representing 20% of all mature B-cell neoplasms.[1] In India, it is relatively rare and accounts for 2% to 3% of adult leukemias.[2] Two-thirds of all CLL patients are diagnosed in the early stage and require only a close follow-up. Patients presenting with advanced stages or those who progress to an advanced stage during follow-up merit treatment for CLL. Overall, 80% of all patients require therapy during the course of the disease.[3] Although the Rai staging system predicts overall survival (OS), it does not predict the patients who will progress and require therapy.[4] The traditional prognostic parameters, like clinical stage, lymphocyte doubling time, β-2 microglobulin levels, and lactate dehydrogenase levels are useful, but they might not accurately predict progression for an individual patient. In the past few years, the focus of research in CLL has changed from clinical to biological prognostic factors, the immunoglobulin (Ig)VH mutational status, CD38 and zeta associated protein (ZAP)-70 expression, to name a few.[5,6] Their implication and utility has been demonstrated in a few recent studies.[7,8] Wierda et al. have incorporated clinical (lymph node size and number) and biological prognostic factors (florescence in situ hybridization [FISH] analysis for 17p, 11q, and IgVH mutation

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status) in their prognostic model for CLL.[7] One recent study by Falchi et al. analyzed these modern biological factors in 2 different ethnic groups and determined their implications, highlighting the racial disparity in the prognosis.[8] Based on these data, it seems prudent to use these newer markers for CLL patients but the estimation of these markers is labor-intensive, expensive, and demands technical expertise not available in most centers. Therefore, there is a need for easier, simple, yet reliable methods, which can be used to prognosticate these patients in a resource-limited setting.

Smudge cells are ruptured B cells present on routine blood smears of all CLL patients. Smudge cell estimation, determined using a microscopic evaluation of Wright–Giemsa-stained blood smears, has recently been reported as a simple and inexpensive predictor of disease progression and OS in CLL.[9-11] The aim of our study was to evaluate the prognostic and predictive potential of percentage of smudge cells on peripheral blood smear of untreated CLL patients.

Materials and Methods

The study population consisted of 222 patients with CLL, diagnosed and registered between January 2000 and December 2011 at the Medical Oncology department of the All India Institute of Medical Sciences, New Delhi. The diagnosis of CLL was made using clinical and hematological examination that included peripheral blood smear examination and flow cytometry. Patients were diagnosed on the basis of an absolute lymphocyte count greater than 5×10^9/L on at least 2 occasions 1 month apart and demonstration of monoclonality using flow cytometry and a panel comprising CD19, CD20, CD5, CD23, CD79b, FMC7, and surface Ig. Staging was done according to the Rai staging system.[4] National Cancer Institute 1996 criteria were used for treatment decisions and response assessment.[12] Patients were treated if they had persistent fatigue unrelated to a coexistent disease, persistent or progressive systemic symptoms (fever, night sweats, weight loss), increasing bone marrow failure (anemia, thrombocytopenia), autoimmune hemolytic anemia or thrombocytopenia not responding to steroids, short lymphocyte doubling time (> 50% over 2 months), and disease-related recurrent bacterial infections and progressive enlargement of the lymph nodes, liver, or spleen. Complete remission was defined as all of the following for at least 2 months: (1) no organomegaly and lymphadenopathy on clinical examination; (2) no constitutional symptoms; (3) normal blood counts (hemoglobin > 11 g/dL without transfusions, absolute neutrophil count > 1.5×10^9/L, platelet count > 100×10^9/L); and (4) less than 30% of nucleated cells being lymphocytes in the bone marrow. Partial remission was defined as: (1) at least 50% reduction in the size of lymph nodes and/or hepatosplenomegaly less than the pretreatment value; (2) at least 50% reduction in absolute lymphocyte count, or (3) at least 50% improvement over pretreatment values. Progressive disease was defined as 50% or greater increase in the size of spleen, liver, absolute lymphocyte count, or the sum of lymph node products or the appearance of new lymph nodes. Stable disease was defined when none of these criteria were met. We calculated smudge cell percentages (ratio of smudged to intact cells plus smudged lymphocytes) on blood smears of all 222 untreated patients. The blood smears were prepared from finger prick using a manual wedge method. A total of 200 lymphocytes and smudge cells were counted on each slide and the results were expressed as a percentage of the total lymphocytes (intact and smudged). The smudge cell percentage was calculated as the percentage of total lymphocytes (intact and smudged) as previously described.[9-11] Each slide was evaluated by 3 hematopathologists and smudge cells were defined as broken cells with no intact cytoplasm, and a disrupted nuclear membrane. Study end points were OS, defined as the time from diagnosis to death from any cause or lost to follow-up, and progression-free period (PFP), defined as the time from diagnosis to progression or indication to start treatment in untreated patients with Rai stage 0, I, II (early and intermediate risk).

Statistical Methods

Quantitative variables were summarized as a median with standard deviation and qualitative variables as proportions. Baseline categorical variables were analyzed using the χ^2 test or Fisher exact test. PFP and OS distributions were estimated using the Kaplan-Meier method. Log rank test was used to compare survival between different stages. Cox regression test was used for multivariable analysis to identify independent predictors ($P < .05$). STATA (version 11.1) was used for statistical analysis.

Results

The clinical characteristics of the study cohort are given in Table 1. The median age was 60 years (range, 28-90 years). One hundred sixty-seven patients were male and 55 patients were female. Ten patients were asymptomatic and diagnosed incidentally during routine examination. The common symptoms were lymphadenopathy in 40% of cases, followed by fatigue and fever, which were observed in 30% and 20% of patients, respectively. The median total leukocyte, total lymphocyte, hemoglobin, and platelet counts were 48×10^9/L, 42×10^9/L, 11.8 g/dL, 160×10^9/L, respectively. Rai stage distribution was: stage 0, 6%, stage I, 19%; stage II, 39%; stage III, 15%; and stage IV, 20%. Median smudge cell percentage was 29.6%. There was no correlation of proportion of

Table 1 Clinicohematological Parameters of All Patients (n = 222)

Baseline Characteristic	Value
Age (Range), Years	60 (28-90)
Male Sex, n (%)	167 (75)
Female Sex, n (%)	55 (25)
Hepatomegaly, n (%)	78 (35)
Splenomegaly, n (%)	100 (45)
Hemoglobin (Range), g/dL	11.80 (4-15)
Total Leukocytes Count, × 10^9/L	48
Absolute Lymphocyte Count, × 10^9/L	42
Platelet Count (Range), × 10^9/L	160 (10-420)
Rai Stage, n (%)	
0	13 (6)
I	43 (19)
II	87 (39)
III	35 (15)
IV	44 (20)