The Prognostic Significance of Combined Expression of ZAP-70 and CD38 in Chronic Lymphocytic Leukemia

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ABSTRACT

Background: Following gene expression profiling which compared the two well established prognostic markers in CLL, ZAP-70 and CD38 with unmutated and mutated IgVH, ZAP-70 has emerged as the most promising surrogate marker for the IgVH mutation status. CD38 expression has also been suggested as a surrogate marker for the IgVH mutation status.

Aim: We aimed to investigate the impact of ZAP-70 and CD38 expressions as well as their combined expressions on the treatment outcome and survival of our CLL patients.

Patients and Methods: This study included 50 CLL patients as well as 10 normal volunteers as a control group. All patients were subjected to complete work up and immunophenotyping to confirm the diagnosis. ZAP-70 and CD38 expressions were studied in (CD19+, CD5+) B cells. Results were expressed as percent expression and mean fluorescent index (MFI). Results were correlated to the treatment outcome and survival as well as to other prognostic markers of CLL including TLC, Hb level, platelets count, modified Rai staging at diagnosis, P53 and BCL2.

Results: A significant association was found between ZAP-70 percent expression and the diffuse pattern of bone marrow infiltration (p<0.002) as well as the P53 percent expression (p=0.005). A significant increase in serum levels of LDH and B2M in ZAP-70 positive as compared to negative groups was detected (p=0.049 and 0.007 respectively). A higher number of non-responding patients was reported in the ZAP70 positive as compared to ZAP70 negative group (p=0.015). ZAP-70 percent expression was significantly associated with shorter time to disease progression (TDP) and shorter overall survival (p=0.025 and 0.029 respectively).

A significant increase in serum levels of B2M in CD38 percent positive as compared to negative group was encountered (p=0.045). CD38-MFI showed a significant association to advanced modified Rai staging at diagnosis (p=0.019) and to higher serum levels of both LDH and B2M (p=0.03 and 0.05 respectively). CD38, either expressed as a percentage or as MFI, showed a significant association with the non-responders (p=0.034 and 0.006 respectively). There was a significant inverse relation between CD38 expression and time to disease progression (p=0.033) while no significant relation was encountered with overall survival (p=0.197).

Combined expression of both markers, ZAP-70+/CD38+ was reported in 5 patients (10%) while ZAP-70-/CD38- was encountered in 24 patients (48%). Patients with either ZAP-70+ or CD38+ represented 42% of the cases (21 patients). There were significant differences between the three groups and the initial response to chemotherapy (p<0.001) and the pattern of bone marrow infiltration (p=0.015), while no significant relation was found with age, sex, modified Rai staging at diagnosis or BCL2 percent expression.

Patients with combined expression of ZAP-70 and CD38 had significantly shorter TDP and overall survival (p=0.001 and 0.03 respectively).

Conclusion: ZAP-70 is one of the most important prognostic markers in CLL, it appears to be more predictive of disease progression and poor outcome than CD38 expression. Semi quantification of the CD38 antigen by flowcytometry greatly improves the prognostic value of its expression. The combination of ZAP-70 and CD38 increases the prognostic power of either alone.

Key Words: CLL – Prognostic factors – ZAP 70 and CD38

INTRODUCTION

B-cell chronic lymphocytic leukemia (B-CLL) is a heterogeneous disorder characterized by a variable clinical course [1]. Some patients have an aggressive disease requiring early therapy, whereas other patients exhibit a more stable, indolent disease with no benefit from palliative
In a continual effort to identify patients with poor prognosis and to facilitate the clinical management of B-CLL, several prognostic markers have been identified during the last two decades [3].

At first, clinical staging systems based on leukemia cell burden were developed. Those systems have delineated the clinical presentation and natural history of B-CLL and have allowed predicting survival and treatment requirements [4,5]. However, the staging systems lack the ability to distinguish prospectively patients with early stage B-CLL that will rapidly progress to aggressive disease from patients destined to remain in early stage for a long time [1]. Therefore, other parameters related to the genetics and biology of B-CLL, such as genomic aberrations and immunoglobulin variable heavy chain (IgV_H) mutation status, are increasingly used for prediction of disease prognosis [3].

