Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Evaluation of blood components and their association with Interleukin-18 in chronic myeloid leukemia

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Article Info

Article history:

Received: January 03, 2025
Accepted: March 07, 2025
Published: April 30, 2025

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Abstract

This study investigated the association between Chronic Myeloid Leukemia (CML), Interleukin-18 (IL-18), and blood components. A case-control, multi-center trial was conducted from November 12, 2023, to August 8, 2024, including 134 CML patients and 44 healthy controls. Results indicated a statistically significant difference between the control group and CML patients in IL-18 levels, platelet count (PLT), and white blood cell count (WBC) ($p = 0.048$, 0.033, and 0.029, respectively). A significant age difference was also observed between the control group and patients ($p = 0.0441$). Furthermore, there was a highly significant difference in age distribution (>40, 40-60, <60 years) between the two groups ($p = 0.0001$). Significant differences were also found in PDW, RBC, MCHC, RDW-CV, MCH, HCT, and PCT levels ($p = 0.0001$). MPV and RDW-SD also showed significant differences between groups ($p = 0.0006$ and 0.0498, respectively). Finally, a significant difference was observed in age distribution (less than 40, 40-60, and more than 60 years) between the two groups ($p=0.048$). These findings suggest that IL-18 and specific blood components may play a role in the pathogenesis of CML.

Keywords: CML, IL-18, Blood components, Hematological Parameters, Leukemia

1. Introduction

Chronic myelogenous leukemia (CML), sometimes referred to as chronic myeloid leukemia, is a white blood cell (WBC) malignancy. It is characterized by increased and uncontrolled myeloid cell growth in the bone marrow, their accumulation in the blood, and the proliferation of mature granulocytes (neutrophils, eosinophils, and basophils). The rise in basophils is a clinically significant feature of this myeloproliferative neoplasm. Because CML can often skip stages, how the disease manifests depends on the stage upon diagnosis. About 90% of patients receive their diagnosis when they are in the chronic stage. In some situations, a high WBC count may coincidentally lead to a diagnosis [1].

Tyrosine-kinase inhibitors (TKIs), which are targeted medications used to treat CML, have significantly increased long-term survival rates since 2001. Males are more often than females to be diagnosed with CML, and the median age at diagnosis is 65 years old. A risk factor for these people is ionizing radiation exposure, with the peak exposure effects appearing around ten years later. The Philadelphia chromosome translocation, a blatant genetic anomaly, was the first malignancy to be connected to CML [2].

CML is commonly divided into three stages based on test results and clinical characteristics. In the absence of treatment, CML often begins in the chronic phase, progresses over several years into an accelerated phase, and ends in a blast crisis. The final stage of chronic myeloid leukemia, called blast crisis, presents clinically as acute leukemia. A full blood count with differential should be part of laboratory blood tests at the time of diagnosis. Before choosing a first-line treatment, further assessment involves calculating the risk score if chronic phase CML is confirmed. It's important to distinguish CML from other causes of granulocytic leukocytosis, like infections or medications. Additionally, there won't be any basophilia. Leukocytosis and thrombocytosis can be symptoms of other myeloproliferative diseases such as polycythemia vera (PV) and chronic neutrophilic leukemia (CNL) [3].

Interleukins (ILs) are a class of cytokines, which are secreted proteins and signal molecules, expressed and secreted by WBCs (leukocytes) and certain other bodily cells. Over fifty interleukins and associated proteins are encoded in the human genome. Interleukins are essential for the immune system's operation, and unusual deficits

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Doi: http://dx.doi.org/10.14715/cmb/2025.71.4.10

of some of them have been identified; these include autoimmune disorders and immunological deficiencies. For example, interleukin 10 (IL-10), or human cytokine synthesis inhibitory factor (CSIF), is a cytokine that reduces inflammation. IL10 is encoded by the human interleukin-10 gene. The human IL-18 gene encodes the protein (IL-18) [4].

