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Laboratory analysis of 124 chronic lymphocytic leukemia cases: Single center study

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Abstract:

BACKGROUND: Chronic lymphocytic leukemia (CLL) is a disease characterized by an uncontrolled proliferation of malfunctioning mature B-lymphocytes. It represents a variable range of clinical presentation and prognoses. Understanding the demographic and cellular characteristics of CLL patients can aid in better disease management and treatment outcomes.

OBJECTIVES: To identifying hematological parameters and patterns and potential markers that could assist in better diagnosis and of CLL in the local population.

PATIENTS, MATERIALS AND METHODS: A retrospective analysis of 124 cases of newly diagnosed CLL was conducted. Data were collected from reports over 2 years (April 2022-April2024), including demographics, Hb levels, WBC counts, platelet counts and expression of CD markers (5, 19, 20, 23, 200, FMC7, LAIR1 and others) using flow cytometry on peripheral blood specimens. Based on FC findings, patients were categorized into two groups for better assessment; definite diagnosis CLL and possible diagnosis CLL; then further subdivided into poor prognosis CLL and good prognosis CLL.

RESULTS: Male patients represented 59.5% of cases and females were 40.5% with a median age of 61 years at diagnosis. The mean Hb level was 109 ± 2 g/L, the mean WBC count was 58.0 ± 25.0 × 10^9/L, and the mean platelet count was 190 ± 40 × 10^9/L. CD5 was positive in 97.6% of cases, with CD23 in 96%.

CONCLUSION: This study provides an overview of the demographic and cellular marker characteristics of CLL patients in FC unit. The findings underscore the heterogeneity of CLL immunophenotype and the importance of detailed characterization in improving patient management protocols; however, further research is warranted to correlate these findings with other parameters of disease burden and prognosis to optimize the therapeutic approaches for CLL patients.

Keywords:

Chronic lymphocytic leukemia, flow cytometry

Introduction

Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in adulthood.[1] The main underlying pathophysiology includes uncontrolled proliferation of functionally incompetent B cell lymphocytes in the blood, bone marrow (BM), and lymphoid tissues.[2] The heterogeneity of CLL can be attributed to a range of factors including patient demographics, percentage of leukemic cells, the expression of specific cellular markers such as cluster of differentiation (CD) antigens as well as molecular markers. In the context of demographic variations, this disease predominantly affects older adults with a median age of diagnosis of around 70 years with higher incidence in males compared to female patients.[3,4] On the other hand, CD markers are found to play a significant role in the diagnosis and prognosis of CLL. These markers such as CD5, CD19, CD20, and CD23 are used to identify and classify the neoplastic cells

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and to estimate prognosis and probable molecular abnormalities such as del (17p) and del (11q), providing essential information for the diagnosis and therapeutic decision-making.[4] CD20 expression can impact the effectiveness of treatments like anti-CD20 (rituximab).[5] On the other hand, the percentage of neoplastic cells can provide insights into the disease burden and progression which also play a role in patient stratification and management.[6-8] This study retrospectively analyzes data from newly diagnosed CLL cases, which included gender, age, hemoglobin (Hb) level, white blood cell (WBC) count, absolute lymphocyte count (ALC), platelet count, and percentage of neoplastic cells and CD markers. By examining these parameters, the study aims to contribute to the understanding of CLL demographic and biological characteristics in the studied population. There remains a need for localized studies to understand the epidemiological and clinical characteristics of CLL within specific populations. Baghdad Medical City Complex serves as a crucial health-care provider in Iraq, and the data from its flow cytometry (FC) unit can offer valuable insights into the presentation of CLL in this region. This analysis might help in identifying patterns and potential markers that could assist in better diagnosis and management of CLL in the local population.

Patients, Materials and Methods

Study design

This retrospective study aimed to characterize CLL patient cases as per demographics and cellular markers using data from the FC unit in Baghdad-Medical City. It was intended to analyze the demographic distribution (gender and age) of CLL patients, Hb level, WBC count, and platelet count (referred to as complete blood count [CBC] parameters in this article) in both genders; assess the percentage of neoplastic cells; and evaluate the expression of CD markers and their implications for diagnosis and prognosis.

Data collection

Data from 124 CLL cases of newly diagnosed over a period 2 years (April 2022 to April 2024) were retrospectively reviewed. Patient specimens were peripheral blood (PB) samples collected in 2 mL K3-EDTA tubes made in China (13 × 75 mm each). Information gathered included patient demographics (gender and age), CBC parameters and laboratory data of CD markers, and the percentage of leukemic cells among CD45-positive cells. To ensure that our sample was randomly selected and free from patient selection bias, our data were sourced from FC unit where all patients diagnosed with CLL over the past 2 years (April 2022 to April 2024) were recorded and a computer-generated random number list was used. An independent auditor verified the random selection process to confirm its adherence to the study protocol. To ensure the study had sufficient power to detect patterns and differences, we aimed for a sample size that would provide a power of 80% with a significance level of 0.05. This calculation was based on previous studies and literature that provided baseline data on the variability of the parameters of interest. CBC analyzers in our unit are Sysmex-330 6-part differential hematology analyzer made in Japan. PB samples were run on the BD FACS-Canto System, using FACS CANTO Diva software (Betcon-Dickinson) made in the USA.

