Prognostic Significance of Bone-marrow Pattern and Immunophenotypic Score in B-chronic Lymphocytic Leukemia at Diagnosis

Azra Jahic1, Ermina Iljazovic2, Aida Arnautovic-Custovic1, Alma Halilbasic1, Vlastimir Simendic1, Aida Zabic3

Clinic for Oncology, Hematology and Radiotherapy, Department of Hematology, University Clinical Center Tuzla, Tuzla, BiH 1
Policlinic for Laboratory Diagnostics, Pathology Department, University Clinical Center Tuzla, Tuzla, BiH 2
Policlinic for Blood Transfusion, University Clinical Center Tuzla, Tuzla, BiH 3

1. INTRODUCTION

B-CLL is a neoplastic, lymphoproliferative disease characterized by the accumulation of small, mature B-lymphocytes in the blood, bone marrow and lymphoid tissues. B-CLL is the most common adult leukemia in the Western world with the incidence of 3.9/100,000 in the U.S. (1). The older age and family history of leukemia or lymphoma are additional risk factors (2, 3). The mean age of occurrence B-CLL is in the range of 64-70 years (4). Some studies show no difference in survival by gender, while others demonstrate a better survival for women (5). According to Binet, the disease clinically develops in three stages, Binet A, Binet B, and Binet C (6). The clinical course and outcome of B-CLL is various and so far unpredictable. Defining prognostic parameters potentiating division of patients in groups with favorable and unfavorable prognosis which could help the benefit assessment of early treatment, improve treatment effects, and potentiate treatment modification for each patient. Aim: To analyze the bone-marrow (BM) pattern and immunophenotypic score at diagnosis of B-CLL and determine the correlation of BM pattern with the clinical stage of disease and immunophenotypic score.

Methods: A sample of 40 untreated patients with B-CLL was divided into two groups: group with clinical stage Binet A and group with clinical stages Binet B and Binet C. BM patterns were observed as a diffuse, interstitial, nodular and mixed. BM immunophenotyping included CD5, CD23, CD22, and CD20 as an indirect indicator of FMC7. Results: The overall sample mean age was 62.88 years ± 11.10, without significant difference in the age of two compared groups (63.15 ± 10.53 years vs. 62.60 ± 11.50 years) (t = 0.16, df = 38, p = 0.88). Proportion of men was significantly higher in stages Binet B and C (12/20) compared to stage Binet A (5/20) (Z=2.24, p=0.025). The percentage of women was higher than men in Binet A stage (75% vs. 25%). The BM patterns in Binet A stage were observed as follows: mixed 50% (10/20), interstitial 30% (6/20), nodular 15% (3/20) and diffuse 5% (1/20). The BM patterns in Binet B and C stages were observed as follows: diffuse 50% (10/20), mixed 40% (8/20), interstitial 5% (1/20) and nodular 5% (1/20). Clinical stage and the BM patterns were significantly associated (c2=8.02, p=0,005). The chance for non-diffuse patterns was 19 times higher in stage Binet A compared to stages Binet B and C, respectively, analyzing 95% CI at least 2 times higher (95% CI: 2.02-866.6). Immunophenotypic score in total sample was observed as follows: score 4: 5% (2/40), score 3: 72.5% (29/40), score 2: 20% (8/40) and score 1: 2.5% (1/40). Immunophenotypic score 3 and > 3 had 77.5% of patients (31/40), but there was no significant association between the immunophenotypic score and the BM patterns (c2=0.76, p=0.38).

Conclusions: Diffuse BM pattern was significantly associated with the clinical stages Binet B and C, compared to non-diffuse BM patterns which were significantly associated with the clinical stage Binet A. Diffuse BM pattern represent the parameter of progressive disease compared to the non-diffuse BM patterns which are more often represented in stable disease. Immunophenotypic score improves diagnostic accuracy of B-CLL, but should not be used as a prognostic parameter of B-CLL.