In this instance, the IL-18 gene lacks this signal peptide, much like other members of the IL-1 family. The most prevalent form of autoimmune hypothyroidism, Hashimoto's thyroiditis (H.T.), is linked to IL-18 as an inflammatory mediator. In response to interferon-gamma, IL-18 is elevated. Additionally, it has been discovered that IL-18 increases the synthesis of amyloid-beta in human neuron cells, which is linked to Alzheimer's disease. Additionally linked to the excretion of urine proteins, IL-18 may serve as a marker for evaluating the course of diabetic nephropathy. When compared to healthy individuals and diabetic patients with normoalbuminuria, this interleukin was likewise markedly higher in patients with microalbuminuria and macroalbuminuria. Following an intracerebral hemorrhage, IL-18 plays a role in the neuroinflammatory response [5].

The blood test determines of volume percentage (vol %) of red blood cells (RBCs) in blood, as is known as the haematocrit (Ht or HCT). Where determine the measurement occur by the size and quantity of red blood cells. The range from 36.1 to 44.3% for women and 40.7 to 50.3% for men is typical. Together with haemoglobin concentration, WBC count, and PLT count, it is a component of an individual's complete blood count findings [6].

The mean cell volume (MCV) is a measurement of the average volume of a red blood cell. The measurement is computed by dividing the volume of erythrocytes by the blood volume multiplied by the percentage of cellular blood. The mean corpuscular volume is a common component of a full blood count. The MCV measurement is what enables the classification of anaemia patients as having either macrocytic anaemia (MCV above normal range), normocytic anaemia (MCV within normal range), or microcytic anaemia (MCV below normal range). It can be used to determine the red blood cell distribution width (RDW) for additional specification. The RDW is a statistical computation that represents the size variability and is produced by automated analyzers [7].

The amount of hemoglobin in a specific volume of densely packed red blood cells is measured by the mean corpuscular hemoglobin concentration or MCHC. Thus, it is a molar concentration or mass. Nevertheless, MCHC is frequently measured as a percentage (%), just like a mass fraction (mHb / mRBC). The range of variation in red blood cell (RBC) volume that is measured as part of a normal complete blood count is known as the red blood cell distribution width (RDW). There are several variants of RDW, including RCDW, RDW-CV, and RDW-SD. Even in healthy blood, the typical volume of red blood cells varies, ranging from 80 to 100 femtoliters. However, there is a markedly greater range in cell size in some illnesses. Greater size variation is indicated by higher RDW values. RDW-CV in human red blood cells typically falls between 11.5% and 15.4%. The presence of fragments, agglutination groups, and/or aberrant red blood cell shape can all contribute to high RDW [8].

Platelets, also called thrombocytes, are a component of blood that, in conjunction with the coagulation factors, react to bleeding from blood vessel damage by clumping together, forming a blood clot. Platelets, which are cytoplasmic fragments generated from megakaryocytes, lack a cell nucleus of the lung or bone marrow which subsequently make their way into the bloodstream. Mammals are the only animals with platelets [9].

Included in blood tests as part of the complete blood count (CBC), mean PLT volume (MPV) is a machine-calculated assessment of the average size of platelets present in blood. The MPV test results can be utilized to draw conclusions regarding PLT production in bone marrow or PLT degradation issues since the average PLT size increases as the body produces more platelets. A higher mean PLT volume (MPV) raises the chance of developing heart disease, when platelets are destroyed, MPV may increase. This can be observed in Bernard-Soulier syndrome, myeloproliferative disorders, and immune thrombocytopenic purpura (ITP). It might also have anything to do with recovering from temporary hypoplasia and pre-eclampsia. Platelets are crucial for the immune response, inflammation, and coagulation activities. A measure of PLT function and activation, PLT distribution width (PDW) represents variations in PLT size [10].

Therefore, this study aims to investigate the relationship between IL-18 levels and blood components in patients with CML, compared to healthy controls, to better understand their potential roles in the pathogenesis and progression of the disease.

2. Materials and methods

2.1. Apparatus

The following instruments and kits were utilized for the analysis of serum IL-18 levels and hematological parameters. These tools were essential for accurate sample processing, measurement, and data acquisition. A comprehensive list of the apparatuses and kits, along with their respective manufacturers, is presented in Table 1.

2.2. Patient Recruitment and Data Collection for a Study on CML

From November 12, 2023, to August 8, 2024, patients attending hospitals for chronic myeloid leukemia (CML) assessment at the National Center of Hematology in Baghdad, Iraq, were included in this study. Ethical clearance was obtained from the center prior to sample collection.

