# Peripheral Blood Smudge Cells Percentage in De Novo CLL: A Comparison with Other Established Laboratory Prognostic Markers

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**Abstract:** Chronic lymphocytic leukemia (CLL) is the most common form of leukemia in the Western world. The timing as to when a patient will require treatment is highly unpredictable. Thus, there has been great interest in identifying prognostic markers that can be used to distinguish patients who may have an aggressive form of CLL and might benefit from early intervention. Recently developed molecular markers are costly and often require a high level of technological expertise. Recent data give evidence for the prognostic relevance of peripheral blood smudge cells percentage in CLL. In our study, we investigated the prognostic potential of smudge cell percentage in 180 de novo CLL patients referring to the National Cancer Institute, Cairo University, Egypt and correlated the smudge cell percentage with established prognostic markers; including age, sex, ZAP 70 and CD 38 expression, pattern of marrow infiltration, Beta-2 microglobulin, Lactate dehydrogenase and lymphocyte doubling time. Our results showed that smudge cells percentage correlated inversely with markers of bad prognosis and correlated positively with hemoglobin and lymphocyte doubling time, which confer a better prognosis. We concluded that peripheral blood smudge cells percentage could be used as a simple, inexpensive and independent prognostic marker that can predict the outcome and survival in de novo CLL patients.
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**Key words:** Smudge cells, Peripheral blood, CLL

## 1. Introduction

B-cell chronic lymphocytic leukemia (B-CLL) is the most common type of adult leukemia in Western countries. Each year, 15,000 to 19,000 individuals are diagnosed with this disease in the United States [1]. It shows a remarkable heterogeneity, with some patients having an almost normal lifespan, while others having only several years of survival in spite of intensive chemotherapy [2]. With the widespread use of automated blood cell counters and flow cytometric immunophenotyping, up to 80% of patients with CLL are diagnosed at an early stage of the disease [3]. However, approximately 50% of patients with early stage disease have accelerated disease progression, while many of the rest survive for more than a decade without even requiring therapy [4]. The prognosis of CLL patients had been accordingly linked to many factors, the most important of which are the immunoglobulin heavy chain gene mutation status, leukemic cell expression of CD38 and ZAP 70 associated protein, chromosome analysis by fluorescence in situ hybridization (FISH) and others, which identify patients with biologically aggressive disease and shorter survival time[5]. Unfortunately, most of such recently developed prognostic tests are costly and often require a high level of technologic expertise[6] and despite this progress, many patients have limited access to these laboratory tests, which require highly sophisticated instruments and a high degree of technical expertise and are costly to perform. In addition, because of the technical complexity of some of the assays, a considerable effort is necessary to ensure reproducibility between the laboratories [7-9].

Smudge cells are ragged lymphoid cells found mainly in peripheral blood smears of CLL patients and which are ruptured during smear preparation of virtually all CLL patients. For nearly a century, smudge cells were thought to be merely an artifact of slide preparation [10]. The interpatient variability in the percentage of smudge cells on a peripheral blood smear is well recognized and is independent of the absolute lymphocyte count and also of the staining technique [11].

It has been discovered that smudge formation is related to the content of the cytoskeletal protein vimentin present in leukemic cells [12]. It was shown that CLL patients with high vimentin content have a low percentage of smudge cells. In addition, it was found that high vimentin expression is associated with a

shortened time to initial therapy in early-stage CLL [5]. Because vimentin expression was found to be a prognostic factor in early-stage CLL, we hypothesize that there could accordingly be an association between smudge cell percentage and prognosis in patients with CLL.

**Aim of work:**

This study was performed to investigate the possible prognostic implication of the percentage of peripheral blood smudge cells, as an easy and cheap technique in de novo CLL patients, through comparing it with other established laboratory prognostic tests.

