Clinical Staging and Flowcytometric CD38 and Zap-70 Prognostic Indicators in Sudanese Patients with Chronic Lymphocytic Leukemia

Enaam Abdelrhman Abdelgader1,2, Nada Hassan Eltayeb3, Tasniem Ahmed Eltahir2, Osama Ali Altayeb4, Eman Abbass Fadul4, Soaad Fadlallah Ali5, Eldirdiri M. Abdelrhman2

1. Department of Pathology, Faculty of Medicine, Alneelain University, Sudan
2. Khartoum Oncology Hospital, Sudan
3. Department of Physiology, Faculty of Medicine, Alneelain University, Sudan
4. Flow Cytometry Laboratory for Leukemia & Lymphoma Diagnosis, Sudan
5. Department of Pathology, Faculty of Medicine, Gezira University, Sudan

Abstract

Background: The clinical course of chronic lymphocytic leukemia is highly variable. The determination of ZAP70 and CD38 is increasingly utilized as prognostic factor for chronic lymphocytic leukemia. The aim of conducting this study was to investigate the frequency of CD38 and ZAP70 expression among Sudanese Chronic lymphocytic leukemia (CLL) patients and to relate them to the Binet and Rai clinical staging systems.

Method: A total of 93 patients (mean age; 62.29 ± 11.68, sd) were enrolled in this cross-sectional study. CD38 and ZAP70 expression levels were measured with four color flowcytometry using the cut-off values of 20% for ZAP70 and 30% for CD38 expression. Staging was assessed by using clinical examination and CBC for all patients. Data were analyzed using the Statistical Package for Social science for Windows (SPSS), version 22.

Results: There were 93 CLL patients and the median age of the group was 63 years (36–95 years). About 71% of the patients presented with lymphadenopathy, 53.8% with splenomegaly, 73.1% with anemia, and 45.2% with thrombocytopenia. There was higher frequency of Binet stage C and Rai stage IV (62 [66.6%] patients and 34 [36.5%] patients, respectively). In addition, CD38 and ZAP70 showed higher frequency among Binet and Rai advance stages. ZAP70 and CD38 positivity were detected in 21 patients (22.6%) and 31 patients (33.3%), respectively. There was no statistically significant association between ZAP70 and CD38 and clinical staging systems (p > 0.05).

Conclusion: No significant association was observed between Flowcytometric (CD38 and Zap70) Prognostic Indicators and clinical staging systems.

Key words: Chronic lymphocytic Leukemia; Flowcytometry; ZAP70; CD38; clinical staging systems

Introduction

Chronic lymphocytic leukemia (CLL) follows a remarkably heterogeneous course ranging from mild disease without ever requiring treatment to aggressive and...
drug-resistant disease [1]. The Rai and Binet [2, 3] clinical staging systems are valuable in classifying CLL patients into broad prognostic subgroups. Clinical stages, however, have some limitations and this has led to a search for novel parameters with improved predictive power. Prognostic predictions in B-CLL at early clinical stage are based on biological disease parameters, such as ZAP-70 and CD38 protein levels, genomic aberrations as well as immunoglobulin variable heavy chain gene (IgVH) mutation status [4]. DNA microarray studies have shown that B-CLL cells with unmutated IgVH genes can be distinguished from those with mutated IgVH genes by the differential expression of a small number of genes, one of which encodes the 70-kDa zeta associated protein (ZAP-70) [5, 6]. ZAP-70, a member of the Syk-ZAP-70 protein tyrosine kinase family, is a key signaling molecule for T lymphocytes and natural killer cells. While ZAP-70 is not expressed in normal B lymphocytes, it is associated with increased intracellular signaling via the immunoglobulin receptor in B-CLL cells [7, 8]. Considering three recently published studies [9–11], ZAP-70 is the most promising surrogate marker for the IgVH mutation status. In contrast to the technically demanding IgVH analysis, ZAP-70 protein expression is conveniently measured by flow cytometry [9, 12, 13]. CD38 is a type II transmembrane glycoprotein that acts as a complex ecto-enzyme and receptor molecule with signaling functions in B-CLL cells [14]. Detection of CD38 cell surface expression can be conveniently performed by flowcytometry, and, together with ZAP-70 [11], CD38 analysis may prove a valuable adjunct in the diagnostic work-up of B-CLL patients. CD38 expression also has been suggested as a surrogate marker for the two important IgVH mutated and unmutated subgroups of B-CLL [15]. At present, both ZAP-70 and CD38 are regarded as independent prognostic variables in B-CLL [9, 16]. The main objective of this study is to determine the frequency of CD38 and ZAP-70 expression and the relationship between clinical staging system and flowcytomtery prognostic markers (CD38 and Zap-70) in Sudanese patients with CLL.