Table 1. Various apparatuses and kits used in this study.

Apparatus	Company/Country
Bench centrifuge	VEB/Germany
ELISA reader	Calabasas/USA
IL-18 estimation	Bioworld Technology, Inc. (USA)
CBC	Diagon D-Cell 60 Hematology Analyzer

The study included 134 CML patients (61 men and 73 women) and 44 healthy controls (14 men and 30 women). Data were collected through direct patient interviews and review of hospital records and medical reports. In cases where medical records were unavailable, patient self-reports were used as an alternative information source. Controls were randomly selected from apparently healthy individuals with no prior CML diagnosis. Informed consent was obtained from all participants (patients and controls), including information on age, sex, and family history of CML.

2.3. Collection of samples

From each participant, 5 mL of venous blood was collected. 2 mL was collected in EDTA tubes for blood component analysis, and 3 mL was collected in plain tubes. Serum was then obtained from the plain tubes via centrifugation and used to measure IL-18 levels in patients and controls.

2.4. Serological parameters

To determine serum IL-18 concentrations, an enzyme-linked immunosorbent assay (ELISA) kit was employed. The kit utilizes a pre-coated 96-well plate and a series of reagents for the quantitative measurement of IL-18. A comprehensive list of the kit's components, including the specific volumes and concentrations, is provided in Table 2.

2.4.1. Additional materials and equipment

The ELISA assay required the following materials and equipment, which were not included in the kit: a microplate reader capable of measuring absorbance at 450 nm; adjustable pipettes and pipette tips suitable for dispensing volumes from 2 µl to 1 ml; adjustable pipettes for reagent preparation, covering a range of 1-25 ml; 100 ml and 1-liter graduated cylinders; absorbent paper; distilled or deionized water; a computer and software for ELISA data analysis; and tubes for preparing standard and sample dilutions.

2.4.2. Serum sample preparation

Serum samples were obtained by allowing whole blood to clot in serum separator tubes for approximately 4 hours at room temperature. Samples were then centrifuged at 1000 x *g* for 15 minutes. The serum was either analyzed immediately or aliquoted and stored at -20°C until analysis.

2.4.3. Human IL-18 Standard Preparation

The lyophilized Human IL-18 Standard was reconstituted by adding 1 ml of Standard/Sample Diluent, resulting in a 2500 pg/ml stock solution. The solution was allowed to sit at room temperature for 5 minutes and then gently vortexed to ensure complete mixing. The reconstituted standard was used within one hour. Each ELISA kit included two tubes of the standard (2.5 ng per tube), with one tube used per experiment.

To generate a standard curve, two-fold serial dilutions of the 2500 pg/ml stock solution were performed using the Standard/Sample Dilution Buffer. The resulting standard concentrations ranged from 39 pg/ml to 2500 pg/ml. The Standard/Sample Dilution Buffer was used as the zero standard (0 pg/ml). The dilutions are outlined in Table 3.

Table 2. List of Reagents and Volumes Included in the ELISA Kit for Determination of Serum IL-18 Levels.

Component	Volume
96-well Plate Coated With Anti-Human IL-18 Antibody	12 x 8 Strips
Human IL-18 Standard	2.5 ng x 2
Biotin-Labeled Detection Antibody (100X)	120 µl
Streptavidin-HRP (100X)	120 µl
Standard/Sample Diluent	30 ml
Detection Antibody Diluent	12 ml
Streptavidin-HRP Diluent	12 ml
Wash Buffer (20X)	30 ml
TMB Substrate Solution	12 ml
Stop Solution	12 ml
Plate Adhesive Strips	3 Strips
Technical Manual	1 Manual

Table 3. IL-18 Standard Curve: Serial Dilution and Resulting Concentrations.

Standard Concentration (pg/ml)	Volume of Standard Added	Volume of Standard/Sample Diluent Added (µl)
2500	Stock	-
1250	500 µl (2500 pg/ml)	500
625	500 µl (1250 pg/ml)	500
312	500 µl (625 pg/ml)	500
156	500 µl (312 pg/ml)	500
78	500 µl (156 pg/ml)	500
39	500 µl (78 pg/ml)	500
0		1 ml

2.4.4. Biotin-Labeled Detection Antibody Working Solution Preparation

The Biotin-Labeled Detection Antibody concentrate was diluted 1:100 with the Detection Antibody Diluent and mixed thoroughly. This solution was prepared no more than 2 hours before use.