Inclusion criteria

1. Newly diagnosed CLL cases
2. Meeting the FC diagnostic criteria for CLL based on analysis of PB samples[9]
3. Diagnostic characteristics included PB lymphocytosis (≥5000/uL) for 3 months or more, small mature B cell morphology with many smear cells, light chain restriction,[10] and positive expression of CD5, CD19, CD20, and C23 with CD20 and CD79b expression lower than normal mature B cell. Matutes score[11] was used (CD5 positive, CD23 positive, FMC7 negative or weak, sIg weak expression and CD79b negative or weakly expressed)
4. No treatment received yet.

Data variables

1. Demographics**:** gender and age of patients at the time of diagnosis
2. CBC parameters at the time of diagnosis
3. CD markers: specific CD markers analyzed included smCD3, CD4, CD8, CD56, TCRyd, smIg-lambda, sIgM, LAIR1, CD10, CD11c, CD81, FMC7, CD5, smIg-kappa, CD19, CD20, CD23, CD31, CD39, CD38, CD43, CD79b, CD200, CD27, and IgD
4. Percentage of leukemic cells: the proportion of leukemic cells among CD45-positive cells, expressed as a percentage.

Data analysis

Statistical Package for the Social Sciences version 29 program (IBM Corp., Armonk, NY) was used to detect the effect of difference factors in study parameters. Chi-square and Fisher's exact test were used to estimate the correlation between CDs and study parameters. Independent *t*-test was used to compare means, Mann–Whitney *U*-test was applied to compare medians. Pearson's correlation coefficient was used to estimate the correlation between percentage of abnormal cells in CD45/SS and patient's age.

Ethical considerations

Ethical approval for this retrospective study was obtained from the authority of the FC Unit at Baghdad Medical City Complex. Given the retrospective nature of the study, patient confidentiality and privacy were

strictly maintained, patient identifiers were removed from the records, and each was assigned a unique code (number) and this step was performed by an independent personnel uninvolved in the subsequent analysis. Besides, researchers analyzing the data were blinded to patient identities and other identifying information. Patients were interviewed and orally explained about nature of the study and they gave their informed consent prior to the study.

Results

A total of 124 subjects were studied. The normality of the quantitative data was confirmed using the Shapiro–Wilk test. The mean age for diagnosing CLL (mean ± standard deviation [SD]) was around 61.86 ± 11.53. Higher age for females 63.59 ± 11.82 than males 60.73 ± 11.27 with *P* value of 0.18. In terms of CBC parameters, there was a statistically significant difference between female and male patients and it is demonstrated in Table 1.

Patients were categorized based on their immunophenotyping (IPT) into two main groups: definite CLL and possible CLL. Within the definite CLL group, patients were further categorized based on their prognostic status into good prognosis CLL (GPCLL) and poor prognosis CLL (PPCLL). The distribution of subjects across these groups is presented in Table 2.

The majority of subjects were in the definite CLL group. In addition, the definite CLL group demonstrated higher number of patients with PP. The percentage of distribution of subjects of the study groups is illustrated in Figure 1.

Demographic statistics of the subjects were analyzed. The Shapiro–Wilk test was used to assess the normality of the quantitative data, revealing normal distributions across the study groups [Table 3].

There were no significant differences in age and gender between the definite CLL group and the possible CLL group, with *P* values of 0.83 and 1.00, respectively. Similarly, no significant differences in age and gender were observed between the subgroups of the definite CLL patients, with *P* values of 0.74 and 0.089, respectively.

In the present study, the correlation between the presence of CD antigens on the surface of leukocytes and the distribution of subjects between the definite CLL and possible CLL groups was analyzed. Chi-square and Fisher's exact tests were utilized to measure these correlations, with the *P* value of 95% confidence interval. The results were expressed as numbers and percentages, as shown in Table 4.