**Material and Methods**

**Patients**

This observational cross-sectional (hospital based) study was conducted in Khartoum Oncology Hospital during the period from September 2016 to February 2017. This study was approved by the ethical committee, Sudan Medical Specialty Board (SMSB). Informed consent was obtained from all participants in accordance to the requirements and guidelines of the ethical committee. A total of 93 untreated Sudanese patients with CLL were included in this study. The patients were diagnosed according to the International
CLL Workshop Criteria [18] and the Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia [19]. Demographic and clinical data were collected from the study population using a well-structured questionnaire. 5 ml of venous blood were collected from each patient in ethylene diamine tetra acetic acid (EDTA) vacuum tubes under adequate precautions and according to the standard protocol. Then the samples were transported to the Flow Cytometry Laboratory for Leukemia & Lymphoma Diagnosis (Khartoum2, Sudan), preserved at room temperature (22–24ºC) and processed within 6–24 h of collection.

**Methods**

**Routine Investigations**

Physical examination was done to evaluate bidimensional diameters of the largest palpable lymph nodes in each of the following sites: cervical, axillaries, supraclavicular, inguinal, and femoral. The size of the liver and spleen, were assessed by using Chest radiography and abdominal ultrasound. CBC was carried out using automated cell counter (Sysmex XE-2100™). Physical examination and complete blood count were used for assigning both Rai and Binet clinical staging systems.

**Flowcytometry**

Four color flowcytometer (COULTER EPICS XL-MCLTM Flowcytometer - Miami, Florida - USA) with SYSTEM II software was used to determine the immunophenotyping, surface CD38 expression and intracellular ZAP-70 expression for the study population. The instrument set up was checked daily using QC check beads Flowcytometry (Beckman Coulter, USA). Immunophenotyping of lymphocytes from lysed whole peripheral blood was carried out to confirm the diagnosis of CLL using the following monoclonal antibodies (Beckman Coulter, USA); CD45, CD5, CD19, CD20, CD22, CD23, kappa and lambda light chains, FMC7, CD79b. A marker was considered positive at a cutoff level of 20%. The Matutes scoring system allocates one point each for the expression of weak SmIg, CD5, CD23, and absent or low expression of CD79b and FMC7 [20, 21].

**Surface CD38 expression**

Surface CD38 was analyzed in peripheral blood using IO Test CD38-PC5 Kit (Beckman Coulter, USA) according to the manufacturer protocol. CD38 is measured by flowcytometry and plotted against B-cell marker CD19 expression. The CD38 expression was considered positive at cutoff level of 30%.
Intracellular ZAP-70 expression

Intracellular ZAP70 expression in PB samples was measured using PerFix-nc a fixation and permeabilization kit and IO Test ZAP-70-PE (PN B57658) Kit (Beckman Coulter, USA) according to the manufacturer protocol. The ZAP-70 expression was considered positive at a cutoff level of 20%.

Data Management

Data were sorted, categorized, coded and summarized on master sheet. The collected data were analyzed using the software program of the Statistical Package for Social science for Windows (SPSS), version 22. Frequencies were determined using descriptive statistic. Chi square test was used to investigate the relationship between age, gender, clinical stages, CD38 and Zap70 at $p = 0.05$.