## 2. Patients and Methods:

Our study included 180 CLL patients referring from the Medical Oncology Department to the Clinical Pathology Department, Egyptian NCI, during the period from May 2007 to Dec 2009. These un-selected patients were newly diagnosed after fulfilling the diagnostic criteria of CLL according to the National Cancer Institute-sponsored Working Group [NCI-WG] guidelines [13]. At diagnosis, these patients were subjected to complete blood count (CBC), bone marrow aspiration/biopsy (BMA/BMB), flowcytometric immunophenotyping (IPT), serum beta-2 microglobulin (ß2M), serum lactate dehydrogenase enzyme (LDH) and smudge cells percentage in peripheral blood smears. Lymphocyte doubling time (LDT) was concluded after two check points evaluating the absolute lymphocytic count; the 1st when patients achieved maximum response of treatment (CR-PR-SD) and the 2nd after 12 months. Correlations between smudge cells percentage and all of the above-mentioned laboratory prognostic tests were explored.

Hemograms were done on Cell-Dyn-3700 automated cell counter. Absolute lymphocytic counts (ALCs) were revised and calculated from peripheral blood smears. Leishman-stained BMA smears were examined for percentages of mature (± immature) lymphocytes. Bone marrow biopsy (BMB) cores were fixed, decalcified, processed, embedded, sectioned and H&E-stained according to the well known routine techniques [14]. BMB sections were histologically examined for lymphoid infiltration patterns.

CLL diagnosis was confirmed by immunophenotypic analysis performed on Partec-III flowcytometer using a panel of McAbs [Dako, Denmark and Santa Cruz Biotechnology, USA] including CD3, CD4, CD20, FMC7, HLA-DR, and Kappa light chain conjugated with fluorescein isothiocyanate (FITC); CD5, CD23, CD10, CD22, CD79b, CD8 and Lambda light chain conjugated with phycoerythrin (PE) and CD19 conjugated with phycoerythrin-Cyanine 5(PE-Cya5). For all of these markers, results were expressed as a percentage of cells showing positive surface expression (when the marker was identified in ≥20%). As prognostic markers, the cytoplasmic expression of ZAP-70 and the surface expression of CD38 were further determined in (CD5/CD19) positive B-CLL cells by using anti-ZAP-70-FITC and anti-CD38-PE McAbs [BD Bioscience, Mountain view, California]. ZAP-70 expression was considered as positive when identified in ≥20% of the gated (CD5/CD19) positive B-cells [15], while CD38 expression was considered as positive when identified in ≥30% of the gated (CD5/CD19) positive B-cells [16]. Serum ß2M was estimated on the fully automated Axyum, Abbott, USA, by micro-ELISA technique. ß2M ≤ 3.4µg/ml was considered normal and ß2M > 3.4µg/ml was considered elevated. LDH was estimated on Synchron CX-9-PRO, Beckman Coulter, Inc., USA, by the spectrophotometric technique. LDH ≤ 480 U/L was considered normal and LDH >480 U/L was considered elevated.

**Assessment of Pb smudge cells percentage:**

From an EDTA blood samples, smears were freshly prepared by the manual wedge method using a clean glass-slide with frosted edges. For each case, two stained peripheral blood smears were examined simultaneously in a blinded manner by two hematopathologists and the mean of their readings was obtained. Smudge cells were identified a broken cells with disrupted nuclear membrane and without intact cytoplasm; accordingly, a total of 300 intact lymphoid cells and smudge/basket cells were counted on each smear. Then, the percentage of smudge cells was calculated through dividing the smudge/basket cells count by the sum of intact lymphoid cells and smudge/ basket cells counts times 100.

**Statistical methods:**

Data was analyzed using SPSSwin statistical package version 17 (SPSS inc., Chicago, IL). Chi-square test was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using Mann-Whitney test (non parametric t-test).

Comparison between 3 groups was done using Kruskal-Wallis test (non-parametric ANOVA). Spearman-rho method was used to test correlation between numerical variables. P-value<0.05 was considered significant.