In landmark studies, it has been shown that survival probability in B-CLL is associated with mutational status of IgV_H genes [6,7]. Currently, IgV_H gene mutation status is considered as one of the most powerful prognostic factors, where B-CLL cases with unmutated IgV_H genes are characterized by an unfavorable clinical outcome [1,3].

ZAP-70 is the most promising surrogate marker for the IgV_H mutation status [8,9]. In contrast to the technically demanding IgV_H analysis, ZAP-70 protein expression is conveniently measured by flowcytometry [10,11]. CD38 expression has been also considered as a surrogate marker for the two important IgV_H mutated and unmutated subgroups of B-CLL [12]. Both ZAP-70 and CD 38 are regarded as independent prognostic variables in B-CLL [13]. In this study we aimed to investigate the role of ZAP-70 and CD38 separately and their combined expressions as prognostic markers in CLL and their impact on treatment outcome and survivals.

**PATIENTS AND METHODS**

(A) Patients:

The study was carried out at the Medical Oncology and Clinical Pathology Departments of the National Cancer Institute (NCI), Cairo University.

Fifty patients with CLL were included in the study. At time of sampling, forty patients received previous treatment, while 10 patients were newly diagnosed. Ten normal volunteers were used as a control group.

The diagnosis of CLL was based on the criteria established by the International Workshop on CLL and the National Cancer Institute-Sponsored Working Group Guidelines for CLL [14]. All cases were staged according to modified Rai staging system [15]. The clinical presentations of the patients including treatment histories were reported. Standard criteria were used for the initiation of chemotherapy for all cases [14].

Patients were subjected to:

1- Thorough history taking, full clinical examination, radiological investigation including chest X-ray, abdominal ultrasound, and/or CT scan were done when needed.

2- Complete blood picture, bone marrow aspiration and biopsy.

3- Routine laboratory tests including, liver and kidney function tests, uric acid and Coombs' test.

4- Estimation of serum lactate dehydrogenase (LDH) and B2 microglobulin (B2M).

5- Immunophenotyping using flowcytometer to confirm the diagnosis of CLL with a wide panel of monoclonal antibodies as previously described [16].

6- Immunophenotyping for the expression of P53 and BCL2 on (CD19+/ CD5+) cells.

8- Expression of the studied markers: ZAP-70 and CD38 on (CD19+/ CD5+) cells was evaluated by flowcytometry [16].

All monoclonal antibodies used were tested as surface expression except for, ZAP-70 and P53 that were tested for cytoplasmic expression.

Results were expressed as a percentage of cells showing positive expression and immunophenotyping were done by flowcytometry (partec III).

For most studied markers, positive expression was considered when the marker is identified in more than or equal to 20%. ZAP-70 was defined as positive when identified in more than or equal to 20% of the gated CD19/CD5 positive cells [10]. CD38 was considered positive when
at least 30% of the gated (CD19+, CD5+) B-Cells expressed it [13]. For CD38, results were also expressed as mean fluorescence index (MFI), this was done by dividing the mean fluorescence intensity of the test over that of the isotype control [17].

According to age and performance status, patients were treated by one of the following chemotherapy regimens:

- Chlorambucil (Clb): It was given orally at a dose of 0.2mg/kg/day.
- Cyclophosphamide, Vincristine, Prednisone (CVP regimen): Cyclophosphamide: 400mg/m² IV on days 1-3, Vincristine: 1.4mg/m² IV on day 1 and oral Prednisone 100mg on days 1-5.
- Fludarabine and Cyclophosphamide (FC regimen): Fludarabine 25mg/m² IV on days 1-3 and Cyclophosphamide 250mg/m² IV on days 1-3.

Response to induction chemotherapy was assessed according to the criteria proposed by the National Cancer Institute (NCI) sponsored Working Group Guidelines for CLL [14]. All patients were evaluated for their initial response after 3-6 cycles of the initial chemotherapy and then have been followed-up with an observation period between 2 to 120 months.

**Time to disease progression (TDP):** Duration of response was measured from the date of initial response until disease relapse or progression; or death from any cause, with observation censored at the date of last contact for patients last known to be alive without report of relapse [18].

**Overall survival (OS):** Was measured from the date of presentation to the Medical Oncology Department until death from any cause, with observation censored at the date patients were last known to be alive for those not known to have died [18].