Key words: B-CLL, prognostic parameter, BM pattern, immunophenotypic score

Corresponding author: Azra Jahic, MD, Department of Hematology, Clinic for Oncology, Hematology and Radiotherapy, University Clinical Center Tuzla, 75000 Tuzla, Trnovac b.b., Bosnia and Herzegovina. Tel: +38761 623 933. E-mail: azrasong1@yahoo.com
clinical course and outcome is various and so far unpredictable. One third of patients at diagnosis have an aggressive disease that requires treatment, one third of patients have an “indolent” disease that follows the progression, and the remaining third are the patients who never required treatment of CLL and who die because of other comorbid conditions. Patients in Binet A stage do not require treatment but are followed (“watch and wait” approach) to clinical progression in Binet stage B or C, which require treatment. Histological analysis of bone marrow (BM) is possible to objectify the four patterns of infiltration by malignant B-lymphocytes: nodular, interstitial, diffuse and mixed (nodular-interstitial) pattern (7). Interstitial pattern is associated with better prognosis and/or early stage of the disease, as opposed to the diffuse pattern which is associated with advanced clinical stage and/or aggressive disease (8).

Based on the most common markers profile in CLL (CD5+, CD23+, FMC7-, CD22– or CD79 a–and weakly positive (+/-) of surface immunoglobulin (SmIg)), is proposed Matutes scoring system, whereby each of these markers with the above expression has the value 1, or value 0 in case of inverse expression of these (9). Matutes score may range from a maximum of 5 which is typical for CLL to 0 which is atypical for CLL. Matutes score in chronic lymphocytic leukemia is > 3, while in the case of other lymphoproliferative diseases < 3, which is very important in distinguishing cases of typical CLL from atypical CLL and in the differential diagnosis of B-CLL from other B-lymphoproliferative diseases. FMC7 is a monoclonal antibody that binds to the CD20 epitope of leukemic cells if the CD20 epitope present in high density (10) and typically does not react with CLL cells, thus reflecting the low level of expression of CD20 on CLL cells (11).

The aim of this study was to determine the gender and age distribution of patients with B-CLL, histological BM infiltration pattern and immunophenotypic score at diagnosis and determine the association of BM pattern with and clinical stage of disease and immunophenotypic score.

2. PATIENTS AND METHODS

The study was retrospective-prospective using sample of 40 untreated patients with B-CLL, both male and female, no matter the age, who were hospitalized in the Department of Hematology, Clinic for Oncology, Hematology and Radiotherapy, University Clinical Center Tuzla, from 2006 to 2010. The sample was divided into two groups. The first group (n=20) consisted of patients with a stable disease in clinical stage A according to Binet. The second group consisted of patients with an agressive form of disease in clinical stages B and C according to Binet. To determine the clinical stage was used Binet classification. For histological analysis and immunophenotyping were used bone marrow samples from the Pathology Department, Polyclinic for Laboratory Diagnostics University Clinical Center Tuzla.

All samples were initially fixed in 10% formalin solution and embedded in paraffin. After immunohistochemical analysis of histological slices of tissue samples of 4 μm thickness by threestage immunoperoxidase with streptavidin, and after deparaffinization, samples were incubated for a period of thirty minutes in 1.5% H2O2 to block endogenous peroxidase activity and treated in citrate buffer (10 mM, pH 6.0) at 100 °C for 15 minutes. In the center of the immuno-staining Sequenza Shandon, made all the degrees of incubation. Following the preincubation period with 100% normal bovine serum, there was a three level immunoperoxidase treatment. Positive reaction was considered to be a clear brown staining of cell membrane, cytoplasm or nucleus, in the absence of nonspecific background staining. Hystological BM patterns were observed as a diffuse, interstitial, nodular and mixed.