Results

Demographic Data

The 93 patients enrolled in this study include 63 (67.7%) male and 30 (32.3%) female with a median age of 63 (36-95 years). At the time of diagnosis, mean of patient ages was 62.29 ± 11.68, sd. Most of the patients 55 (59.1%) were in the age group 56 – 75. (Table 1).

Lymph Nodes and Organomegaly

The majority of patients presented with lymphadenopathy in 66 patients (70.9%). On the other hand clinical examination showed presence of splenomegaly in 50 patients (53.8%), hepatomegaly in 23 patients (24.7%) and hepatosplenomegaly in 22 patients (23.7%) (Table 1).

CBC

CBC results showed white blood cell count more than $50 \times 10^9$ in 62 (66.7%) patients, anaemia in 68 (73.1%) patients and thrombocytopenia in 42 (45.2%) patients, (Table 1).
TABLE 1: Characteristics of the Study Population.

| Parameter                        | Frequency no (%) | Parameter                        | Frequency no (%) |
|----------------------------------|------------------|----------------------------------|------------------|
| Leukocyte count (x 10^3/µl)      |                  | Leukocyte count (x 10^3/µl)      |                  |
| ≤50                              |                  | >50                              |                  |
| <50                              |                  | >50                              |                  |
| Scoring system                   |                  | Scoring system                   |                  |
| Score 3                          |                  | Score 3                          |                  |
| Score 3.5                        |                  | Score 3.5                        |                  |
| Score 4                          |                  | Score 4                          |                  |
| Score 4.5                        |                  | Score 4.5                        |                  |
| Score 5                          |                  | Score 5                          |                  |
| Rai staging system               |                  | Rai staging system               |                  |
| Stage 0                          |                  | Stage 0                          |                  |
| Stage I                          |                  | Stage I                          |                  |
| Stage II                         |                  | Stage II                         |                  |
| Stage III                        |                  | Stage III                        |                  |
| Stage IV                         |                  | Stage IV                         |                  |
| Binet staging system             |                  | Binet staging system             |                  |
| Stage A                          |                  | Stage A                          |                  |
| Stage B                          |                  | Stage B                          |                  |
| Stage C                          |                  | Stage C                          |                  |
| CD38 and ZAP-70 Expression       |                  | CD38 and ZAP-70 Expression       |                  |
| CD38 +                           |                  | CD38 +                           |                  |
| CD38 −                           |                  | CD38 −                           |                  |
| ZAP-70 +                         |                  | ZAP-70 +                         |                  |
| ZAP-70 −                         |                  | ZAP-70 −                         |                  |
| CD38 + / ZAP-70 +                |                  | CD38 + / ZAP-70 +                |                  |
| CD38 − / ZAP-70 −                |                  | CD38 − / ZAP-70 −                |                  |
| CD38 + / ZAP-70 −                |                  | CD38 + / ZAP-70 −                |                  |
| CD38 − / ZAP-70 +                |                  | CD38 − / ZAP-70 +                |                  |
| CD38 + / ZAP-70 −                |                  | CD38 + / ZAP-70 −                |                  |
| CD38 − / ZAP-70 +                |                  | CD38 − / ZAP-70 +                |                  |
| Splenomegaly                     |                  | Splenomegaly                     |                  |
| Present                          | 50 (53.8%)       | Absent                           | 43 (46.2%)       |
| Absent                           |                  |                                 |                  |
| Hepatomegaly                     |                  | Hepatomegaly                     |                  |
| Present                          | 23 (24.7%)       | Absent                           | 70 (75.3%)       |
| Absent                           |                  |                                 |                  |
| Haemoglobin (g/dl)*              |                  | CD38 and ZAP-70 Expression       |                  |
| Severe                           | 13 (14.0)        | CD38 +                           | 31 (33.3%)       |
| Moderate                         | 31 (33.3%)       | CD38 −                           | 62 (66.7%)       |
| Mild                             | 24 (25.8)        | ZAP-70 +                         | 21 (22.6%)       |
| Non anaemia                      | 25 (26.9)        | ZAP-70 −                         | 72 (77.4%)       |
| Platelets count (×10^3/µl)       |                  | CD38 + / ZAP-70 +                | 10 (10.8%)       |
| <150                             | 42 (45.2)        | CD38 − / ZAP-70 −                | 51 (54.8%)       |
| 150–450                          | 50 (53.7)        | CD38 + / ZAP-70 −                | 21 (22.6%)       |
| >450                             | 1 (1.1)          | CD38 − / ZAP-70 +                | 11 (11.8%)       |

