Flow Cytometry Study of Biomarkers in Chronic Lymphocytic Leukemia Patients Treated At the Royal Medical Services, Jordan

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ABSTRACT

Introduction: Leukemia, of which there are different types, is a crucial disease that is encountered in clinics. Chronic lymphocytic leukemia (CLL) is the most prevalent type of leukemia among adults. Flow cytometery is a key component to the diagnosis of CLL. Most cases can be identified using a panel of antibodies specific for CD5, CD19, CD20, CD23 and kappa and Lambda Ig light chain.

Study objectives: The main objectives were to study the biomarkers involved in CLL as well as the positivity of these biomarkers according to gender.

Methods and subjects: A retrospective study design was employed to collect data from files of patients with CLL. The study sample included a total of 108 patient files. An Excel spreadsheet was created to record the raw data of all the patients. Statistical analysis was carried out using SPSS version 21. Descriptive statistics were used to describe continuous variables using means and standard deviations, of which age was one. Frequency and percentages were used to describe categorical variables such as gender and the positivity of biomarkers.

Results: 70% of participants were males. The mean age was 66.56 ± 11.20 years. CD19 was the most frequent (100%), followed by CD5 (99.07%). CD22 was lowest frequent biomarker (0.9%). With regard to gender, the mean age of females was 65.5 ± 8.68 years and that of males was 67.03 ± 12.16 years. In female patients with CLL, the most frequent positive biomarker was CD5 in 32 patients (96.97%), followed by CD200 in 29 patients (87.78%). In male patients with CLL, CD5 and CD19 were the most frequent biomarkers.

Conclusions: The results showed that the frequency and distribution of biomarkers in patients with CLL is affected by gender. CD5 and CD200 were the most prevalent biomarkers in females, while CD5 and CD19 were the most frequent among males.

Keywords: CLL, biomarkers, gender, CD, CD5, CD19.

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Introduction

Chronic lymphocytic leukemia (CLL) is one of the most common diagnoses made by flow cytometry laboratories. The diagnosis of CLL is usually suspected when adult found to have absolute lymphocytosis, the presence of ≥5000 B-lymphocytes/μL in the peripheral blood for the duration of at least 3 months is required for diagnosis and work up. There is no consensus on which markers need to be used in flow cytometry for accurate Immunophenotyping [1].

Herein, we investigated the role of markers used in flow cytometry in the distinction between CLL and MCL [2].

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Genetics does not play a role in diagnosing CLL, irrespective of the existence of numerous genetic alterations [2,3].It has been reported that the diagnosis of CLL depends on the existence of permanent B lymphocytes for a duration of more than three months. CLL is characterized by morphological and immunological features in flow cytometry [4,5]. Most cases can be identified using a panel of antibodies specific for CD5, CD19, CD20, CD23, and weak surface membrane immunoglobulins (sIg) typically, only a single immunoglobulin light chain is expressed either kappa or Lamda but not both[6]. The diagnosis of CLL is difficult because of its morphological and immunophenotypical heterogeneity [7, 8]. To overcome these difficulties, a scoring system was developed [9, 10]. Five parameters were included in this system: CD5, CD22, CD23, FMC7, and sIg. Further, CD79b replaced CD22 [11, 12].

Lymphoproliferative disorders (LPDs) are a group of diseases that are characterized by their heterogeneity and classified by the World Health Organization (WHO) as part of the mature B cell neoplasm category, as the CLL is an extremely heterogeneous disease, patients with early stage asymptomatic CLL (Rai stage < 3, Binet A or B) the standard of care is observation rather than immediate treatment. The Rai and Binet staging systems use physical examination and blood counts to stratify patients into three risk groups (low, intermediate and high) to create prognostic information and to identify patients who would benefit from treatment. In our study there were 42 of 108 (39%) kept on watch and wait strategy as they had an early stage disease and there was no indication for treatment at the time of diagnosis. [13, 14].

CLL is likely to develop in elderly patients and has variations in its progression [15]. The peripheral blood of patients with CLL has accumulated forms of mature CD19+, CD5+, and CD23+ B lymphocytes [16]. CLL has been reported to be the most prevalent hematological malignancy in Western countries, and its incidence is approximately 4.5 new cases per 100,000 individuals [16, 17].

CLL mostly occurs in white populations in the United States, while its lowest occurrence is among Eastern Asian populations [18]. Most patients are diagnosed with CLL at 72 years of age, and males are more likely to develop CLL compared to their female counterparts [19, 20]. Although no exact etiology of CLL has been established, it has been proposed that genetical and environmental factors may play significant roles in the development and progression of CLL, including apoptosis regulating genes and B-cell biology [21].