Immunophenotypic score was determined based on the most common marker profile in CLL: CD5, CD23, CD22, and CD20 as an indirect indicator of FMC7. In the case of CD5+, CD23 +, CD20 weakly + and CD22 -, each marker had a point 1. In the case of CD5-, CD23-, CD20 strong + and CD22 +, each marker had a point 0. Immunophenotypic score could range from 4 to 0. Given the lack of technical conditions for the determination of markers SmIg and FMC7 in our study, we analyzed the scoring system that excluded SmIg. Instead of FMC7, we used to determine the degree of CD20 expression on the surface of B-lymphocytes as an indirect indicator of FMC7 expression. Statistical analysis was performed in ARCUS Quick Stat Biomedical. For statistical analysis of data were used basic methods of descriptive statistics (measures of central tendency, measures of dispersion). Categorical variables were compared by χ2 test or, when the frequency of small, by Fisher exact test. Numerical varibles were compared by Student t-test. All tests were done with the significance level of 95% (p <0.05).

3. RESULTS

In total sample of 40 patients, average age was 62.88 years ± 11.10 with a minimum of 42 years and maximum of 82 years. The age was not significantly different in compared groups (63.15 ± 10.53 years vs. 62.60 ± 11.50 years) (\( t \) =0.16, \( df \)=38, \( p \)=0.88). The sample consisted of 57.5% women (23/40) and 42.5% (17/40) of males. There was no significantly difference in gender prevalence within the two observed groups analyzed by hi-square test (\( \chi^2 \)=3.68, \( p \)=0.055). As the value of \( p \) very near \( p \) = 0.05, the calculation was repeated with a stronger, parametric test to compare proportions. This test showed significantly higher proportion of males in agressive disease (Binet B and C) compared to stable disease (Binet A) (12/20 vs 5/20) (\( Z \)=2.24, \( p \)=0.025). The proportion of women was higher than men in the group of stable disease (Binet A) (75% vs. 25%) (Figure 1).

![Figure 1. Comparison of gender representation between both groups](image)
The first group consisted of 50% (20/40) patients in Binet stage A. The second group consisted of 30% (12/40) patients in Binet stage B and 20% (8/40) of patients in Binet stage C. In the group of patients in clinical stage Binet A, BM pattern was represented as follows: diffuse 50% (10/20), interstitial 30% (6/20), nodular 15% (3/20) and diffuse 5% (1/20). In the group of patients with clinical stages Binet B and C, the BM pattern was represented as follows: diffuse 50% (10/20), mixed 40% (8/20), interstitial 5% (1/20) and nodular 5% (1/20) (Figure 2).

By Chi² test, analysis of 2x2 frequency tables, it was determined there was a significant relation between the clinical stage and the BM patterns (Chi2=8.02, p=0.005). Results are shown in Table 1. The chance for non-diffuse BM patterns was 19 times higher in Binet stage A compared to stages Binet B and C, respectively, by analyzing the 95% CI at least 2 times higher (95% CI: 2.02-866.6).

In a total sample immunophenotypic score was observed as follows: score 4: 5% (2/40), score 3: 72.5% (29/40), score 2: 20% (8/40) and score 1: 2.5% (1/40). Immunophenotypic score of 3 and > 3 had a total of 77.5% patients (31/40), of which 42.5% of patients with stable disease (Binet stage A) and 35% of patients with aggressive disease (Binet stages B and C). The results of our study showed that 45% (18/40) of patients had mild to moderately positive expression of CD20 on CLL cells, of which 27.5% (11/40) with stable disease and 17.5% (7/40) with aggressive disease. The intense positive expression of CD20 had 55% of patients (22/40), of which 22.5% (9/40) with stable disease and 32.5% (13/40) with aggressive disease. By Chi² test, analysis of 2x2 frequency tables, there was no significant association between the BM pattern and immunophenotypic score (Chi²=0.76, P=0.38) (Table 2).