**Staging systems**

The study results showed that, there was higher frequency of Binet stage C and Rai stage IV (62 (66.6%) patients, 34 (36.5%) patients respectively) followed by Bient stage B and Rai stage III (21 (22.6%) patients, 29 (31.2%) patients respectively), Binet stage A and Rai stage II 10 (10.8%) patients, 18 (19.4%) patients respectively) and Rai stage 1 and Rai stage 0 (8 (8.6%) patients, 8 (8.6%) patients respectively), (Table 1).

**CD38 expression**

The B-CLL cells were considered CD38 positive when ≥30% expressed the membrane antigen. Based on this cutoff value, 31 patients (33.3%) were CD38 positive and 62 patients (66.7) were CD38 negative (Table 1).
ZAP-70 expression

A CLL population was considered ZAP-70-positive when at least 20% of the gated cells (CD19 positive B cells) expressed it. The leukemic cells were ZAP-70 positive in 21 patients (22.6%) and ZAP-70 negative in 72 patients (77.4), (Table 1).

Association of clinical staging systems with lymph nodes, organomegaly and CBC

Rai and Binet staging system showed highly significant association with lymphadenopathy (\(p = 0.01, 0.00\) respectively), Haemoglobin concentration (\(p = 0.00\) for both) and platelet count (\(p =0.00\) for both). splenomegaly showed highly significant association with Rai staging system (\(p =0.00\)) while no significant association was observed with Binet staging system (\(p = 0.20\)). No significant association was observed of Binet and Rai clinical staging systems with age, sex, hepatomegaly and TWBCs count (\(p > 0.05\), Table 2.

### Table 2: Association Between Clinical Staging Systems and the Study Variables.

| Variables          | Rai Staging System (%) | Binet Staging System (%) | \(p\) value | \(p\) value |
|--------------------|------------------------|--------------------------|--------------|--------------|
| **Age**            |                        |                          |              |              |
| 36–55              | 1 (1.1%)               | 2 (2.2%)                 | 5 (5.4%)     | 10 (10.7%)   | 11 (11.7%)   | 0.91 | 2 (2.2%) | 6 (6.5%) | 21 (22.5%) | 0.61          |
| 56–75              | 3 (3.2%)               | 6 (6.5%)                 | 11 (11.7%)   | 17 (18.3%)   | 18 (19.4%)   | 8 (8.6%) | 13 (14.0%) | 34 (36.5%) |              |
| 76–95              | 0 (0.0%)               | 0 (0.0%)                 | 2 (2.2%)     | 5 (5.4%)     | 0 (0.0%)     | 0 (0.0%) | 2 (2.2%) | 7 (7.5%) |              |
| 96–100             | 0 (0.0%)               | 0 (0.0%)                 | 0 (0.0%)     | 0 (0.0%)     | 0 (0.0%)     | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |              |
| **Sex (%)**        |                        |                          |              |              |
| Male               | 3 (3.2%)               | 4 (4.3%)                 | 11 (11.8%)   | 17 (18.3%)   | 28 (30.1%)   | 5 (5.4%) | 14 (15.0%) | 44 (47.3%) | 0.42          |
| Female             | 1 (1.1%)               | 4 (4.3%)                 | 7 (7.5%)     | 12 (12.9%)   | 6 (6.5%)     | 5 (5.4%) | 7 (7.5%) | 18 (19.4%) |              |
| **Lymphadenopathy**|                        |                          |              |              |
| Not found          | 4 (4.3%)               | 0 (0.0%)                 | 0 (0.0%)     | 0 (0.0%)     | 0 (0.0%)     | 6 (6.4%) | 2 (2.2%) | 19 (20.4%) | 0.00          |
| One site           | 0 (0.0%)               | 1 (1.1%)                 | 3 (3.2%)     | 0 (0.0%)     | 4 (4.3%)     | 1 (1.1%) | 0 (0.0%) | 3 (3.2%) |              |
| Two sites          | 4 (4.3%)               | 0 (0.0%)                 | 2 (2.2%)     | 2 (2.2%)     | 10 (10.7%)   | 3 (3.2%) | 2 (2.2%) | 3 (3.2%) |              |
| Three sites        | 10 (10.7%)             | 1 (1.1%)                 | 0 (0.0%)     | 2 (2.2%)     | 16 (17.2%)   | 0 (0.0%) | 2 (2.2%) | 2 (2.2%) |              |
| More than three sites | 9 (9.6%)             | 2 (2.2%)                 | 2 (2.2%)     | 0 (0.0%)     | 21 (22.5%)   | 0 (0.0%) | 15 (16.1%) | 36 (38.6%) |              |