2.4.5. Streptavidin-HRP Working Solution Preparation

The Streptavidin-HRP concentrate was diluted 1:100 with the Streptavidin-HRP Diluent and mixed thoroughly. This solution was prepared no more than 1 hour before use.

2.4.6. Wash Buffer Working Solution Preparation

The entire contents (30 ml) of the Wash Buffer Concentrate were poured into a clean 1,000 ml graduated cylinder. The final volume was adjusted to 600 ml with distilled or deionized water (1:20 dilution).

2.5. ELISA Assay Procedure

The ELISA assay procedure was performed according to the manufacturer's protocol. The Streptavidin-HRP Working Solution and TMB Substrate Solution were prewarmed at 37°C for 30 minutes before use. All samples and reagents were thoroughly mixed before use. A standard curve was generated for each experiment.

1. 100 µl of each standard and sample was added into appropriate wells.
2. The plate was covered and incubated for 90 minutes at room temperature or overnight at 4°C with gentle shaking.
3. The cover was removed, the solution was discarded, and the plate was washed 3 times with Wash Buffer Working Solution. The Wash Buffer Working Solution was allowed to remain in the wells for 1-2 minutes during each wash. The plate was blotted onto absorbent paper, ensuring that the wells did not dry completely at any time.
4. 100 µl of Biotin-Labeled Detection Antibody Working Solution was added into each well, and the plate was incubated at 37°C for 60 minutes.
5. The plate was washed 3 times with Wash Buffer Working Solution, allowing the solution to remain in the wells for 1-2 minutes during each wash. The Wash Buffer Working Solution was discarded, and the plate was blotted onto absorbent paper.
6. 100 µl of Streptavidin-HRP Working Solution was added into each well, and the plate was incubated at 37°C for 45 minutes.
7. The plate was washed 5 times with Wash Buffer Working Solution, allowing the wash buffer to remain in the wells for 1-2 minutes during each wash. The wash buffer was discarded, and the plate was blotted onto absorbent paper.
8. 100 µl of TMB Substrate Solution was added into each well, and the plate was incubated at 37°C in the dark for 30 minutes.
9. 100 µl of Stop Solution was added into each well to stop the reaction.

For calculation, the relative O.D.450 was determined as follows: (relative O.D.450) = (O.D.450 of each well) - (O.D.450 of Zero well). The standard curve was plotted as the relative O.D.450 of each standard solution (Y) versus the respective concentration of the standard solution (X). The concentration of the samples was then interpolated from the standard curve. If samples were diluted, the dilution factor was multiplied by the concentrations obtained from the interpolation to determine the concentration before dilution.

2.6. Measurement of blood components

Blood components were measured using the Diagon D-Cell 60 Hematology Analyzer. The Standard/Sample Dilution Buffer served as the zero standard (0 pg/ml) in the ELISA. Reconstituted standard solutions were used within 2 hours of preparation. The 2500 pg/ml standard solution was stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Repeated freeze-thaw cycles were avoided.

2.10. Statistical Analysis

The Statistical Analysis System (SAS, 2018) software was used for data analysis. Differences between groups were assessed using the t-test, and post-hoc comparisons were performed using the least significant difference (LSD) test. The Chi-square test was used to compare percentages between groups. A p-value of less than 0.05 was considered statistically significant.

3. Results

Table 4 presents a summary of the demographic characteristics of the study population, including the distribution of CML patients and healthy controls across gender and age groups. Chi-square tests revealed a statistically significant difference in gender distribution between the two groups ($p = 0.0394$), with a higher proportion of females in the control group. While differences in age group distribution were observed, these did not reach statistical significance ($p = 0.0441$).

Table 5 summarizes the mean age and standard error for both the CML patient group (46.83 ± 2.09 years) and

Table 4. Distribution of sample study according to Gender and Age in patients and control groups.