Table 1: Complete blood count parameters of patients included in the study presented as means±standard deviation

Parameter	All patients (*n*=124)	Male (*n*=74)	Female (*n*=50)	Test	*P*
Hb (g/L)	109±20	100±15	120±12	*t*-test	<0.05*
WBC count (×10^9/L)	58.0±25.0	40±15.0	84±20.0	*t*-test	<0.05*
ALC (×10^9/L)	26.0±10	20±8.0	35±9.0	*t*-test	<0.05*
Platelet count (×10^9/L)	190±40	213±35	157±30	*t*-test	<0.05*

*Significant difference. Hb=Hemoglobin, WBC=White blood cell, ALC=Absolute lymphocyte count

Table 2: Patients categorization based on flow cytometry markers-diagnosis

	Definite CLL		Possible CLL
Subjects (*n*)	113		11
	GPCLL	PPCLL	
	42	71	

n=Number of samples, CLL=Chronic lymphocytic leukemia, GPCLL=Good prognosis CLL, PPCLL=Poor prognosis CLL

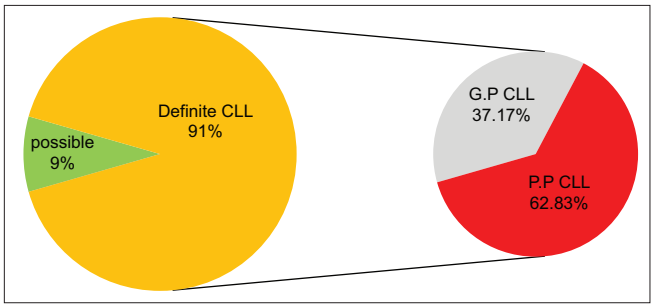

Figure 1: Percentage of patients' distribution into definite and possible chronic lymphocytic leukemia (CLL) groups. Definite CLL group also included good and poor prognosis cases. CLL: Chronic lymphocytic leukemia, GP: Good prognosis, PP: Poor prognosis

A significant positive correlation was found between the presence of CD11c, CD81, CD31, and CD43 antigens on the surface of leukocytes and the distribution of subjects between definite CLL and possible CLL groups. Specifically, CD11c, CD81, CD31, and CD43 antigens were present in 45.1%, 83.2%, 73.5%, and 98.2% of definite CLL patients, respectively. In contrast, these antigens were present in only 9.1%, 36.4%, 36.4%, and 81.8% of possible CLL patients, respectively. No significant correlation was observed for the remaining CD antigens listed in Table 3.

In addition, the study examined the correlation between the presence of CD antigens on the surface of leukocytes and the classification of subjects as GPCLL and PPCLL groups. Chi-square and Fisher's exact tests were used to measure these correlations, with 95% confidence interval. The results were expressed as numbers and percentages [Table 5].

There was a significant positive correlation between the presence of sIgM, smIg-lambda, CD81, smIg-kappa, CD31,

Table 3: Demographic statistics of subjects in the study groups

Variables	Definite CLL		Possible CLL	Test	Value	*P*
Age (years), mean±SD	61.79±11.65		62.54±10.73	*t*	0.21	0.83 (NS)
	GPCLL	PPCLL				
	62.26±11.07	61.52±12.04		*t*	–0.33	0.74 (NS)
Gender, *n* (%)						
Male	68 (60.2)		7 (63.6)	Fisher's exact	0.05	1.00 (NS)
Female	45 (39.8)		4 (36.4)			
Variables	**GPCLL**	**PPCLL**	**Possible CLL**	**Test**	**Value**	***P***
Gender, *n* (%)						
Male	21 (50)	47 (66.2)		Chi-square	2.88	0.089 (NS)
Female	21 (50)	24 (33.8)				

NS=Nonsignificant difference, *n*=Number of samples, CLL=Chronic lymphocytic leukemia, CLL=Chronic lymphocytic leukemia, GPCLL=Good prognosis CLL, PPCLL=Poor prognosis CLL

Table 4: Correlation of cluster of differentiation antigens and patients classification into definite-chronic lymphocytic leukemia and possible-chronic lymphocytic leukemia groups

	Variables		Definite CLL, *n* (%)	Possible CLL, *n* (%)	Test	Value	*P*
CD10	smCD3	Negative	113 (100)	11 (100)	-	-	-
CD19	CD4	Positive	0	0			
CD20	CD8						
TCRyd	CD56						
SIgM		Negative	59 (52.2)	5 (45.5)	Chi-square	0.183	0.66 (NS)
		Positive	54 (47.8)	6 (54.5)			
SmIglambda		Negative	84 (74.3)	9 (81.8)	Fisher's exact	-	0.72 (NS)
		Positive	29 (25.7)	2 (18.2)			

CLL=Chronic lymphocytic leukemia, NS=Nonsignificant, CD=Cluster of differentiation

CD38, and CD79b antigens and the percentage of patients in the PP subgroup. These antigens were present in 59.2%, 32.4%, 93.0%, 50.7%, 93.0%, 28.2%, and 56.3% of PP patients, respectively. Conversely, these antigens were present in only 28.6%, 14.3%, 66.7%, 26.2%, 40.5%, 4.8%, and 14.3% of GP patients, respectively. Furthermore, a significant positive correlation was found between the presence of LAIR1, FMC7, and CD27 antigens and the percentage of patients in the GP subgroup with positivity in 64.3%, 7.1%, and 52.4% of patients, respectively, compared to 28.2%, 0.0%, and 32.4% of PP patients, respectively. No significant correlation was observed for the remaining CD antigens [Table 6]. On the other hand, the study investigated the correlation between the presence of CD antigens and the gender of with PPCLL and GPCLL patients. Chi-square and Fisher's exact tests were used, with 95% confidence interval. The results are shown in Table 7.

