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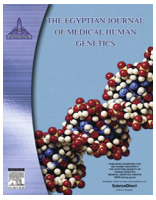

Original article

New insights into smudge cell percentage in chronic lymphocytic Leukemia: A novel prognostic indicator of disease burden

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ABSTRACT

Background: Percentage of smudge cells in CLL patients has recently been reported as a novel prognostic factor.
Objectives: To investigate the impact of smudge cells percentage on the clinicolaboratory data of CLL patients and to evaluate the relationship between it and other prognostic factors in CLL.
Methods: Ninety adults with CLL were enrolled. Smudge cells percentage was calculated by microscopic evaluation of blood smears. Testing of CD38 expression was done by immunophenotyping and detection of ATM, P53 deletions and trisomy 12 were performed using fluorescent in situ hybridization (FISH)
Results: Lower smear cells percentage (<30%) was significantly correlated with age, lymphadenopathy, organomegaly and advanced staging. It was also associated with high TLC, low hemoglobin and platelets count and high absolute and atypical lymphocytic count. Correlation studies with other prognostic factors revealed an association between low smear cells percentage and CD38 expression, short LDT, P53 and ATM deletions. Logistic regression analysis was also done to provide complementary prognostic information identifying the significant independent factors that predict low smear cell percentage.
Conclusion: low percentage of smudge cells (<30%) could be considered as an adverse prognostic predictor being associated with high risk markers in CLL.

1. Introduction

B-cell chronic lymphocytic leukemia (B-CLL) results from accumulation of small mature B lymphocytes that have undergone monoclonal expansion in blood, bone marrow and lymphoid organs which is mainly due to inhibition of apoptosis rather than enhanced cell proliferation [1].

The clinical course of CLL is heterogenous in different patients, some patients die within 2–3 years with refractory disease, whereas others live for decades after diagnosis without need for a therapy [2]. To address this heterogeneity and predict the prognosis of patients, several prognostic markers based on genetic phenotypic or molecular characteristics of CLL B cells have been discovered [3].

Prognostic markers such as expression of specific proteins in or on CLL cells (ie, CD38, Zap70 or CD 49d), cytogenetic abnormalities (del 13q, del 11q, del 17p and trisomy 12) quantified by fluorescent in situ hybridization (FISH) and immunoglobulin heavy chain (IGHV) gene mutation have all been very useful [2].

Despite this progress, many patients have limited access to these laboratory procedures, which require highly sophisticated instruments and a high degree of technical expertise and are costly to perform. Therefore, less expensive prognostic markers are needed. The percentage of smudge cells on routine blood smears has recently been reported as a prognostic test available to patients with CLL especially those in developing countries. It is simple, accessible and inexpensive [4].

Smudge cells are ruptured CLL B cells seen on routine blood smears of virtually all CLL patients. For nearly a century, smudge cells were thought to be an artifact of slide preparation. Many investigators recently discovered that smudge formation is related to the content of the cytoskeletal protein vimentin present in leukemic cells [5].

Smudge cell formation has been demonstrated to be linked to a reduced expression of vimentin in CLL lymphocytes. High vimentin expression (low percentage of smudge cells) has been shown to be associated with poor prognosis and a shortened time to first treatment [6].

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2. Aim of the study

To highlight the role of smudge cells percentage as a novel prognostic marker in CLL, in addition to investigate the impact of smudge cells percentage on the clinicolaboratory data of CLL patients and to evaluate the relationship between it and other prognostic factors in CLL.

3. Subjects and methods

This prospective study was carried out on 90 newly diagnosed adults with CLL recruited from hematology and Oncology unit at Ain Shams University hospital. Their ages ranged from 33 to 85 years (Mean 58. 83 ± 11. 79 years). 51 were males and 39 were females with a male to female ratio of 1. 2: 1. An informed consent was obtained from each patient before participation in the study. The procedures applied in this study were approved by the Ethical Committee of Human Experimentation of Ain Shams University, and are in accordance with the Helsinki Declaration of 1975.