(Continued)


Association of ZAP-70 and CD38 expression with lymph nodes, organomegaly, CBC and clinical staging systems

CD38 and ZAP70 showed higher frequency among Bient and Rai advance stages. The frequency of CD38 in Rai stage III and IV was equal (11 patients (11.8%). ZAP70 frequency in Rai stage III and IV was (8 (8.6%), 7 (7.5%), respectively), (Table 4). The results showed no significant association of Flowcytometric (CD38 and Zap-70) Prognostic Indicators with lymphadenopathy, Organomegaly, TWBCs, haemoglobin concentration, platelet count and clinical staging systems ($p > 0.05$), (Table 3).
### Table 3: Association of Flowcytometer Prognostic Indicators with the Study Variables.

| Variables          | CD38 no (%) | ZAP-70 no (%) | p value | CD38 no (%) | ZAP-70 no (%) | p value |
|--------------------|-------------|---------------|---------|-------------|---------------|---------|
|                    | CD38 +      | CD38 -        |         | ZAP-70 +    | ZAP-70 -      |         |
| **Age**            |             |               |         |             |               |         |
| 36–55              | 7 (7.5%)    | 22 (23.7%)    | 0.40    | 9 (9.7%)    | 20 (21.5%)    | 0.35    |
| 56–75              | 20 (21.5%)  | 35 (37.6%)    |         | 11 (11.8%)  | 44 (47.3%)    |         |
| 76–95              | 4 (4.3%)    | 5 (5.4%)      |         | 1 (1.1%)    | 8 (8.6%)      |         |
| **Sex**            |             |               |         |             |               |         |
| Male               | 22 (23.7%)  | 41 (44.08%)   | 0.64    | 14 (15.1%)  | 49 (52.7%)    | 0.36    |
| Female             | 9 (9.7%)    | 21 (22.6%)    |         | 7 (7.5%)    | 23 (24.7%)    |         |
| **Lymphadenopathy**|             |               |         |             |               |         |
| Not found          | 9 (9.7%)    | 18 (19.4%)    | 0.64    | 6 (6.5%)    | 21 (22.6%)    | 0.42    |
| One site           | 0 (0.0%)    | 4 (4.3%)      |         | 1 (1.1%)    | 3 (3.2%)      |         |
| Two sites          | 2 (2.2%)    | 5 (5.3%)      |         | 0 (0.0%)    | 7 (7.5%)      |         |
| Three sites        | 1 (1.1%)    | 3 (3.2%)      |         | 0 (0.0%)    | 4 (4.3%)      |         |
| More than three    | 19 (20.4%)  | 32 (34.4%)    |         | 14 (15.0%)  | 37 (39.8%)    |         |
| **Splenomegaly**   |             |               |         |             |               |         |
| Present            | 19 (20.4%)  | 31 (33.3%)    | 0.49    | 12 (12.9%)  | 38 (40.9%)    | 0.83    |
| Absent             | 12 (13.0%)  | 31 (33.3%)    |         | 9 (9.7%)    | 34 (36.5%)    |         |
| **Hepatomegaly**   |             |               |         |             |               |         |
| Present            | 10 (10.8%)  | 13 (13.9%)    | 0.40    | 5 (5.4%)    | 18 (19.4%)    | 0.85    |