**Statistical analysis:**

Data were analyzed using the statistical package SPSS version 12. Chi-square test (Fisher’s exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using Mann-Whitney test. Kappa was used as a measure of agreement beyond chance. Receiver Operating Characteristic (ROC) curve was plotted against normal controls for determination of the cut off values for positivity of P53%, MFI-ZAP and MFI CD38 variables. Survival analysis was done using Kaplan-Meier Method. Comparison between two survival curves was done using log-rank test.

Probability (p-value) ≤ than 0.05 was considered significant.

**RESULTS**

The study included 50 patients with CLL, they were 40 males (80%) and 10 females (20%). Their median age was 57 years (Range 37 to 80). There was no significant difference regarding age and sex between the study and the control group.

Table (1) shows the clinical characteristics of the patients.

Nineteen patients (38%) received CVP regimen, 26 patients (52%) received Chlorambucil and 5 patients (10%) received FC regimen. Overall response to initial chemotherapy was reported in 32 patients (64%), (CR=14 and PR=18), while 18 patients (36%) showed no response (SD=11and PD=7). Out of the 32 patients who showed initial response, 16 patients showed progression of their disease later on. Six CLL related deaths (12%) occurred during the study period.

Autoimmune hemolytic anemia (AIHA) with positive Coombs’ test was recorded in two patients (4%) and hypogammaprotienemia in one patient (2%). Richter transformation occurred in one case (2%) and Hepatocellular carcinoma (HCC) was recorded in one patient (2%).

**Laboratory findings:**

Table (2) shows the hematological and serological parameters as well as P53 and BCL2 expression levels in patients compared to the control group. Using the Roc curve, a threshold of 4.95% was found to be appropriate for P53, above which the results were considered positive. P53% was positive in 56% of the cases.

Results of bone marrow biopsy were evaluable for 48 patients and revealed diffuse pattern in 21 patients (43.8%) while 27 cases (56.3%) had non diffuse pattern of infiltration {nodular, interstitial or mixed}. 
**ZAP-70 expression:**

Our studied CLL group had a median ZAP-70% expression of 15% with a range of zero to 60% compared to a median of 4.4% with a range of 1.3 to 6.4 for the control group \( (p=0.001) \). For ZAP 70 MFI, a median of 6.25 with a range of 0.6 to 100 was reported for the CLL group compared to 1.1 with a range of 0.6 to 6.4 for the control group \( (p<0.001) \). Using the ROC curve analysis, area under the curve was equal to 0.839. The best cut off value was 1.33 with a sensitivity of 86% and specificity of 90%, therefore a level of \( \geq 1.33 \) was considered positive for ZAP-70 MFI Using percentage expression, 22/50 patients (44%) were positive while by using the MFI 39 patients (78%) were positive for ZAP-70 expression. Statistically significant association was found between ZAP-70% expressions and the diffuse infiltration pattern of bone marrow, among the ZAP-70 positive cases 15/22 showed diffuse infiltration pattern (68.2%) versus 6/26 (23%) in the ZAP-70 negative group \( (p=0.002) \). There was also significant association between ZAP-70% and P53% expressions, in the ZAP-70 positive group 11/13 cases (84.6%) were positive for P53 versus 6/18 (33.3%) in the ZAP-70 negative group (kappa =0.5, \( p=0.005 \)).

Initial response to chemotherapy (CR and PR) was reported in 24/28 (85.7%) of the ZAP70 negative group compared to only 8/22 (36.4%) of the ZAP70 positive patients \( (p=0.001) \).

**CD38 expression:**

The median CD38% expression in the studied group was 7.95% with a range of zero to 58.5% compared to a median of 2.18% and a range of 1.1 to 3.47 for the control group \( (p<0.001) \). Positive percent expression was detected in 9 cases (18%). Using CD38 MFI expression, a median of 11.35 (range 0.6 to 35.6) was reported compared to a median of 1.8 (range 0.6-3.1) for the control group. Using ROC curve analysis a cut off value of 2.25 was found to be appropriate to discriminate between positive and negative cases. Twenty nine patients (58%) were positive using CD38 MFI expression. There was no significant relation between CD38% expressions on one hand and either age, sex, bone marrow infiltration pattern or modified Rai stage at diagnosis on the other hand. Also by measuring CD38% expression no significant differences were found for TLC, Hb, PLT, LDH, P53 and BCL2 between CD38 positive and negative groups. There was significant increase in serum levels of B2M in CD38 positive patients with a median of 3.65mg/l (Range 2.4 - 6.1) as compared to negative group with a median of 2.9 (Range 1.6-6.4) \( (p=0.045) \).