Factor		Patients No. (%)	Control No. (%)	P-value
Gender	Male	61 (45.52%)	14 (31.82%)	0.0394 *
	Female	73 (54.48%)	30 (68.18%)	
	Total	134	44	---
Age group (year)	<40 yr.	52 (38.81%)	17 (38.64%)	0.0441 *
	40-60 yr.	46 (34.33%)	15 (34.09%)	
	>60 yr.	36 (26.87%)	12 (27.27%)	
	Total	134	44	---

*** (P≤0.05).**

Table 5. Comparison between patients and control groups in Age.

Group	Means ±SE of Age (year)
Patients	46.83 ±2.09
Control	27.23 ±3.26
T-test	8.101 **
P-value	0.0001
** (P≤0.01).	

Table 6. Comparison between patients and control groups in IL-18.

Group	Means ±SE of IL-18 (ng/ml)
Patients	15.96 ±0.78
Control	17.71 ±1.28
T-test	3.070 NS
P-value	0.259

the healthy control group (27.23 ± 3.26 years). The difference in mean age was statistically significant, as determined by a t-test ($p < 0.0001$).

Despite investigating IL-18's potential role in CML, Table 6 reveals no statistically significant difference in mean serum IL-18 levels between CML patients and healthy controls ($p = 0.259$). The means and standard errors for each group are presented.

Table 7 shows a detailed comparison of blood parameters between CML patients and controls. Significant differences ($p < 0.01$) were found in PDW, RBC, MCHC, RDW-CV, RDW-SD, MPV, MCH, HCT, and PCT. In contrast, Lymphocyte count, WBC count, platelet count (PLT), and mean corpuscular volume (MCV) showed no significant differences between the groups. Means and standard errors for all parameters are shown in the table.

Table 8 details the impact of sex on blood parameters in CML patients. The only parameter with a statistically significant difference between males and females was the WBC count, with females exhibiting a higher mean WBC count (9.05 ± 1.40) compared to males (6.93 ± 0.49; $p = 0.026$). No significant sex-based differences were observed for other measured parameters.

Table 9 details the effects of age group on blood parameters and IL-18 in CML patients. IL-18 levels, platelet count, and plateletcrit showed statistically significant differences across age groups ($p<0.05$). Specifically, IL-18 was highest in the >60 yr age group, while platelet count and platelet crit were highest in the 40-60 yr age group. Different letters indicate statistically significant differences between specific age groups for each parameter based on post-hoc LSD analysis.

Our study found no significant difference in WBC levels between CML patients and controls ($p = 0.662$; Table 7). However, gender significantly affected WBC levels ($p = 0.026$; Table 8), while age did not ($p = 0.341$; Table 9). We observed a highly significant difference in mean corpuscular hemoglobin concentration (MCHC) between CML patients and controls ($p = 0.0001$; Table 7), with no significant differences based on gender ($p = 0.169$; Table 8) or age ($p = 0.692$; Table 9). Red cell distribution width-CV (RDW-CV) differed significantly between patients and controls ($p = 0.0001$; Table 7), with no significant differences for gender ($p = 0.468$; Table 8) or age ($p = 0.779$; Table 9). Lastly, red cell distribution width-SD (RDW-SD) was significantly different between patients and controls ($p = 0.0498$; Table 7), with no significant differences for gender ($p = 0.914$; Table 8) or age ($p = 0.506$; Table 9). Our study revealed no significant differences between CML patients and controls in platelet (PLT) ($p = 0.216$; Table 7) or IL-18 levels ($p = 0.259$; Table 6). However, MCH, HCT, and PCT levels were significantly different between the groups ($p < 0.0001$). Age (less than 40, 40-60, more than 60) also showed a significant difference ($p = 0.048$; Table 6). No significant differences were observed based on gender ($p = 0.283$) or MCV level ($p = 0.965$).

Figure 1 displays the distribution of serum IL-18 levels (ng/ml) in CML patients and healthy controls using boxplots. The boxplots show the median, interquartile range, and outliers for each group. A t-test revealed a significant difference in IL-18 levels between patients and controls ($P\leq0.05$).

Figure 2 presents a boxplot comparison of platelet distribution width (PDW) in CML patients and healthy controls. The boxplots depict the median, interquartile range, and outliers for each group. A t-test revealed a significant increase in PDW in CML patients compared to controls ($P\leq0.05$).