Study objectives

The main objectives were to study the biomarkers involved in CLL and as well as the positivity of these biomarkers according to gender.

METHODS AND SUBJECTS

Study design and setting

A retrospective study design was employed to collect data from the files of patients with CLL. Files of patients who received treatment at The Royal Medical Services Clinics from February 2005 to May 2019 were included in the study.

Study sample

The sample of this study included the files of 108 patients with CLL. Files that contained complete information about the patients were included.

Study procedure

An ethical approval was obtained from the Institutional Review Board of Royal Medical Services prior to conducting the study. After reviewing the patient files, an Excel spreadsheet was created to record the raw data of all the patients. Statistical analysis was carried out using SPSS version 21. Descriptive statistics were used to describe continuous variables using means and standard deviations. Frequency and percentages were used to describe categorical variables such as gender and the positivity of biomarkers.

RESULTS

Demographic variables of participants

(Table I) shows the study sample, which included 75 (about 69%) males and 33 (about 31%) females. The mean age of participants was 66.56 ± 11.2 years.

Table I: Demographic variables of participants

Variable	Description
Gender (frequency, %):	
- *Male*	75 (69.4%)
- *Female*	33 (30.6%)
Age (M ± SD) years	66.56 ± 11.20

Frequency of positive biomarkers in CLL

As indicated in ***(Table II),*** the incidence of several biomarkers was investigated in patients with CLL using flow cytometry. CD19 was the most frequent (100%), followed by CD5 (99.07%). CD22 was the least frequent biomarker (0.9%). The remaining biomarkers were distributed in the range of 0.9% –100%.

Table II: Frequency of positive biomarkers in CLL

Biomarker	Frequency (N)	Percentage (%)
CD5	107	99.07%
CD19	108	100%
CD23	96	88.89%
CD20	88	81.48%
CD25	59	54.63%
CD200	100	92.60%

CD11C	27	25%
CD22	1	0.9%
CD79b	15	13.88%
CD10	27	25%
FMC7	55	50.93%
SIgM	37	34.26%
Anti-Kappa	15	13.88%
Anti-Lambda	20	18.52%

The mean age of study participants according to gender

As seen in ***(Figure 1)***, the mean age of females was 65.5 ± 8.68 years and that of males was 67.03 ± 12.16 years.

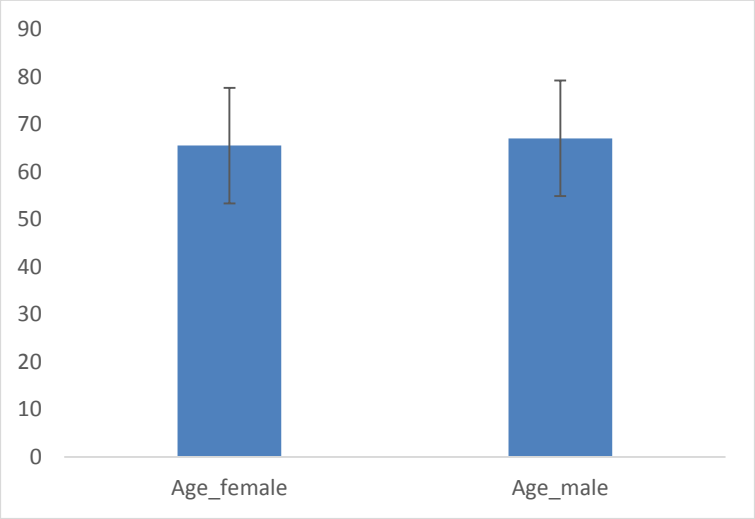

Figure 1: The mean age of study participants

Approximate positivity of CLL in female participants

As shown in (***Figure 2)***, the approximate positivity of CLL among female patients was 50% –60% in 14 patients. The positivity of 20% and 67% was seen in one patient each. The positivity of 40% occurred in two patients. The positivity in the range of 70% –75% was shown in eight patients. The positivity of 80% was seen in five patients. The highest level of positivity (85%) was seen in two patients.