**3. Results:**

Our study was carried out on 180 patients with de novo CLL, 126 males (70%) and 54 females (30%). Their age ranged from 35 to 79 years with a median of 57 years and a mean of 57.9±8.3 years. Table (1) describes the data of the numerical laboratory parameters of the 180 patients included in the study. It shows that the peripheral blood smudge cells percentage was ranging from 3 to 75 with a median of 27 and a mean of 30.1±19.7

**Table (1):** Descriptive data for the numerical laboratory parameters of the 180 patients included in this study

| Parameter | Patients number = 180 | | | | |
|---|---|---|---|---|---|
| | Median | Minimum | Maximum | Mean | ±SD |
| Hb (gm/dl) | 10 | 5.1 | 15.3 | 9.8 | 2.5 |
| TLC (x10³/Cmm) | 80.8 | 6.6 | 514 | 105 | 89.1 |
| Pb (L) % | 83 | 38 | 99 | 80.1 | 13.8 |
| Pb (IL) % | 0 | 0 | 12 | 1.2 | 2.9 |
| Pb ALC (x10³/Cmm) | 66.23 | 5.02 | 493.44 | 90.08 | 82.55 |
| BMA (L) % | 79 | 12 | 97 | 75.1 | 15.8 |
| BMA (IL) % | 0 | 0 | 9 | 1 | 2.1 |
| Serum B2M (mg/L) | 3.7 | 1.6 | 7.3 | 4 | 1.5 |
| Serum LDH (U/L) | 572 | 282 | 1538 | 632 | 261 |
| Pb SCs % | 27 | 3 | 75 | 30.1 | 19.7 |

**Hb=** hemoglobin**, TLC=** total leucocytic count**, L=** lymphocyte, **IL=** immature lymphocyte**, ALC=** absolute lymphocytic count, **BMA=** bone marrow aspiration, **ß2M=** beta 2 microglobulin, **LDH=** lactate dehydrogenase, **Pb=** peripheral blood, **SCs=** smudge cells

Figure (1) shows a peripheral blood smear with high smudge cells percentage (70%) and figure (2) shows another smear with low smudge cells percentage (4%) in 2 de novo CLL patients.

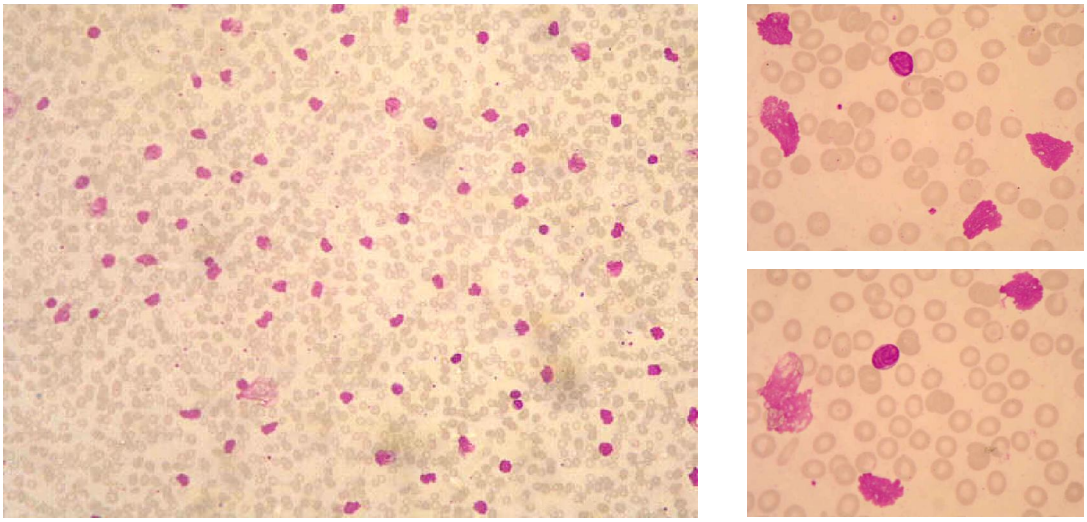

**Fig. (1):** Peripheral blood smear shows high smudge cells percentage (70%) in de novo CLL patient, Leishman's stain, x20, x100 and x100, respectively.