Table 7. Comparison between patients and control groups in Blood parameters.

Parameters	Means ±SE		T-test	P-value
	Patients	Control		
PDW	16.24 ±0.07	15.60 ±0.08	0.243 **	0.0001
Lymph.	3.11 ±0.65	2.23 ±0.17	2.247 NS	0.437
R.B.C	3.99 ±0.08	4.87 ±0.13	0.322 **	0.0001
W.B.C.	8.07 ±0.79	7.46 ±0.41	2.775 NS	0.662
MCHC	334.66 ±1.73	29.28 ±0.36	5.954 **	0.0001
RDW-CV	0.166 ±0.01	14.92 ±0.54	0.617 *	0.0001
RDW-SD	50.36 ±1.59	45.10 ±1.01	4.576 *	0.0498
PLT	274.80 ±17.45	235.41 ±16.85	62.90 NS	0.216
MPV	9.14 ±0.18	7.96 ±0.16	0.661 **	0.0006
MCH	28.98 ±0.55	24.52 ±0.76	2.091 **	0.0001
HCT	0.341 ±0.01	40.72 ±1.54	1.762 **	0.0001
MCV	86.29 ±1.34	83.05 ±1.88	5.096 NS	0.210
PCT	2.42 ±0.14	0.184 ±0.01	0.497 **	0.0001

* (P≤0.05), ** (P≤0.01), NS: Non-Significant.

Table 8. Effect of Sex in parameters study of patients1 groups.

Parameters	Means ±SE		T-test	P-value
	Male	Female		
IL-18 (ng/ml)	16.45 ±1.13	15.53 ±1.09	3.083 NS	0.384
PDW	16.25 ±0.08	16.23 ±0.10	0.268 NS	0.887
Lymph.	2.39 ±0.21	3.73 ±1.19	2.638 NS	0.298
R.B.C	3.86 ±0.10	4.11 ±0.12	0.332 NS	0.079
W.B.C.	6.93 ±0.49	9.05 ±1.40	1.168 *	0.026
MCHC	337.26 ±2.41	332.43 ±2.42	6.962	0.169
RDW-CV	0.161 ±0.01	0.169 ±0.01	0.024 NS	0.468
RDW-SD	50.17 ±2.36	50.52 ±2.18	6.461 NS	0.914
PLT	263.40 ±28.57	284.57 ±21.48	67.636 NS	0.332
MPV	9.13 ±0.28	9.14 ±0.25	0.759 NS	0.961
MCH	29.62 ±0.77	28.43 ±0.78	2.252 NS	0.308
HCT	0.334 ±0.01	0.348 ±0.01	0.028 NS	0.283
MCV	87.61 ±1.91	85.16 ±1.89	5.481 NS	0.374
PCT	2.34 ±0.26	2.49 ±0.15	0.560 NS	0.861

* (P≤0.05), NS: Non-Significant.

Table 9. Effect of Age groups in parameters study of patients1 groups.

Parameters	Means ±SE			L.S.D.	P-value
	<40 yr.	40-60 yr.	>60 yr.		
IL-18 (ng/ml)	14.73 ±1.34 b	15.18 ±1.16 ab	18.83 ±1.46 a	3.822 *	0.048
PDW	16.23 ±0.07	16.26 ±0.14	16.23 ±0.13	0.333 NS	0.978
Lymph.	3.86 ±1.61	2.75 ±0.26	2.42 ±0.37	3.271 NS	0.610
R.B.C	3.94 ±0.12	4.03 ±0.14	4.02 ±0.18	0.412 NS	0.840
W.B.C.	8.86 ±1.89 a	8.46 ±0.57 a	6.36 ±0.61 b	1.928	0.341
MCHC	335.38 ±2.67	333.04 ±2.61	335.64 ±4.1	8.632 NS	0.692
RDW-CV	0.163 ±0.01	0.171 ±0.01	0.162 ±0.01	0.031 NS	0.779
RDW-SD	50.06 ±2.06	52.64 ±3.46	47.88 ±2.71	8.011 NS	0.506
PLT	251.00 ±17.06 ab	334.59 ±41.86 a	233.82 ±23.19 b	83.861 *	0.033
MPV	9.03 ±0.20	9.26 ±0.39	9.14 ±0.40	0.941 NS	0.872
MCH	29.13 ±0.90	29.08 ±0.82	28.63 ±1.25	2.792 NS	0.965
HCT	0.339 ±0.01	0.349 ±0.01	0.335 ±0.02	0.035 NS	0.625
MCV	86.54 ±2.17	87.14 ±2.08	84.81 ±2.96	6.795 NS	0.861
PCT	2.24 ±0.15 ab	2.92 ±0.34 a	2.04 ±0.19 b	0.694 *	0.029