Nonsignificant correlation was found between the expression of CD antigens and the gender of both PP and GP patients, with the exception of smIg-kappa as a significant positive correlation was observed for it, where positive expression was seen in 61.7% of male patients compared to 29.2% of female patients in the PPCLL subgroup. In addition to that, the current study analyzed patients age with respect to CD antigens expression in the 2 groups. A *t*-test was used to compare the means, with a 95% confidence interval. The results were expressed as mean ± SD [Table 8].

Nonsignificant difference was found in CD markers expression regarding patients age. Positive CD antigens expression was seen in both GP and PP groups. An exception for this was observed for the smIg-kappa antigen in the GPCLL subgroup. The mean age of patients with the smIg-kappa antigen expression was significantly higher than those without this antigen, with a *P* value of 0.03. In addition, a significant decrease in the mean age was noted for patients with positive IgD expression in the PPCLL subgroup compared to those without the IgD marker, also with a *P* value of 0.03. Finally, the percentage of abnormal cells in the CD45/SSC gate dot plot was compared between the PP and the GP subgroups using a *t*-test showing a nonsignificance difference (PP 0.67 ± 0.15 and GP 0.65 ± 0.17 with *P* value of 0.51). This parameter was also assessed in comparison between male and female patients (male patients 0.67 ± 0.15, female patients 0.65 ± 0.17), showing a nonsignificant difference. The percentage of abnormal cells in CD45/SSC gate dot blot in relation to patients age was studied using Pearson's correlation coefficient and showed a nonsignificant difference (r = 0.014 and *P* = 0.876).

Discussion

CLL is a chronic clonal disorder characterized by uncontrolled proliferation and cessation of apoptosis of mature B lymphocyte, diagnosed based on the criteria

Table 5: Correlation of leukocytes cluster of differentiation antigens and patients distribution into definite chronic lymphocytic leukemia and possible chronic lymphocytic leukemia groups

Variables	Definite CLL, *n* (%)	Possible CLL, *n* (%)	Test	Value	*P*
LAIR1					
Negative	66 (58.4)	8 (72.7)	Fisher's exact	-	0.35 (NS)
Positive	47 (41.6)	3 (40.3)			
CD11c					
Negative	62 (54.9)	10 (90.9)	Fisher's exact	-	0.021*
Positive	51 (45.1)	1 (9.1)			
CD81					
Negative	19 (16.8)	7 (63.6)	Fisher's exact	-	<0.001*
Positive	94 (83.2)	4 (36.4)			
FMC7					
Negative	110 (9.3)	10 (90.9)	Fisher's exact	-	0.31 (NS)
Positive	3 (2.7)	1 (9.1)			
CD5					
Negative	1 (0.9)	0	Fisher's exact	-	1.00 (NS)
Positive	112 (99.1)	11 (100)			
SmIg-kappa					
Negative	66 (58.4)	9 (81.8)	Fisher's exact	-	0.19 (NS)
Positive	47 (41.6)	2 (18.2)			
CD23					
Negative	2 (1.8)	1 (9.1)	Fisher's exact	-	0.24 (NS)
Positive	111 (98.2)	10 (90.9)			
CD31					
Negative	30 (26.5)	7 (63.6)	Fisher's exact	-	0.016*
Positive	83 (73.5)	4 (36.4)			
CD39					
Negative	66 (58.4)	8 (72.7)	Fisher's exact	-	0.52 (NS)
Positive	47 (41.6)	3 (27.3)			
CD38					
Negative	91 (80.5)	11 (100)	Fisher's exact	-	0.21 (NS)
Positive	22 (19.5)	0			
CD43					
Negative	2 (1.8)	2 (18.2)	Fisher's exact	-	0.039*
Positive	111 (98.2)	9 (81.8)			
CD79b					
Negative	67 (59.3)	7 (63.6)	Fisher's exact	-	1.00 (NS)
Positive	46 (40.7)	50 (40.3)			
CD200					
Negative	1 (0.9)	1 (9.1)	Fisher's exact	-	0.17 (NS)
Positive	112 (99.1)	10 (90.9)			
CD27					
Negative	68 (60.20)	9 (81.8)	Fisher's exact	-	0.20 (NS)
Positive	45 (49.8)	2 (18.2)			
IgD					
Negative	83 (73.5)	7 (63.6)	Fisher's exact	-	0.49 (NS)
Positive	30 (26.5)	4 (36.4)			