Table (2) shows the results of categorical laboratory parameters obtained in this study. They included immunophenotyping for common prognostic markers [namely ZAP 70 and CD38], LDT and BM biopsy infiltration pattern. On the flowcytometer, 83 patients (46.1%) showed positive ZAP 70 expression, 58 patients (32.2%) showed positive CD38 expression and 33 patients (18.3%) showed ZAP70/CD38 co-expression. Twenty five patients (13.9%) were ZAP 70 negative and CD38 positive, 50 patients (27.8%) ZAP 70+ CD38- and 72

patients (40%) were both ZAP70 and CD38 negative. On BM histological examination, 95 patients (52.8%) showed diffuse pattern of lymphoid infiltration, 11 patients (6.1%) showed nodular pattern and 74 patients (41.1%) showed mixed pattern of lymphoid infiltration. Out of the 180 patients, 61 were missed and could not be followed for more than 12 month, while, 119 patients could be followed for estimating the lymphocyte doubling time (LDT). Thirty nine patients (32.8%) had LDT less than 12 months and 80 ones (67.2%) had LDT more than 12 months.

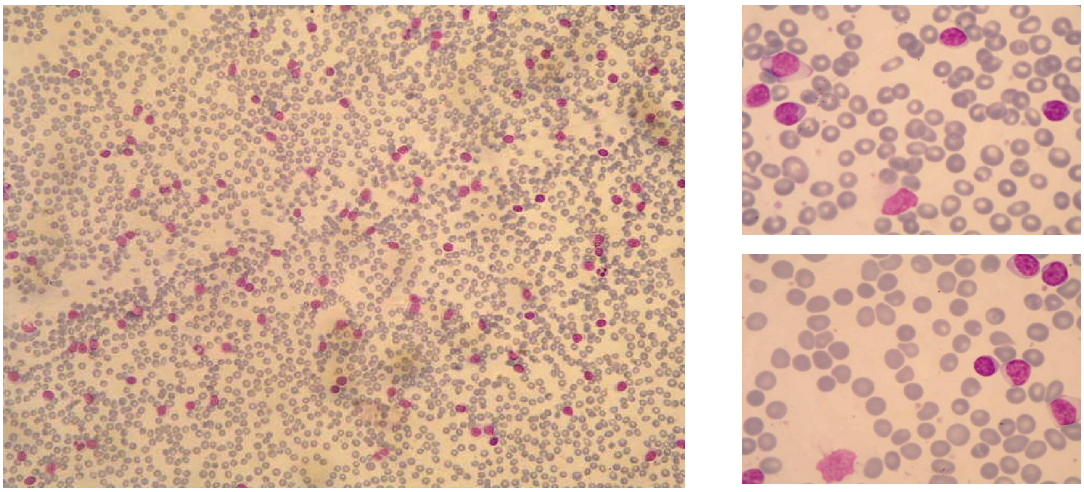

**Fig. (2):** Peripheral blood smear shows low smudge cells percentage (4%) in de novo CLL patient, Leishman's stain, x20, x100 and x100, respectively.

**Table (2):** Results of categorical laboratory parameters of the 180 patients including: (a) IPT for prognostic markers (b) BM biopsy pattern and (c) LDT.

| Parameter | | Patients number = 180 | |
|---|---|---|---|
| | | Positive | Negative |
| (a) | CD38 expression | 58 (32.2 %) | 122 (67.8%) |
| | ZAP-70 expression | 83 (46.1%) | 97 (53.9%) |
| | CD38/ ZAP-70 co-expression | 33 (18.3%) | 147 (81.7%) |

| | | | | |
|---|---|---|---|---|
| (b) | BM biopsy infiltration pattern | Diffuse | Nodular | Mixed |
| | | 95 (52.8%) | 11(6.1%) | 74(41.1%) |

| | | Patients number (valid) = 119* | |
|---|---|---|---|
| (c) | Lymphocyte Doubling Time | < 12 month | ≥ 12 month |
| | | 39 (32.8%) | 80 (67.2%) |