| Absent             | 21 (22.6%)  | 49 (52.7%)    |         | 16 (17.2%)  | 54 (58.0%)    |         |
| **Haemoglobin concentration (g/dl)** |           |               |         |             |               |         |
| Severe             | 6 (6.5%)    | 7 (7.5%)      | 0.68    | 3 (3.2%)    | 10 (10.8%)    | 0.99    |
| Moderate           | 11 (11.8%)  | 20 (21.5%)    |         | 7 (7.5%)    | 24 (25.8%)    |         |
| Mild               | 7 (7.5%)    | 17 (18.3%)    |         | 5 (5.4%)    | 19 (20.4%)    |         |
| No anaemia         | 7 (7.5%)    | 18 (19.4%)    |         | 6 (6.5%)    | 19 (20.4%)    |         |
| **TWBCs count x 10^9** |           |               |         |             |               |         |
| ≤50                | 10 (10.8%)  | 21 (22.6%)    | 0.88    | 5 (5.4%)    | 26 (27.9%)    | 0.29    |
| >50                | 21 (22.6%)  | 41 (44.0%)    |         | 16 (17.2%)  | 46 (49.5%)    |         |
| **Platelet count x 10^9** |         |               |         |             |               |         |
| <150               | 16 (17.2%)  | 26 (28.0%)    | 0.56    | 9 (9.7%)    | 33 (35.5%)    | 0.83    |
| 150–450            | 15 (16.1%)  | 35 (37.6%)    |         | 12 (12.9%)  | 38 (40.8%)    |         |
| >450               | 0 (0.0%)    | 1 (1.1%)      |         | 0 (0.0%)    | 1 (1.1%)      |         |

### Table 4: Association Between Flowcytometer Prognostic Indicators and Clinical Staging Systems.

| Parameter        | CD38 no (%) | ZAP-70 no (%) | p value | CD38 no (%) | ZAP-70 no (%) | p value |
|------------------|-------------|---------------|---------|-------------|---------------|---------|
|                  | Positive    | Negative      |         | Positive    | Negative      |         |
| **Rai Staging System** |           |               |         |             |               |         |
| Stage 0          | 1 (1.1%)    | 3 (3.2%)      | 0.69    | 1 (1.1%)    | 3 (3.2%)      | 0.91    |
| Stage I          | 1 (1.1%)    | 7 (7.5%)      |         | 1 (1.1%)    | 7 (7.5%)      |         |
| Stage II         | 7 (7.5%)    | 11 (11.8%)    |         | 4 (4.3%)    | 14 (15.1%)    |         |
| Stage III        | 11 (11.8%)  | 18 (19.4%)    |         | 8 (8.6%)    | 21 (22.6%)    |         |
| Stage IV         | 11 (11.8%)  | 23 (24.7%)    |         | 7 (7.5%)    | 27 (29.0%)    |         |
(Continued)
Association between ZAP-70 and CD38 expression

The study results showed that, there was higher frequency of CD38 (31 patients, (33.3)) than ZAP-70 (21 patients, (22.6)) among patients. Ten of 93 patients (10.8%) were concordant ZAP-70 positive CD38 positive and 51 patients (54.8%) were concordant ZAP-70 negative CD38 negative (Table 1). No significant statistical association was observed between ZAP-70 and CD38 expression among Sudanese CLL patients ($p = 0.05$).