Patients were diagnosed on the basis of: i) complete history taking and through clinical examination; ii) laboratory investigations including: complete blood count (CBC) using LH 750 (Bechman Coulter), examination of Leishman stained peripheral blood (PB) films laying stress on the percentage of Smudge cells (ratio of smudged to intact cells plus smudged lymphocytes) according to *Johnston* et al. [7] who considered 30% of Smudged cells as a cut off level to differentiate between low and high risk group. The same cut off value was used in two studies by *Nowakowski* et al. [5,8]. In addition to bone marrow (BM) examination, flowcytometric immunophenotyping was performed using EPICS XL Coulter flowcytometer. FISH analysis using locus-specific identifier (LSI) probes for detection of ataxia telangiectasia mutated (ATM) and protein 53(P53) genes deletions. Centromeric enumeration probe (CEP) for trisomy 12 detection was also used. Two age-matched healthy volunteers were used as controls; to check the intensity of signals of the used probes.

Staging of the patients was done according to the Rai staging system [9]

3.1. Sample collection

PB and BM samples were collected on ethylene diaminetetraacetic acid (EDTA) (1. 2 mg/ml) for morphological and immunophenotyping. BM aspirates were collected in sterile preservative-free lithium heparin coated vacutainer tubes for cytogenetic analysis.

3.2. FISH technique

The FISH analysis was performed on BM aspirates using LSI for detection of ATM and P53genes deletions as well as CEP for trisomy 12. At least 100 interphase nuclei and/or 20 metaphases were scanned under fluorescence microscope for the detection of ATM, P53 deletions and trisomy 12. In monoallelic ATM deletion, one red signal was reported in >10% of examined cells and biallelic deletion was reported by absence of red signals in at least 10% of examined interphase cells. P53 deletion was reported if 1 red signal was noticed in >10% of cells. Trisomy 12 was reported if 3 red signals were observed in at least >10% of interphase cells and/or >2% of metaphase.

3.3. Statistical analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS™) version 20. Qualitative data were presented as numbers and percentages while quantitative data were entered into Kolmogorov-Smirnov test of normality and parametric distribution data were presented as mean, standard deviations and ranges while non parametric distribution data were presented as median with interquartile range (IQR). In order to compare parametric quantitative variables between two groups, Student *t*-test was applied. For comparison of non-parametric quantitative variables between two groups, Mann-Whitney test was used. The comparison between two groups with qualitative data was done using Chi-square test. Logistic regression analysis was employed to determine variables affecting low smear cell percentage.

4. Results

Descriptive and laboratory data of the studied patients are shown in Table 1. The prognostic markers described in this study to predict the disease progression and to assess the tumor burden are listed in Table 2. The impact of the studied cytogenetic abnormalities on demographic, clinical and laboratory data of the patients was shown in Tables 3.The relation between high risk cytogenetic abnormalities and other studied prognostic factors are demonstrated in Table 4.

Table 1
Demographic, clinical and laboratory data of all the studied patients.

Parameter	Patient (n = 90)
Age (years), Mean ± SD Range	58. 83 ± 11. 79 33–85
Sex, n (%) Female Male	39 (43. 3%) 51 (56. 7%)
Lymphadenopathy, n(%)	36 (40. 0%)
Splenomegaly, n(%)	54 (60. 0%)
Hepatomegaly, n (%)	51 (56. 7%)
Staging, n(%) Low grade(I,II) High grade(III,IV)	21 (23. 3%) 69 (76. 7%)
TLC(x10^9/L), Median (IQR) Range	36. 3 (22. 5–105) 11. 2–270
Hemoglobin (g/dL), Mean ± SD Range	10. 76 ± 2. 82 5. 6–18. 5
Platelets (×10^9/L), Mean ± SD Range	188. 27 ± 92. 81 48–382
PB Lymphocytes (×10^9/L), Median (IQR) Range	31. 9 (19. 5–96. 6) 9. 5–231. 1
Atypical lymphocytes (%), Median (IQR) Range	9 (7–10) 5–36
Prolymphocytes (%), Mean ± SD Range	3. 03 ± 1. 44 1–6
BM Lymphocytes (%), Median (IQR) Range	66 (35–75) 16–96
Immunophenotyping score, n(%) Atypical Typical	15 (16. 7%) 75 (83. 3%)

TLC: total leucocytic count; PB: peripheral blood; BM: Bone marrow, IQR: interquartile range, SD: standard deviation.

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