Means having with the different letters in same row differed significantly. * (P≤0.05), NS: Non-Significant.

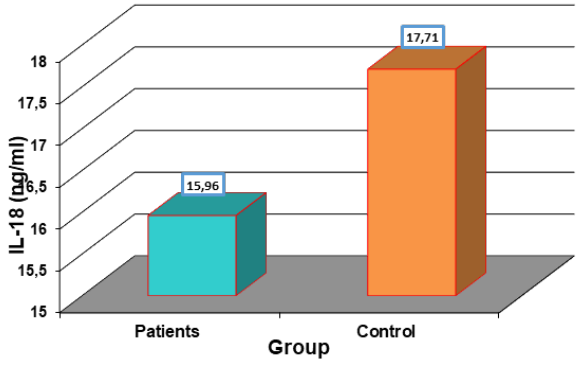

Fig. 1. Comparison between patients and control groups in IL-18.

Figure 3 compares lymphocyte counts (Lympho) between CML patients and healthy controls. Despite the importance of lymphocytes in immune function, there was no statistically significant difference in lymphocyte counts between the two groups (p = 0.437), suggesting that Lympho may not be a primary differentiating factor in this study.

Figure 4 displays the distribution of red blood cell (RBC) counts in CML patients and healthy controls using boxplots. The boxplots show the median, interquartile

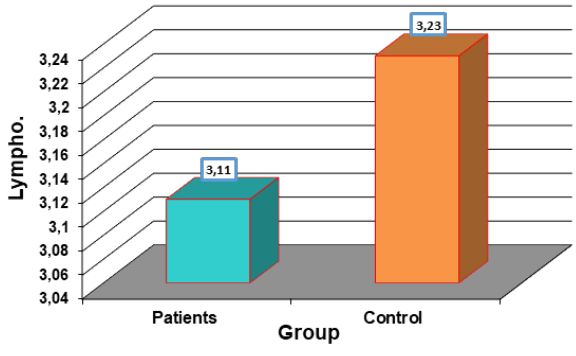

Fig. 2. Comparison between patients and control groups in PDW

range, and outliers for each group. There was no significant difference observed in RBC count between the two groups RBC counts are expressed in $x10^6/\mu L$.

Figure 5 presents a boxplot comparison of WBC counts in CML patients and healthy controls. The boxplots display the median, interquartile range, and outliers for each group. No significant difference in WBC count was observed between the two groups. WBC counts are expressed in $x10^3/\mu L$.

Figure 6 illustrates the mean corpuscular hemoglobin concentration (MCHC) in CML patients compared to healthy controls. MCHC was significantly higher in CML patients ($P \leq 0.05$). MCHC is expressed in g/L.

Figure 7 compares red cell distribution width - coefficient of variation (RDW-CV), a measure of anisocytosis (variation in red blood cell size), between CML patients and healthy controls. The significantly lower RDW-CV observed in CML patients ($P \leq 0.01$) suggests increased variation in red blood cell size.

To provide a comprehensive overview of the hematological changes observed in this study, the following section details additional findings. These results are presented in text form, supplementing the information conveyed in the figures above.

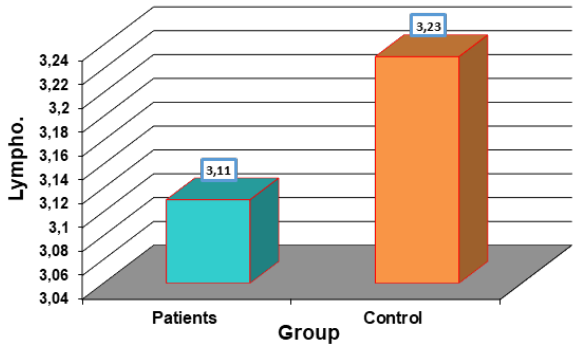

Fig. 3. Comparison between patients and control groups in Lympho.