**IPT**= immunophenotyping, **BM**= bone marrow and **LDT**= lymphocyte doubling time
*Out of total 180 patients, 119 were valid to be followed and 61 were missed and could not be followed

Peripheral blood smudge cell % was lower with high ZAP 70 expression, high CD38 expression, high ZAP70/CD38 coexpression and diffuse pattern of marrow infiltration (P<0.001 for each).

Peripheral blood smudge cell % was negatively correlated with Beta-2 microglobulin level (P=0.029), LDH level (P=0.002), bone marrow lymphocyte % (P<0.001) and bone marrow immature lymphocytes % (P=0.008). It was also negatively correlated with TLC (P=0.035) and peripheral blood absolute lymphocytic count (P=0.039).

Peripheral blood smudge cells % was positively correlated with Hb (P<0.001) and LDT (P<0.001). There was no correlation with peripheral blood lymphocyte % (P=0.287) peripheral blood immature lymphocyte % (p=0.061), age (P=0.82) or sex (p=0.566). Table (3) shows different correlations between peripheral blood smudge cells percentage and numerical laboratory parameters included in this study.

Table (3): Correlations between the peripheral blood SCs percentage and other numerical laboratory parameters

| | | Peripheral blood | | | | | BMA | | Serum | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Hb | TLC | L % | IL % | ALC | L % | IL % | ß2M | LDH |
| **Pb SCs %** | r-value | 0.406 | - 0.158 | - 0.08 | - 0.140 | - 0.154 | - 0.288 | - 0.198 | - 0.374 | - 0.322 |
| | *p*-value | **0.001** | **0.035** | 0.287 | 0.061 | **0.039** | **0.001** | **0.008** | **0.001** | **0.001** |

**Pb=** peripheral blood, **SCs=** smudge cells**, Hb=** hemoglobin**, TLC=** total leucocytic count**, L=** lymphocyte, **IL=** immature lymphocyte**, ALC=** absolute lymphocytic count, **BMA=** bone marrow aspiration, **ß2M=** beta 2 microglobulin, **LDH=** lactate dehydrogenase

## 4. Discussion:

The appearance of smudge cells on a peripheral-blood smear is a characteristic feature of CLL, with virtually all patients demonstrating at least some degree of smudging [5]. Since their description in 1896 by **Gumprecht** on blood smears of patients with lymphocytic leukemia, smudge cells which are also known as Gumprecht or basket cells, were thought to be just an artifact of slide preparation resulting from the fragility of CLL cells [17]. In 1959 **Heinivaara** made two important observations, first that the percentage of smudge cells was not dependent simply on the degree of lymphocytosis or the slide stain method and second that smudging appeared to be patient specific [5].

Studies demonstrated that smudge cells formation is inversely correlated with CLL B cell content of vimentin, a cytoskeletal protein critical for rigidity and integrity of lymphocytes [17]. High vimentin expression has been shown to be associated with poor prognosis and metastatic potential in breast [18] and colon cancer [19].

In the present study, we hypothesized that the calculated smudge cells percentage on a blood smear would have prognostic value in CLL, based on the studies that proved that the percentage of smudge cells inversely correlates with vimentin expression, which by its turn was proven to confer bad prognosis [17].

While clinical staging systems have been used to stratify patients into risk categories, they lack the ability to predict disease progression or response to therapy. Recent advances in the understanding of the biology of CLL have led to the identification of numerous cellular and molecular markers with potential prognostic and therapeutic significance. We correlated the percentage of peripheral blood smudge cells with such established markers of prognosis. Several studies demonstrated that age and gender [20], ZAP 70 [21,22], CD38 [23,24], Beta-2 microglobulin [25], serum lactate dehydrogenase [26], lymphocyte doubling time [27] and bone marrow infiltration pattern [22] are important prognostic factors in CLL.