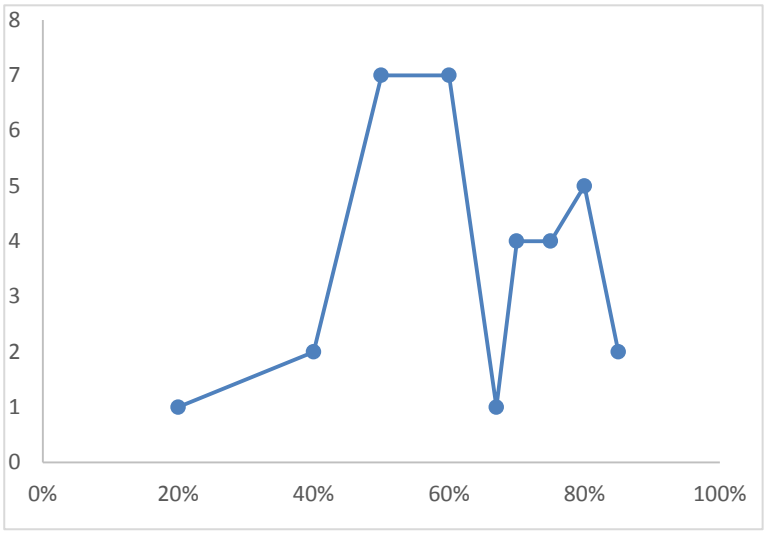

Figure 2: Approximate positivity of CLL in study female participants

Approximate positivity of CLL in male participants

As seen in (***Figure 3)***, positivity rates of 30%, 55%, and 69% were reported in one patient each, and positivity rates of 15% and 20% were each reported in two patients. Positivity of 50%, 60%, and 95% was reported in four patients each. Positivity of 35%, 65%, 75%, and 85% was reported in five patients each. Positivity of 80% and 90% was reported in six patients each. Positivity of 40% was reported in 11 patients, and the most frequent positivity of 70% was reported in 13 patients.

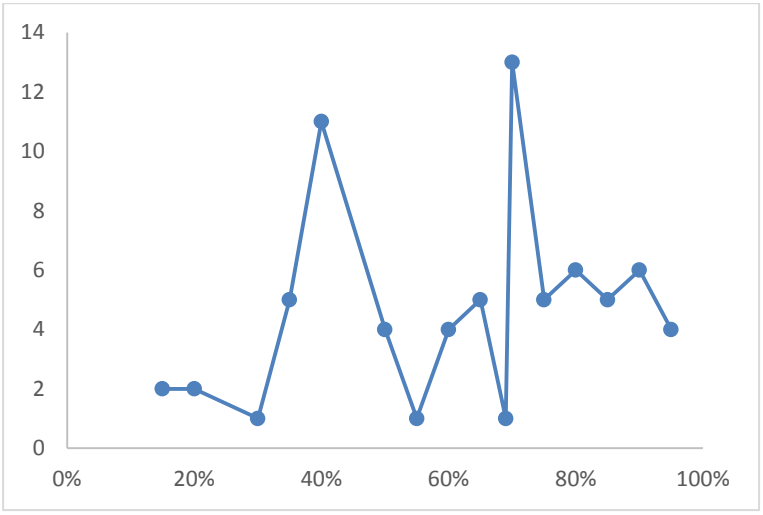

Figure 3: Approximate positivity of CLL in males

Positive biomarkers in female patients with CLL

As shown in (***Figure 4)***, the most frequent positive biomarker was CD5 in 32 patients (96.97%), followed by CD200 in 29 patients (87.78%), CD20 in 27 patients (81.81%), and CD23 in 26 patients (78.78%). The least frequent biomarker was CD22 in 1 patient (3.03%).

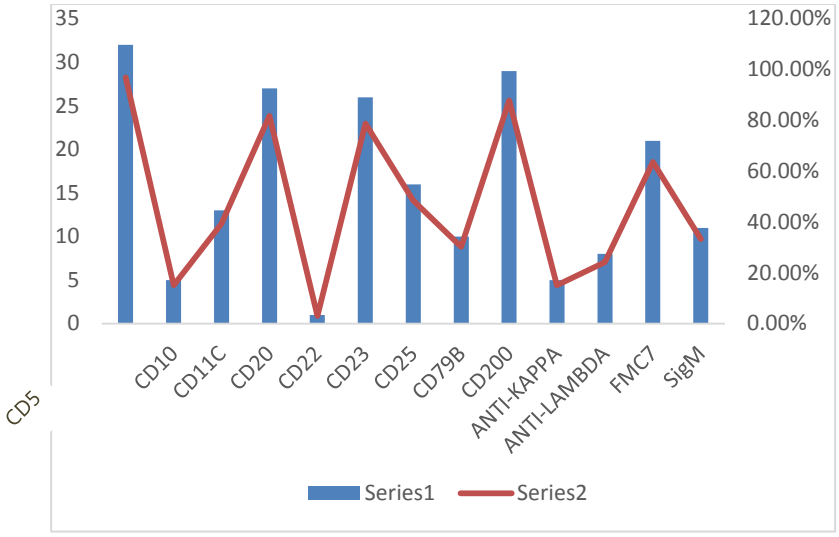

Figure 4: Positive biomarkers in female patients with CLL. Series 1 represents the frequency of positive biomarkers; series 2 represents the percentage of positive biomarkers