4. DISCUSSION

B-CLL is the most common adult leukemia in the Western world. According to data from the registry, the incidence of CLL in the U.S. is 3.9/100 000 with a two times higher incidence rate in men compared to women (4). The older age and family history of leukemia or lymphoma are an additional risk factors (2, 3). The mean age of occurrence CLL moving in the range of 64-70 years (4), and rarely occurs in persons younger than 25 years. The results of our study showed the average age of patients with B-CLL in a total sample was 62.88 ± 11.10 years with a minimum of 42 years and a maximum of 82 years, thus the age distribution of our patients with CLL did not differ from the reported age for this disease.

The patients with stable disease were not on average significantly older compared to the patients with aggressive disease (t=0.16, df=38, p=0.88). Women were proportionally more represented compared to men in the group of stable disease (75% vs. 25%). The proportion of men in stages Binet B and C (12/20) was significantly higher compared to stage Binet A (5/20) (Z=2.24, P=0.025), suggesting significant difference in the frequency of gender in stable and aggressive disease. Men were proportionally more significantly represented compared to female in the group of patients with aggressive disease (Binet B and C), suggesting that men have more aggressive clinical course compared to women. In most cases, the incidence is higher in men compared to women (M:F=1.5) (1). Influence of gender on prognosis is controversial, since some studies show no difference in survival by gender, while others demonstrate a better survival for women. The most common explanation for the better survival of women with B-CLL has a longer life expectancy for women in the general population, and the fact that this disease in women tend to present fewer unfavorable characteristics in relation to men (5).

Histological analysis of bone marrow is possible to objectify the four patterns of BM infiltration by malignant B-lymphocytes: nodular, interstitial, diffuse and mixed (nodular-interstitial) (7). The results of our study showed that 50% (10/20) of patients in Binet stages B and C had diffuse BM pattern compared to only 5% (1/20) of patients in Binet stage A, which was significant (Chi²=8.02, P=0.005). Also, 30% of patients (6/20) in Binet stage A had interstitial BM pattern, while this BM pattern in B-CLL is associated with early stage of the disease and with better prognosis, in contrast to the diffuse pattern associated with advanced clinical stage and aggressive disease (8, 12). Our results are consistent with results of other authors (8, 12) in terms of significant presence of non-diffuse BM patterns in...
stable disease and diffuse BM pattern in advanced disease, suggesting a way to diffuse BM pattern correlates with advanced clinical stage and represent an unfavorable prognostic parameter. Diffuse BM pattern in CLL is predominantly associated with the unmutated status of IGHV gene and increased expression of ZAP-70 (13). The status of unmutated IGHV gene and increased expression of ZAP-70 represent an adverse prognostic parameters (13), which further confirms the negative prognostic value of diffuse BM pattern. BM pattern (diffuse vs. non-diffuse) was also declared one of the strongest predictors of survival by other researchers (14, 15).

In the total sample immunophenotypic Matutes score 3 and > 3 had a total of 77.5% (31/40) patients, of which 54.84% (17/31) of patients with stable disease (Binet stage A) and 45.16% (14/31) of patients with aggressive disease (Binet stages B and C). BM infiltration pattern and immunophenotypic score were not significantly associated. Although most patients in our study have had Matutes score 3 and > 3, which is characteristic of CLL and it is in accordance with literature data, research findings indicate certain specific regions. Relatively low or borderline Matutes score in our study can be partly explained by the significant representation of aggressive stages of disease, or atypical forms of CLL.

Immunophenotypic score in our study was determined based on the most common markers profile in CLL: CD5, CD23, CD22, and CD20 as an indirect indicator of FMC7. Given the lack of technical conditions for the determination of SmIg and FMC7, we analyzed the scoring system that excluded SmIg, thus scoring system could have a maximum of 4 points instead of 5 points under the original scoring system of Matutes (16). Instead of FMC7, we used to determine the degree of expression of CD20 on the surface of B-lymphocytes as an indirect indicator of FMC7 expression. Since the expression of SmIg and FMC7 are the most reliable immunophenotypic markers (16) and that they did not analyzed, there is a possibility to influence the aforementioned on not found association between immunophenotypic score and BM pattern, and the existence of immunophenotypic score lower than 3 in 22.5% of patients in a total sample.