Discussion

In order to investigate the frequency of ZAP-70 and CD38 and their relationship with clinical staging systems we performed a cross-sectional study in 93 cases of CLL. To our knowledge this is the first study to examine the frequency of ZAP-70 and CD38 and their relationship with clinical staging systems in Sudan. Clinical staging systems are the most robust and established prognostic factors in CLL. Recent studies have pointed out some of the limitation in these traditional staging systems [9]. Not all early-stage patients fall into the same prognostic category. A study from Vroblová et al [22] showed that the classic staging systems by Rai and Binet are not able to determine an individual patient’s ongoing clinical course at the time of diagnosis, particularly in early stages. Most of our patients presented in advance stages (Blent stage C and Rai stage IV (71.2%, 40.4% respectively)). This may be explained by; poor health services distribution and lack of screening program and awareness. Rai and Binet staging system showed highly significant association with lymphadenopathy ($p = 0.01$, 0.00 respectively), Haemoglobin concentration ($p = 0.00$ for both) and platelet count ($p = 0.00$ for both). splenomegaly showed highly significant association with Rai staging system ($p = 0.00$) while no significant association was observed with Binet staging system ($p = 0.20$). No significant association was observed between both Rai and Binet staging systems and age, sex, hepatomegaly and TWBCs count ($p > 0.05$), (Table 2).

Various studies have reported ZAP-70 positivity ranging from 25% to 57% and CD38 positivity in CLL ranging from 29% to 60% as given in (Table 5).
Our study results showed that, there was higher frequency of CD38 than ZAP-70, among 93 patients who were tested 33.3% (31 patients) were CD38 positive and 22.6% (21 patients) were ZAP-70 positive. Crespo et al, investigated ZAP-70 and CD38 among 56 patients with CLL. 57% of patients were positive for ZAP-70 and 60% positive for CD38 [23]. This in agreement with our study. Also our findings are in accordance with a study by Gogia et al which examined ZAP-70 and CD38 expression among 80 CLL patients, they found that ZAP-70 was positive in 25% and CD38 was positive in 36% [24]. In contrast a study by Hus et al reported positive expression for ZAP-70 in 36% of 156 patients and CD38 in 33% [4]. This is inconsistent with our findings. Our results are incompatible with a study carried out by Schoroer et al among 252 patients. Their results revealed that 46% of patients were ZAP-70 positive and 29% were CD38 positive [25]. One hundred and fifty seven patients were enrolled in a study by D’Arena et al, their results reported that ZAP-70 was positively expressed among 36% of patients and CD38 among 29%.This in disagreement with our results [26].

Flowcytometric (CD38 and Zap-70) Prognostic Indicators show no significant association with age, sex, lymph Nodes, Organomegaly, CBC and clinical staging systems ($p > 0.05$). A study by Del Poeta et al., and Hus et al. indicated significant correlation between high ZAP-70 levels and advanced Rai stage and splenomegaly [4, 27]. This in disagreement with our results. Our findings goes in line with a study done by Gogia et al from India, reported no association of ZAP-70 and CD38 positivity with age, sex, lymphadenopathy, organomegaly, and Rai staging [24]. The contrast in our results may be contributed to biological factors, ethnic heterogeneity and environmental factors.

**Conclusion**

In conclusion, CD38 (31 patients, (33.3)) show higher frequency than ZAP-70 (21 patients, (22.6)) among patients. There was no significant association of ZAP-70 and CD38 positivity with age, sex, lymphadenopathy, organomegaly, Bient and Rai staging.
systems. Further studies are recommended to develop a standardized flowcytometry protocol that will allow comparison of ZAP-70 and CD38 measurements between different laboratories.

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Conflict of Interest

The authors have no conflict of interest to declare.

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