Our results showed that lower smudge cells percentage was correlated with markers of bad prognosis, such as ZAP 70 expression (P<0.001), CD38 expression (P<0.001) and ZAP70/CD38 coexpression (P<0.001). We agree in this respect with the results reported by **Nowakowski *et al.,*** [17] and **Johansson *et al.,*** [6]. It was also correlated with high B-2 microglobulin level (P=0.029), high LDH level (P=0.002), diffuse pattern of marrow infiltration (P<0.001), high TLC (P=0.035), high peripheral blood absolute lymphocytic count (P=0.039), high bone marrow lymphocyte % (P<0.001) and high bone marrow immature lymphocyte % (P=0.008).

On the other hand, higher peripheral blood smudge cells percentage was correlated with higher Hb (P<0.001) and lymphocyte doubling time of more than 12 months (P<0.001), which confer a better prognosis in CLL patients.

To the best of our knowledge, no other studies correlated peripheral blood smudge cells percentage with Beta-2 microglobulin, LDH level, pattern of marrow infiltration, absolute lymphocytic count, immature lymphocyte % or lymphocyte doubling time.

Further investigation to define a precise cut off for the peripheral blood smudge cells percentage might be a helpful parameter to discriminate the lower bad prognostic values from the higher good ones, which might have different impacts on the clinical outcome.

We conclude that peripheral blood smudge cells percentage, estimated by microscopic examination of routine blood smears, could be used as a simple, inexpensive and independent prognostic marker that can predict the outcome and survival in de novo CLL patients.