*Significant correlation. NS=Nonsignificant correlation, *n*=Number of samples, CLL=Chronic lymphocytic leukemia, LAIR1=Leukocyte-associated immunoglobulin-like receptor-1, CD=Cluster of differentiation

of having ≥5 × 10^9/L clonal B lymphocytes in PB sustained for at least 3 months; the clonality of these B lymphocytes needs to be confirmed by demonstrating immunoglobulin light chain restriction using FC.[9] In

Table 6: Correlation between cluster of differentiation antigens and patient classification into good prognosis and poor prognosis chronic lymphocytic leukemia subgroups

Variables	PPCLL, *n* (%)	GPCLL, *n* (%)	Test	Value	*P*
SIgM					
Negative	29 (40.8)	30 (71.4)	Chi-square	9.89	0.002*
Positive	42 (59.2)	12 (28.6)			
SmIglambda					
Negative	48 (67.6)	36 (85.7)	Chi-square	4.53	0.033*
Positive	23 (32.4)	6 (14.3)			
LAIR1					
Negative	51 (71.8)	15 (35.7)	Chi-square	14.17	<0.001*
Positive	20 (28.2)	27 (64.3)			
CD11c					
Negative	35 (49.3)	27 (64.3)	Chi-square	2.39	0.12 (NS)
Positive	36 (50.7)	15 (35.7)			
CD81					
Negative	5 (7.0)	14 (33.3)	Chi-square	13.04	<0.001*
Positive	66 (93.0)	28 (66.7)			
FMC7					
Negative	71 (100)	39 (92.9)	Fisher's exact	-	0.049*
Positive	0	3 (7.1)			
CD5					
Negative	0	1 (2.4)	Fisher's exact	-	0.37 (NS)
Positive	71 (100)	41 (97.6)			
smIg-kappa					
Negative	35 (49.3)	31 (73.8)	Chi-square	6.52	0.017*
Positive	36 (50.7)	11 (26.2)			
CD23					
Negative	1 (1.4)	1 (2.4)	Fisher's exact	-	1.00 (NS)
Positive	70 (98.6)	41 (97.6)			
CD31					
Negative	5 (7.0)	25 (59.5)	Chi-square	37.27	<0.001*
Positive	66 (93.0)	17 (40.5)			
CD39					
Negative	45 (63.4)	21 (50.0)	Chi-square	1.94	0.16 (NS)
Positive	26 (36.6)	21 (50.0)			
CD38					
Negative	51 (71.8)	40 (95.2)	Chi-square	9.22	<0.002*
Positive	20 (28.2)	2 (4.8)			
CD43					
Negative	2 (2.8)	0	Fisher's exact		0.52 (NS)
Positive	69 (97.2)	42 (100)			
CD79b					
Negative	31 (43.7)	36 (85.7)	Chi-square	19.33	<0.001*
Positive	40 (56.3)	6 (14.3)			
CD200					
Negative	0	1 (2.4)	Fisher's exact	-	0.37 (NS)
Positive	71 (100)	41 (97.6)			
CD27					
Negative	48 (67.7)	20 (47.6)	Chi-square	4.39	0.04*
Positive	23 (32.4)	22 (52.4)			
IgD					
Negative	55 (77.5)	28 (66.7)	Chi-square	1.57	0.20 (NS)
Positive	16 (22.50	14 (33.3)			

*Significant correlation. NS=Nonsignificant correlation, *n*=Number of samples, CLL=Chronic lymphocytic leukemia, GPCLL=Good prognosis CLL, PPCLL=Poor prognosis CLL, LAIR1=Leukocyte-associated immunoglobulin-like receptor-1, CD=Cluster of differentiation

Table 7: Correlation between cluster of differentiation markers and patients gender in poor and good prognosis groups