A significant relation was found between CD38% expression and the initial response to chemotherapy. Among the CD38% negative group 29/41 (70.7%) showed CR or PR versus 3/9 (33.3%) in the CD38% positive cases \( (p=0.034) \). This significant relation was also confirmed by CD 38 MFI where initial response to chemotherapy was significantly higher in CD 38 MFI negative compared to positive group \( (p=0.006) \).

By measuring CD38 MFI, a significant association was found between positive CD38 MFI and advanced modified Rai staging at diagnosis \( (p=0.019) \). The median TLC was significantly higher in CD38 MFI positive versus negative groups \( (124X10^9/L \text{ versus } 69X10^9/L \text{ respectively, } p=0.02) \) and the median Hb level was 10.2 versus 11.5 g/dl in both groups respectively \( (p=0.015) \). The median LDH was 952 versus 510 IU/L in CD38 MFI positive compared to negative groups \( (p=0.03) \) while B2M median level was 3.8 versus 2.3mg/l in both groups respectively \( (p=0.05) \).

The impact of combined expression of ZAP-70 and CD38: Table (3).

Our CLL patients were classified according to the combined expression of ZAP-70% (cut off 20%) and CD38% (cut off 30%) into 3 main groups:

The first is the concordant positive group (ZAP-70+/CD38+), which included 5 patients (10%), the second with discordant expression (ZAP-70+/CD38-) or (ZAP-70-/CD38+), which included 21 patients (42%) and the third is the concordant negative (ZAP-70-/CD38–), which included 24 patients (48%). Significant relations were encountered between the different groups and the initial response to chemotherapy \( (p=0.001) \) (Fig.1) and the pattern of B M infiltration \( (p=0.015) \), Table (3), while no significant relation was found with age, sex, modified Rai staging at diagnosis or BCL2% expression.
Table (1): The clinical characteristics of 50 chronic lymphocytic leukemia patients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years):</strong></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>57.5</td>
</tr>
<tr>
<td>Range (37-80)</td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>40 (80)</td>
</tr>
<tr>
<td>F</td>
<td>10 (20)</td>
</tr>
<tr>
<td><strong>Previous treatment</strong></td>
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</tr>
<tr>
<td>No</td>
<td>10 (20)</td>
</tr>
<tr>
<td>yes</td>
<td>40 (80)</td>
</tr>
<tr>
<td><strong>B Symptoms</strong></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>11 (22)</td>
</tr>
<tr>
<td>Negative</td>
<td>39 (78)</td>
</tr>
<tr>
<td><strong>Performance status (P.S)</strong></td>
<td></td>
</tr>
<tr>
<td>(0, I)</td>
<td>24 (53.3)</td>
</tr>
<tr>
<td>(II, III, IV)</td>
<td>21 (46.7)</td>
</tr>
<tr>
<td><strong>Lymph node enlargement</strong></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>47 (94)</td>
</tr>
<tr>
<td>No</td>
<td>3 (6)</td>
</tr>
<tr>
<td><strong>Splenomegaly</strong></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>36 (72)</td>
</tr>
<tr>
<td>No</td>
<td>14 (28)</td>
</tr>
<tr>
<td><strong>Hepatomegaly</strong></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>32 (64)</td>
</tr>
<tr>
<td>No</td>
<td>18 (36)</td>
</tr>
<tr>
<td><strong>Modified Rai stage at diagnosis</strong></td>
<td></td>
</tr>
<tr>
<td>Low and intermediate risk (0, I, II)</td>
<td>26 (52)</td>
</tr>
<tr>
<td>High risk (III, IV)</td>
<td>24 (48)</td>
</tr>
</tbody>
</table>