Positive biomarkers in male patients with CCL

As indicated in ***(Figure 5)***, CD5 and CD 19 were the most frequent biomarkers in all male patients with CLL. CD23 was positive in 69 patients (92%), CD200 in 61 patients (81%), CD20 in 51 patients (68%), FMC7 in 35 patients (47%), CD25 in 34 patients (45%), SIgM in 25 patients (33%), CD10 in 22 patients (29%), anti-kappa in 18 patients (24%), CD11C in 14 patients (19%), CD79B in 13 patients (17%), and anti-lambda in 12 patients (18%).

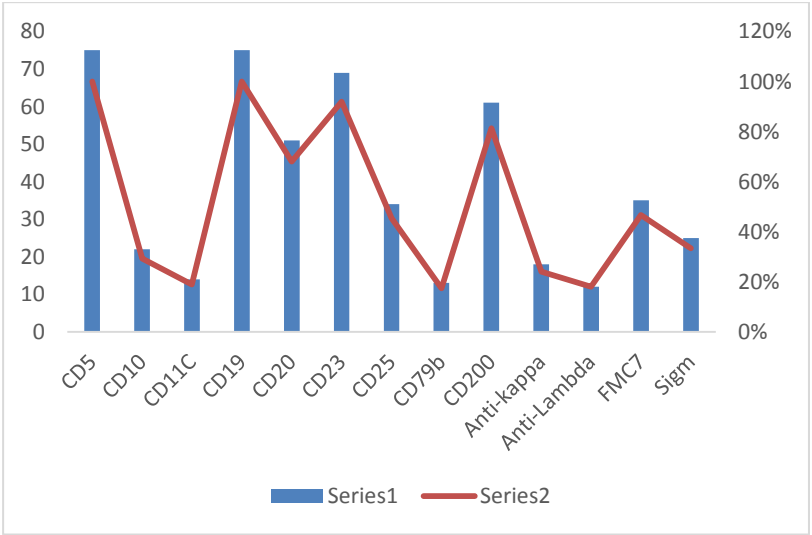

Figure 5: Positive biomarkers in male patients with CLL. Series 1 represents the frequency of positive biomarkers; series 2 represents the percentage of positive biomarkers

DISCUSSION

The results of this study showed that male patients are more likely to have CLL as compared to female ones, and the mean age lies in the elderly age. These findings are consistent with previous studies that showed males are more involved in CLL in addition to the onset of disease at an elderly phase [18, 19].

The results of the present study showed that each of the biomarkers CD5, CD19, and CD200 were found in almost all cases. CD20 and CD23 existed as well, but to a lesser extent. These biomarkers confirm the diagnosis of CLL as well as characterize its immunophenotypic features [2]. However, the results of this study are not completely consistent with other studies, which showed CD23 as the most characterized biomarker for cases of CLL [22, 23].

When patients with CLL were divided according to gender, the results revealed that males and females were both close to the elderly age group. This confirms that CLL is likely to occur in the elderly age range irrespective of gender [18, 19].

Regarding the approximate positivity of CLL among females (Figure 2), 70% positivity was most frequently reported. Among males, 70% positivity was the most frequent proportion as well. It seems that gender does not have an impact on the pathogenesis of CLL. In contrast, previous studies have revealed gender as a significant predictor for the outcome of CLL [24, 25]. However, the present study did not explore the same variables as reported in [24].

Positive biomarkers exhibited different biological behaviors among patients with CLL according to gender. Among females, the most prevalent biomarkers were CD5 and CD200, while CD22 was the least frequent biomarker (Figure 4). On the other hand, among males, CD5 and CD 19 were the most frequent biomarkers, while CD79B and anti-lambda were the least. These variations in biomarkers according to gender have not been well-established in literature. Some authors reported such a trend among patients with CLL for other biomarkers such as CD38 and p53 [24,26,27]. These variations could potentially explain whether the outcome of CLL is in favor of females; however, more studies are required for this.

CONCLUSION

The present study showed that patients with CLL have the main positive immunophenotyping patterns of CD5, CD19, and CD200. Considering gender, CD5 and CD200 were the most prevalent biomarkers in females, while they were CD5 and CD 19 among males.

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