Variables	PPCLL		Test	Value	*P*	GPCLL		Test	Value	*P*
	Male, *n* (%)	Female, *n* (%)				Male, *n* (%)	Female, *n* (%)			
SmIglambda										
Negative	35 (74.5)	13 (54.2)	Chi-square	2.99	0.08 (NS)	18 (85.7)	18 (85.7)	Fisher's exact		1.00 (NS)
Positive	12 (25.5)	11 (45.8)				3 (14.3)	3 (14.3)			
sIgM										
Negative	17 (36.2)	12 (50.0)	Chi-square	1.25	0.26 (NS)	15 (71.4)	15 (71.4)	Chi-square	0.00	1.00 (NS)
Positive	30 (63.8)	12 (50.0)				6 (28.6)	6 (28.6)			
LAIR1										
Negative	31 (66)	20 (83.3)	Chi-square	2.37	0.12 (NS)	8 (38.1)	7 (33.3)	Chi-square	0.10	0.74 (NS)
Positive	16 (34)	4 (16.7)				13 (61.9)	14 (66.7)			
CD11c										
Negative	22 (46.8)	13 (54.2)	Chi-square	0.34	0.55 (NS)	11 (52.4)	16 (76.2)	Chi-square	2.59	0.10 (NS)
Positive	25 (53.2)	11 (45.8)				10 (47.6)	5 (23.8)			
CD81										
Negative	3 (6.4)	2 (8.3)	Fisher's exact		1.00 (NS)	8 (38.1)	6 (28.6)	Chi-square	0.42	0.51 (NS)
Positive	44 (93.6)	22 (91.7)				13 (61.9)	15 (71.4)			
FMC7										
Negative	47 (100)	24 (100)	Chi-square	0.00	1.00 (NS)	21 (100)	18 (85.7)	Fisher's exact		0.06 (NS)
Positive	0	0				0	3 (14.3)			
CD5										
Negative	0	0	Chi-square	0.00	1.00 (NS)	1 (4.8)	0	Fisher's exact		1.00 (NS)
Positive	47 (100)	24 (100)				20 (95.2)	21 (00)			
smlg-kappa										
Negative	18 (38.3)	17 (70.8)	Chi-square	6.72	0.009*	14 (66.7)	17 (81)	Chi-square	1.10	0.29 (NS)
Positive	29 (61.7)	7 (29.2)				7 (33.3)	4 (19)			
CD23										
Negative	1 (2.1)	0	Fisher's exact		1.00 (NS)	0	1 (4.8)	Fisher's exact		1.00 (NS)
Positive	46 (97.9)	24 (100)				21 (100)	20 (95.2)			
CD31										
Negative	3 (6.4)	2 (8.3)	Fisher's exact		1.00 (NS)	11 (52.4)	14 (66.7)	Chi-square	0.88	0.34 (NS)
Positive	44 (93.6)	22 (91.7)				10 (47.6)	7 (33.3)			
CD39										
Negative	29 (61.7)	16 (66.7)	Chi-square	0.16	0.68 (NS)	11 (52.4)	10 (47.6)	Chi-square	0.095	0.75 (NS)
Positive	18 (38.3)	8 (33.3)				10 (47.6)	11 (52.4)			
CD38										
Negative	31 (66.0)	20 (83.3)	Chi-square	2.37	0.12 (NS)	20 (95.2)	20 (95.2)	Fisher's exact		1.00 (NS)
Positive	16 (34.0)	4 (16.7)				1 (4.8)	1 (4.8)			
CD43										
Negative	2 (4.3)	0	Fisher's exact		0.54 (NS)	21 (100)	21 (100)	Chi-square	0.00	1.00 (NS)
Positive	45 (95.7)	24 (100)				0	0			
CD79b										
Negative	16 (34.0)	15 (62.5)	Chi-square	5.23	0.37 (NS)	17 (81.0)	19 (90.5)	Fisher's exact		0.66 (NS)
Positive	31 (66.0)	9 (37.5)				4 (19.0)	2 (9.5)			
CD200										
Negative	0	0	Chi-square	0.00	1.00 (NS)	1 (4.8)	0	Fisher's exact		1.00 (NS)
Positive	47 (100)	24 (100)				20 (95.2)	21 (100)			
CD27										
Negative	29 (61.7)	19 (79.2)	Chi-square	2.21	0.13 (NS)	11 (52.4)	9 (42.9)	Chi-square	0.38	0.53 (NS)
Positive	18 (38.3)	5 (20.8)				10 (47.6)	12 (57.1)			
IgD										
Negative	36 (76.6)	19 (79.2)	Chi-square	0.06	0.80 (NS)	14 (66.7)	14 (66.7)	Chi-square	0.00	1.00 (NS)
Positive	11 (23.4)	5 (20.8)				7 (33.3)	7 (33.3)			

*Significant correlation. NS: Nonsignificant correlation, CLL=Chronic lymphocytic leukemia, GPCLL=Good prognosis CLL, PPCLL=Poor prognosis CLL, CD=Cluster of differentiation, LAIR1=Leukocyte-associated immunoglobulin-like receptor-1

Table 8: Correlation between cluster of differentiation markers and patients age in poor prognosis and good prognosis subgroups