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**References:**

1. Zent CS, Kyasa MJ; Evans R, *et al.* (2001). Chronic lymphocytic leukemia incidence is substantially higher than estimated from tumor registry data. Cancer. 92:1325–1330.
2. Xu W, Li J, Wu y, *et al.* (2009). CD38 as a prognostic factor in Chinese patients with chronic lymphocytic leukemia. Leuk Res.; 33:237-43.
3. Call T, Phyliky R, Noel P *et al.* (1994). Incidence of chronic lymphocytic leukemia in Olmsted County, Minnesota, 1935 through 1989, with emphasis on changes in initial stage at diagnosis. Mayo Clin Proc.; 69: 323-328.
4. Chiorazzi N, Rai K and Ferrarini M(2005). Chronic lymphocytic leukemia. N Engl J Med.; 352: 804-815.
5. Nowakowski GS, Hoyer JD, Shanafelt TD, *et al.* (2007). Using smudge cells on routine blood smears to predict clinical outcome in chronic lymphocytic leukemia: A universally available prognostic test. Mayo Clin Proc.; 82:449–453.
6. Johansson P, Eisele L, Klein-Hitpass L, *et al.* (2010). Percentage of smudge cells determined on routine blood smears is a novel prognostic factor in chronic lymphocytic leukemia. Leuk Res.; 7: 892-898.
7. Letestu R, Rawstron A, Ghia P, *et al.* (2006). Evaluation of ZAP-70 expression by flow cytometry in chronic lymphocytic leukemia: A multicentric international harmonization process. Cytometry B Clin Cytom.; 70:309–314.
8. Peková S, Baran-Marszak F, Schwarz J, *et al.* (2006). Mutated or non-mutated? Which database to choose when determining the IgVH hypermutation status in chronic lymphocytic leukemia? Haematologica.; 91: e11-e12.
9. Wilhelm C, Neubauer A and Brendel C. (2006). Discordant results of flow cytometric ZAP-70 expression status in B-CLL samples if different gating strategies are applied. Cytometry B Clin Cytom.; 70:242–250.
10. Macdonald D, Richardson H and Raby A(2003). Practice guidelines on the reporting of smudge cells in the white blood cell differential count. Arch Pathol Lab Med.; 127:105.
11. Heinivaara O.(1959). Smudge cells in lymphocytic leukemia. Ann Med Intern Fenn.; 48: 69-75.
12. Nowakowski GS, Lee Y, Bone ND, *et al.* (2005). Proteomic analysis of chronic lymphocytic leukemia cells identifies vimentin as a novel prognostic factor for aggressive disease. Blood.; 106:707.
13. Cheson BD, Bennett JM, Grever M(1996). National Cancer Institute-sponsored working group guidelines for chronic lymphocytic leukemia: Revised guidelines for diagnosis and treatment. Blood.; 87: 4990-4997.
14. Bain BJ, Clark DM, Lampert IA, Wilkins BS(2001). Technical methods applicable to trephine biopsy specimens. Appendix. Bone Marrow Pathology. 3rd edition, London, Blackwell Science Ltd.; pp. 474-482.
15. Durig J, Nuckel H, Cremer M, Fuhrer A, Halfmeyer K, Fandrey J. ZAP-70 expression is a prognostic factor in chronic CLL. Leukemia. 2003; 17: 2426-2434.
16. Hamblin TJ, Orchard JA, Ibbotson RE, *et al.* (2002). CD38 expression and immunoglobulin variable region mutations are independent prognostic variables in CLL, but CD38 expression may vary during the course of the disease. Blood.; 99: 1023-1029.
17. Nowakowski GS, Hoyer JD, Shanafelt TD, *et al.* (2009). Percentage of Smudge Cells on Routine Blood Smear Predicts Survival in Chronic Lymphocytic Leukemia. J Clin Oncol.; 27: 1844–1849.
18. Domagala W, Lasota J, Dukowicz A, *et al.* (1990). Vimentin expression appears to be associated with poor prognosis in node-negative ductal NOS breast carcinomas. Am J Pathol.; 137: 1299-1304.
19. Ngan CY, Yamamoto H, Seshimo I, *et al.* (2007). Quantitative evaluation of vimentin expression in tumour stroma of colorectal cancer. Br J Cancer.; 96: 986-992.
20. Catovsky D, Richards S, *et al.* (2007). Assessment of fludarabine plus cyclophosphamide for patients with chronic lymphocytic leukaemia (the LRF CLL4 Trial): a randomised controlled trial. *Lancet*.; 370: 230-23.
21. Crespo M, Bosch F, Villamor N, *et al.* (2003). ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. *N Engl J Med*.; 348: 1764-1775.
22. Schade U., Vornhusen S., Jager A., *et al.* (2006). Bone marrow infiltration pattern in B-cell chronic lymphocytic leukemia is related to immunoglobulin heavy chain variable region mutation status and expression of 70-kd z associated protein (ZAP-70). Hum Path.; 37: 1153-1161.
23. Jelinek DF, Tschumper RC, Geyer SM, *et al.* (2001). Analysis of clonal B-cell CD38 and immunoglobulin variable region sequence status in relation to clinical outcome for B-chronic lymphocytic leukaemia. *Br J. Haematol*.; 115: 854-861.
24. Parker T. and Strout M *(2011)*. Chronic lymphocytic leukemia: Prognostic factors and impact on treatment. *Discov Med.;* **11: 115-123.**
25. Gentile M, Cutrona G, Neri A, *et al.* (2009). Predictive value of beta2-microglobulin (beta2-m) levels in chronic lymphocytic leukemia since Binet A stages. *Haematologica*.; *9*: 887-888.
26. Jahrsdörfer B., Wooldridge J E. , Blackwell S E. *et al.* (2005). Good prognosis cytogenetics in B-cell chronic lymphocytic leukemia is associated *in vitro* with low susceptibility to apoptosis and enhanced immunogenicity. Leukemia., 19: 759-766.
27. Viñolas N, Reverter JC, Urbano-Ispizua A, *et al.* (1987). Lymphocyte doubling time in chronic lymphocytic leukemia: an update of its prognostic significance. Blood cells.; 12: 457-70.

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