Table (2): Comparison of the hematological and the serological results as well as P53 and BCL2 between CLL patients and the control group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patient N= 50</th>
<th>Control N=10</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Range</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>TLC X10³/L</td>
<td>101.3±76</td>
<td>20-373</td>
<td>8.3 ±3.3</td>
</tr>
<tr>
<td>Hb g/dl</td>
<td>10.6±2.5</td>
<td>4.2-15</td>
<td>13.42±1.0</td>
</tr>
<tr>
<td>PLT X10³/L</td>
<td>181±391</td>
<td>37-536</td>
<td>278.6±72</td>
</tr>
<tr>
<td>LDH IU/L</td>
<td>603.4±218</td>
<td>311-1515</td>
<td>340.5±35.7</td>
</tr>
<tr>
<td>B2M mg/L</td>
<td>3.1±1.1</td>
<td>1.6-6.4</td>
<td>1.7±0.37</td>
</tr>
<tr>
<td>P53 (%)</td>
<td>13.0±17.8</td>
<td>0.0-85</td>
<td>1.45±0.84</td>
</tr>
<tr>
<td>BCL2 (%)</td>
<td>37.2±33.0</td>
<td>0.0-95</td>
<td>0.87±0.26</td>
</tr>
</tbody>
</table>

Table (3): Comparison of clinical and laboratory data between ZAP-70% and CD38% concordant and discordant groups in 50 B-CLL patients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concordant both positive</th>
<th>Discordant One positive, one negative</th>
<th>Concordant Both negative</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>5 (10%)</td>
<td>21 (42%)</td>
<td>24 (48%)</td>
<td></td>
</tr>
<tr>
<td><strong>Rai stage diagnosis:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0, I, II) %</td>
<td>2/5 (40%)</td>
<td>9/21 (42.8%)</td>
<td>15/24 (62.5%)</td>
<td>0.396</td>
</tr>
<tr>
<td>(III, IV) %</td>
<td>3/5 (60%)</td>
<td>12/21 (57.1%)</td>
<td>9/24 (37.5%)</td>
<td></td>
</tr>
<tr>
<td><strong>Initial response:</strong></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(CR, PR) %</td>
<td>0/5 (0%)</td>
<td>11/21 (52%)</td>
<td>21/24 (87.5%)</td>
<td></td>
</tr>
<tr>
<td>(SD, PD) %</td>
<td>5/5 (100%)</td>
<td>10/21 (48%)</td>
<td>3/24 (12.5%)</td>
<td></td>
</tr>
<tr>
<td><strong>Bone marrow biopsy:</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.015</td>
</tr>
<tr>
<td>Diffuse %</td>
<td>4/5 (80%)</td>
<td>12/21 (57.1)</td>
<td>5/22 (22.7%)</td>
<td></td>
</tr>
<tr>
<td>Non-diffuse %</td>
<td>1/5 (20%)</td>
<td>9/21 (42.8%)</td>
<td>17/22 (77.2%)</td>
<td></td>
</tr>
</tbody>
</table>
**Time to disease progression (TDP) in relation to combined ZAP70/CD38 expressions:**

Response to initial chemotherapy (CR&PR) was reported in 32 patients (64%). The follow-up period for TDP ranged between 2 and 100 months (Median 10 months).

The progression free survival (PFS) at 1 year was 79% for ZAP-70 negative compared to 42% in ZAP-70 positive patients ($p=0.020$) (Fig. 2A).

For the CD38 negative patients, PFS at one year was 88%, which was significantly higher when compared to 33% in CD38 positive patients, ($p=0.033$) (Fig. 2B).

No patients in the ZAP70+/CD38+ group responded to the initial chemotherapy. In the ZAP-70-/CD38- group, the PFS was 86%, the median TDP was 29 months. In the discordant (ZAP-70+/CD38- or ZAP-70- /CD38+) group the PFS was 41% and the median TDP was only 12 months. The PFS was significantly longer for the ZAP-70-/CD38- group than in either ZAP70+ or CD38+ group. ($p<0.001$) (Fig. 2C).
Overall survival in relation to combined ZAP70/CD38 expressions:

The follow-up period ranged between 2 to 120 months with a median of 18 months.

The cumulative overall survival was 89%. At the end of the study 88% of the cases were still alive. The OS at 18 months was 96% for ZAP-70 negative compared to 81% for ZAP-70 positive patients ($p=0.029$) (Fig. 3A).

The CD38 negative patients had an OS of 92% compared to 76% for CD38 positive patients, this difference was statistically insignificant ($p=0.197$) (Fig. 3B).