Variables	*n* (%)	PPCLL	*t*	*P*	GPCLL	*t*	*P*
		Age (mean±SD)			Age (mean±SD)		
SmIglambda							
Negative		60.95±12.22	0.57	0.57 (NS)	62.22±11.09	0.05	0.95 (NS)
Positive		62.69±11.85			62.50±12.02		
sIgM							
Negative		60.37±14.40	0.66	0.51 (NS)	60.76±12.39	1.88	0.06 (NS)
Positive		62.30±10.21			66.00±5.59		
LAIR1							
Negative		60.19±13.09	0.36	0.71 (NS)	60.06±9.66	1.01	0.31 (NS)
Positive		62.35±9.07			63.48±11.78		
CD11c							
Negative		62.68±12.20	−0.80	0.42 (NS)	62.40±11.41	−0.11	0.91 (NS)
Positive		60.38±11.95			62.00±10.82		
CD81							
Negative		61.00±16.91	0.073	092 (NS)	62.64±10.77	−0.15	0.87 (NS)
Positive		61.56±11.77			62.07±11.41		
FMC7							
Negative		61.52±12.04	-	-	62.12±10.92	0.20	0.85 (NS)
Positive		-			64.00±15.58		
CD5							
Negative		-	-	-	-	-	-
Positive		61.52±12.04			62.43±11.15		
smlg-kappa							
Negative		61.11±12.50	0.27	0.78 (NS)	60.48±11.71	2.21	0.03*
Positive		61.91±11.74			67.27±7.37		
CD23							
Negative		-	-	-	-	-	-
Positive		61.35±12.05			62.31±11.21		
CD31							
Negative		63.8±12.61	0.42	0.69 (NS)	61.64±11.65	0.44	0.65 (NS)
Positive		61.34±12.08			63.17±10.44		
CD39							
Negative		61.28±10.83	0.19	0.84 (NS)	60.76±12.92	0.87	0.38 (NS)
Positive		61.92±14.12			63.76±8.93		
CD38							
Negative		61.13±12.10	0.42	0.67 (NS)	62.47±11.29	−1.28	0.33 (NS)
Positive		62.50±12.14			58.00±4.24		
CD43							
Negative		68.00±2.82	−2.68	0.09 (NS)	-	-	-
Positive		61.33±12.16			62.26±11.07		
CD79b							
Negative		61.45±11.60	−0.04	0.96 (NS)	61.61±11.68	1.54	0.14 (NS)
Positive		61.57±12.52			66.16±5.41		
CD200							
Negative		-	-	-	-	-	-
Positive		61.52±12.04			62.36±11.19		
CD27							
Negative		61.87±11.96	0.35	0.72 (NS)	60.35±11.94	1.06	0.29 (NS)
Positive		60.78±12.45			64.00±10.19		
IgD							
Negative		62.85±12.64	2.16	0.03*	60.96±11.68	1.14	0.26 (NS)
Positive		56.938.52			64.85±9.62		

*Significant deference. NS=Nonsignificant deference, CLL=Chronic lymphocytic leukemia, GPCLL=Good prognosis CLL, PPCLL=Poor prognosis CLL, LAIR1=Leukocyte-associated immunoglobulin-like receptor-1, CD=Cluster of differentiation