Immunophenotyping allows more precise classification of chronic lymphoproliferative diseases. Matutes score in CLL is > 3, while in the case of other lymphoproliferative diseases <3, which is very important in distinguishing cases of typical CLL from atypical CLL and in the differential diagnosis of B-CLL from other B-lymphoproliferative diseases. Typical cases of CLL are diagnosed in the majority of Binet stage A, which does not require treatment, while the atypical CLL cases mostly presented in advanced stages that require treatment and have a shorter survival. Matutes et al. (16) analyzed circulating cells from 666 cases, including CLL, prolymphocytic leukemia and hairy cell leukemia. On the basis of common immunophenotype marker profile in CLL (CD5 +, CD23 +, FMC7–and weak expression (+/-) of SmIg and CD22-) is proposed a scoring system. In considering each marker individually, it is not possible on the basis of one marker differ the CLL from other chronic lymphoproliferative diseases, although the expression of SmIg and FMC7 are the most reliable (16).

Moreau et al. (17) found that replacement of CD22 with CD79a in the original scoring system increases the potential for resolution of CLL from other B-cell lymphoproliferative diseases. Other studies that have attempted to correlate immunophenotype with prognosis have not given satisfactory results (18, 19). In a series of patients with B-CLL who meet strict immune criteria, high expression of CD20 significantly correlates with atypical morphology and poorer prognosis (20).

Therefore, quantitative immunophenotyping would allow better analysis of the biological heterogeneity of the disease, although it is difficult to transfer the results in to the prognosis.

The results of our study showed that 45% (18/40) of patients had mild to moderately positive expression of CD20 on CLL cells, of which 27.5% (11/40) in group with stable disease and 17.5% (7/40) in group with aggressive disease. The intense positive expression of CD20 had 55% (22/40) of patients, of whom 22.5% (9/40) of patients with stable disease and 32.5% (13/40) of patients with aggressive disease. The results showed that patients with stable disease generally had mild to moderate expression of CD20 in contrast to patients with advanced disease who have had more intense expression of CD20. High expression of CD20 on the surface of leukemic cells significantly correlates with atypical morphology and poorer prognosis (20). Intense expression of CD20 was proportionally more represented in the group of patients with aggressive disease, what could be the reason that in the total sample were 22.5% of patients with immunophenotypic score of less than 3, and the correlation of immunophenotypic score and BM pattern was not found.

5. CONCLUSIONS
The average age of the total sample was 62.88 years ± 11.10. The average age did not differ in the two observed groups. The gender distribution was significantly different in stable and aggressive disease. Women were proportionally more represented than men in the group of stable disease (75% vs. 25%). The proportion of men in aggressive disease (Binet B and C) (12/20) was significantly higher compared to the proportion of men in stable disease (Binet A) (5/20).

BM pattern was significantly associated with the clinical stage of disease. Diffuse pattern was significantly associated with clinical stages Binet B and C, compared to the non-diffuse pattern which was significantly associated with clinical stage Binet A. Matutes immunophenotypic score of 3 and > 3 had a total of 77.5% (31/40) patients, of which 54.84% (17/31) of patients with stable disease (Binet stage A) and 45.16% (14/31) of patients with aggressive disease (Binet stages B and C), but there was no significant correlation between the BM infiltration pattern and immunophenotypic score.

The results of our study suggest that the diffuse BM infiltration pattern represent the parameter of poor prognosis and aggressive disease compared to non-diffuse BM patterns. Immunophenotypic Matutes score improves diagnostic accuracy of B-CLL, but should
not be considered as a prognostic parameter of B-CLL.

REFERENCES