In ZAP-70+/CD38+ (both positive group), the OS was 80% while it was 81% in the either positive (ZAP-70+ or CD38+) group. In ZAP-70-/CD38- (both negative group) the cumulative survival was 100% since all patients in this group were alive at the end of the study ($p=0.031$) (Fig. 3C).

**DISCUSSION**

**Clinical parameters:**

The male to female ratio in our study was 4:1. This confirmed the male predominance that was reported by other Egyptian studies [19,20]. B symptoms were positive in 11/50 and this was near to what was reported by Abbott 2006 [21] as 25% of his patients had B symptoms. The ECOG performance status (PS) at diagnosis was ($<$II) in 53.3% and ($\geq$II) in 46.7% and this is different from that reported by Hsi et al. [18] who recorded PS of 97% and 3% respectively and this could be explained by the fact that 24/50 (48%) of our patients were at an advanced Rai stage at diagnosis.
The most common physical findings in our patients were lymphadenopathy (94%), splenomegaly (72%) and hepatomegaly (64%) which were higher than what was reported by Abbott 2006 [21], who recorded 87%, 54% and 14% respectively and similar to the high frequency reported in our previous study showing lymph-adenoopathy, splenomegaly and hepatomegaly in 95.1%, 87.1% and 64.5% of the patients respectively [19].

Results of the modified Rai staging showed, low and intermediate risk stage in 52% and high risk stage in 48%. This is comparable to the 53% and 47% respectively that was reported by Hsi et al. [18].

Response to induction chemotherapy (CR and PR) was reported in 32/50 (64%). These results are in accordance with Ghia et al. [22] who reported that 19/30 (63.6%) responded to the initial chemotherapy.

ZAP-70 expression:

ZAP-70 has demonstrated an equivalent clinical utility to IgH mutational status in correlation with disease progression and survival [23].

In this study, when using the percentage expression for interpretation, ZAP-70 was positive in 22 patients (44%). This result is similar to what was reported by others [24,25]. However, a higher percentage of positivity was detected when using MFI, suggesting a higher sensitivity for this method.

Significant associations were found between ZAP-70 % expression and some of B-CLL poor prognostic parameters (LDH and B2M). This is in accordance with what was reported earlier [24,26,27]. Our results showed a significant association between ZAP-70 % expression and the diffuse pattern of bone marrow infiltration. This is in agreement with other studies which reported that the expression of ZAP-70 was related to the infiltration type [24,25]. Our study showed a significant association between ZAP-70% and P53% expressions; this was also reported by others [28,29]. However no significant relation with BCL2 was encountered and this is in accordance with previous studies [29,30].

We demonstrated a significant relation between ZAP-70% and the initial response to chemotherapy and these results are in accordance with what was previously reported [29,31,32]. In our study, the TDP and PFS were significantly less in ZAP-70 positive compared to ZAP-70 negative group. This is in agreement with previous reports [10,24,26,33].

Six CLL-related deaths had occurred during the observation period. Five out of those were ZAP-70+ (83.6%). In the ZAP-70 positive group 5/22 (22.7%) died in contrast to only 1/28 (3.5%) patients in the ZAP-70 negative group. Our results are in agreement with that of Hus et al. [27], who reported that, 15/156 CLL-related deaths occurred during their observation period and 12/15 patients of this group were ZAP-70+ (80%) in contrast to only 3/15 patients in the ZAP-70- group (20%) [27].

At a median follow up period of 18 months, the OS of the ZAP-70 negative patients was significantly longer as compared to the ZAP-70 positive cases and this is in accordance with other studies [10,24,26,33].

• CD38 expression:

In B-CLL, CD38 was considered of prognostic value when ≥30% of (CD19+, CD5+) B-CLL cells expressed this membrane antigen [34].

Based on the current study using this cut off value, 18% of the studied patients were positive, the low percentage of CD38 positivity may be attributed to the fact that most of our patients (80%) had received chemotherapy before the time of testing. Previous studies have reported that CD38 expression can change with time and under different conditions and chemotherapy selectively eliminates the CD38 positive clone [13]. It was therefore suggested, for the accurate assessment of the prognostic significance of CD38 positivity to ensure that only samples close to or at the time of presentation are tested [17]. Our results are more or less similar to previously reported data [26,34]. This can be expected as most of their patients had, as well, received chemotherapy at the time of analysis. In contrast, a different ratio was obtained where CD38 positivity was observed in 89% of the studied CLL cases, none of them had received chemotherapy at the time of sampling [24].