CLL, B lymphocytes express the surface antigen CD5 along with the B-cell antigens CD19, CD20, and CD23. The levels of surface immunoglobulin, CD20, and CD79b are characteristically low compared with those found on normal B cells.[10-12] A recommended approach using markers to refine diagnosis in borderline cases (CD43, CD79b, CD81, CD200, CD10, and ROR1) has been suggested.[13] This study supported the established knowledge in literature that CLL occurs more frequently with increasing age, more frequently in males and is diagnosed based on the aforementioned CD markers. In this observational laboratory study which involved the analysis of 124 cases of CLL, the average age of diagnosing CLL was around 60 years agreeing with a study in Kerbala[14] and with the average age of CLL diagnosis in Iraq.[15] Male patients represented around 59% of all patients, while female patients were around 41%, indicating a higher incidence in males.[1] There has been a significant difference in values of CBC parameters (Hb, WBC, platelets, and ALC) between female and male patients as shown in Table 1. In this study, male patients have shown an Hb value less than those of female patients, while WBC count and ALC were lower. The higher Hb level for females despite higher WBC and lymphocyte count can be attributed to different factors, it is possible that female patients had a slower disease progression allowing for the maintenance of higher Hb level despite higher WBC and lymphocyte counts and this could reflect a different interaction between disease burden and BM function in females compared to males. Second, females may have had a better BM reserve or compensatory mechanisms allowing them to maintain higher Hb levels. The average age for diagnosing CLL was a menopause age with the absence of menstrual blood loss. Moreover, higher WBC and ALC may not indicate a more aggressive or acute phase of CLL in females; the disease might have been more chronic and indolent, thus not having a significant impact on Hb levels. The elevated WBC and ALC might have triggered a compensatory increase in erythropoiesis in some females, and finally, the observed difference might also be due to variability within the sample, a larger sample size or a more detailed stratification might reveal that this difference is not statistically significant, as a study in Pakistan[16] or is limited to a specific subset of patients. Platelet count was normal for both genders and was higher for male patients than female patients and platelet count was generally within the normal range, higher in female patients in the Pakistani study. In 2001 by Mukiibi *et al*. showed a lower platelet count,[17] and Sengul *et al*.[18] had median WBC count and ALC 70,600/µl and 51,490/µl, respectively, with anemia in 26% of patients and thrombocytopenia in 18%. Indian study in 2007 showed anemia in 26% of participants and thrombocytopenia in 18%.[19] In Sudan, a study demonstrated that WBC count was inversely correlated with age and highest means for ALC were seen in young males and lowest in elder females with no significant correlation with age and gender, thrombocytopenia was seen in 39.1% of patients and anemia in 34.5% of patients.[20] In the current study, patients were divided into two main groups depending only on IPT-based diagnosis: definite CLL and possible CLL groups [Table 2 and Figure 1]. Definite diagnosis had + 4 points on Matutes score,[11] while possible CLL cases got less than 4 points, presented with atypical morphology or demonstrated unusual IPT, needing other markers to confirm the diagnosis and exclude other possibilities as monoclonal B lymphocytosis MBL, mantle cells lymphoma MCL, splenic B-cell lymphoma/leukemia with prominent nucleoli (SBLLPN) previously known as prolymphocytic leukemia PLL, through total clonal cell count for MBL,[21] immunohistochemical and genetic testing of MCL,[22] and morphology and clinical features for SBLLPN.[23,24] Furthermore, the definite CLL patients were categorized into GP and PP [Table 3 and Figure 1], also depending only on FC finding. High sIgM levels have been associated with shorter response to treatment,[25] CD38 with aggressive disease,[26] CD79b with early disease progression,[27] and LAIR1 (CD 305) is higher in patients with low-risk CLL, being an independent prognostic marker for GPCLL cases.[28-30] The intensity of CD20 expression correlated with FMC7 and low scores.[31] FMC7 higher expression has been associated with more indolent disease course and better prognosis agreeing with El-Sebaie *et al*.[32] This kind of classification provided a structured framework for understanding the heterogeneity within CLL patient population. Despite their significant prognostic importance, ZAP70 and CD38 were not studied due to the shortage in stock resulting from fluctuating financial resources. On the other hand, the categorization of CLL cases and its prognostic variations have been shown not to influenced by demographic factors as age gender between the definite CLL and possible CLL groups, nor between the GP and PP subgroups, agreeing with Catovsky D's *et al*.[33] The correlation between CD antigens and CLL groups is demonstrated in Tables 4 and 5. In this study, it was noted that CD11c, CD81, CD31, and CD43 antigens were present in 45.1%, 83.2%, 73.5%, and 98.2% of definite CLL patients, respectively; meanwhile, these antigens were present in only 9.1%, 36.4%, 36.4%, and 81.8% of possible CLL patients, respectively. This significant positive correlation indicates that these antigens may serve as reliable markers for confirming a diagnosis of CLL, enhancing diagnostic accuracy. CD81 as well as bright CD43 differentiates CLL from MCL,[22,34] while CD11c expression in CLL has been associated with prolonged immune and coagulation stilmulation, increased inflammation and with lymphadenopathy and splenomegaly.[35,36] On the other hand, CD31 expression has been associated with better prognosis in CLL patients

as it serves as a physiological ligand for CD38.[37] The presence of CD markers in both GP and PPCLL groups has been studied. Chi-square and Fisher's exact tests were used to measure the correlations, with 95% confidence interval *P* value. There was a significant positive correlation between the presence of sIgM, smIg-lambda, smIg-kappa, CD81, CD31, CD38, and CD79b antigens the percentage of patients in the PPCLL subgroup, in addition to a significant positive correlation between presence of LAIR1, FMC7, and CD27 antigens patients number the GP CLL subgroup. CD27 has a higher expression in ZAP-70-positive cases whose levels correlate with CLL capacity to adhere to stromal cells with antibody blockade of CD27 impairs CLL binding to stromal cells[38] besides, FMC7 being of greater diagnostic value than CD20 for the purpose of distinguishing CLL from other B-cell disorders.[31] Interestingly, nonsignificant correlation was found in the expression of most CD antigens and patients gender and age in both PP and GP subgroups, except for smIg-kappa where male patients with PPCLL showed a higher prevalence of smIg-kappa compared to female patients, which may suggest a gender-specific expression pattern for this antigen in the context of PP, and smIg-kappa in the GP CLL subgroup and IgD in the PPCLL subgroup in respect to age [Tables 7 and 8]. In the GP group, the mean age of patients with smIg-kappa was significantly higher, while in the PP group, patients with IgD expression were significantly younger. These age-related differences suggest potential variations in the disease's biological behavior based on antigen expression. Further studies are needed.

Conclusion

This observational laboratory study characterized CLL patients in Baghdad, focusing on demographic, hematological, and immunophenotypic parameters, offering valuable insights for understanding and managing CLL in the region. The randomized sampling method minimized selection bias, ensuring a representative sample from a reputable diagnostic institution. However, limitations include the potential lack of generalizability to other regions, reliance on existing records, and a smaller sample size. The study's scope was also limited by the absence of key prognostic factors, suggesting the need for future multicenter and longitudinal studies incorporating genetic, molecular analyses, and staging for more comprehensive insights.

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Conflicts of interest

There are no conflicts of interest.

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