In our study, no significant relation was found between CD38% expression and age, sex, TLC, the infiltration pattern of bone marrow or modified Rai staging at diagnosis. Also, no
significant relation was found between CD38% and P53% or BCL2% expressions and these results are in accordance with others [17].

Our results showed a significant increase in serum level of \( B_{2}M \) in CD38 positive as compared to CD38 negative. These results are in accordance with previous results [17,26] and in discordance with an earlier study [35].

A significant relation was found between CD38% expression and the initial response to chemotherapy; only 33.3% of CD38% positive cases responded to chemotherapy. These results are in agreement with other studies [29,34] and in contrast with another study that reported no significant differences in CD38% expression between responders and non-responders to the initial chemotherapy [27].

In CD38 positive group, the median TDP was 3 months and the PFS was 33% in contrast to CD38 negative group where they were 24 months and 88% respectively. Although there was an observed difference yet, statistical significance was not achieved. These results are in accordance with other studies [10,17,24,26,34,36]. CD38% expression was reported as a marker to distinguish patients who required treatment early versus late, albeit less well than IgV\(_H\) mutation status or ZAP-70 expression (Wiestner et al.) [37]. In our study, the OS was 92% for CD38 negative compared to 76% for CD38 positive patients. This difference was statistically insignificant and this could be explained by the small number of CD38% positive cases. Our results are in accordance with other studies [10,17,24,26,34,36].

By using the MFI for measuring CD38, a significant relation was found between CD38 MFI on one hand and advanced modified Rai Staging at diagnosis and the initial response to chemotherapy on the other hand.

Also CD38 MFI positive patients showed significantly increased TLC, decreased Hb, increased LDH and B2M as compared to CD38 MFI negative cases. As a result, we can conclude that, semiquantification of the CD38 antigen by flowcytometry greatly improves the prognostic value of the percentage expression and this was also recomended by other reports [17,36].

**Combined ZAP-70 and CD38 expression:**

Combined analysis of ZAP-70 and CD38 allowed separation of our patients cohort into 3 subgroups, that were, concordant negative ZAP-70-/CD38- (42%), concordant positive ZAP-70+/CD38+ (10%) and patients with discordant results (Either ZAP-70+, CD38- or ZAP-70-, CD38+) (48%). In our study, we found that the combined analysis of ZAP-70 and CD38 expressions increased the prognostic power of either alone. Deaglio et al. [38] tried to find a molecular explanation of why combined CD38 and ZAP-70 assessment provides a more accurate identification of patients with high-risk CLL. They concluded that CD38 and ZAP-70 are functionally linked and mark CLL cells with high migratory potential.

A significant relation was found between the three subgroups with the initial response to chemotherapy and pattern of bone marrow infiltrations.

There was no relation between the three subgroups and sex, age or the advanced modified Rai staging at diagnosis. These results are in accordance with other reports [10,26,27,33].

A significant relation was found between the OS and the combined expression of both markers. These results are in accordance with previous researches [10,24,26,27]. Also a significant relation was found between TDP and the combined expression of both markers. The TDP was 29 versus 12 months for the concordant both negative and the discordant both markers groups respectively. The PFS was 86% versus 41% for both groups respectively. The shortest TDP was found in the concordant positive group where no patients in this group responded to the initial chemotherapy. These results are in agreement with other studies [24,27].

In conclusion: ZAP-70 expression is associated with poor response to chemotherapy, more advanced disease stage, decreased overall survival and shorter TDP. In addition, ZAP-70 is a stable marker not affected by chemotherapy. This suggests that ZAP-70 assay is a powerful predictor of outcome that can be applied at any time point. For the accurate assessment of the prognostic significance of CD38 positivity, it should be applied on chemonaive cases and consider the use of MFI in association with percentage positivity as a better predictor of
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disease progression and outcome than the percentage alone. The prognostic information obtained from ZAP-70 and CD38 expression is complementary. Combined analysis of these two markers allows the identification of more aggressive B-CLL patient subgroups, and therefore could be used to guide treatment decisions.

REFERENCES