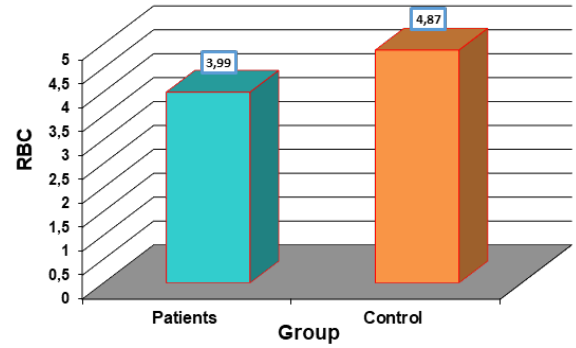

Fig. 4. Comparison between patients and control groups in RBC.

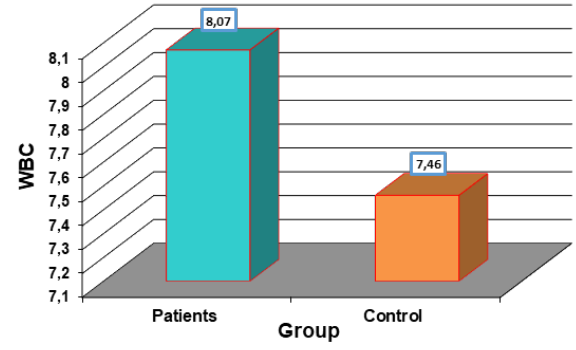

Fig. 5. Comparison between patients and control groups in WBC.

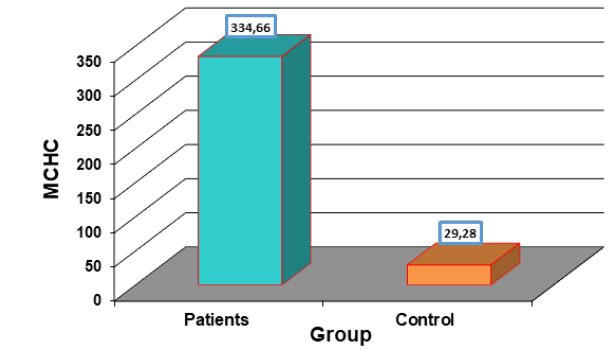

Fig. 6. Comparison between patients and control groups in MCHC.

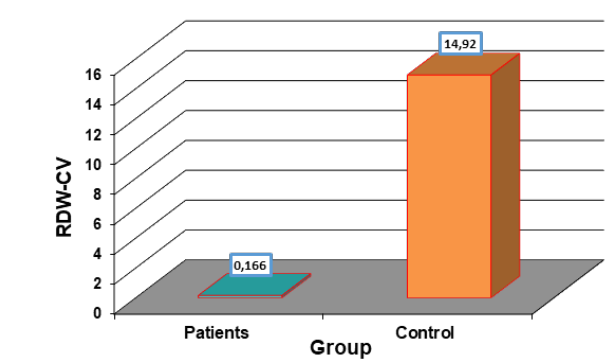

Fig. 7. Comparison between patients and control groups in RDW-CV.

RDW-SD, a measure of red blood cell size variability, showed no significant difference between the patient and control cohorts ($p > 0.05$). A statistically significant increase in platelet (PLT) counts was observed in CML patients relative to healthy controls ($p \leq 0.05$). Analysis of MPV revealed no significant difference between the patient and control groups ($p > 0.05$). The average amount of hemoglobin within red blood cells, as measured by MCH, was significantly greater in CML patients ($p \leq 0.05$). The mean corpuscular volume (MCV) was similar in CML patients and healthy controls ($p > 0.05$). A significant elevation in procalcitonin (PCT) levels was observed in the CML patient group ($p \leq 0.01$).

4. Discussion

Driven by growth-promoting, somatically acquired mutations that lead to aberrant proliferation of hematopoietic stem cells, clonal hematopoiesis of undetermined potential (CHIP) affects approximately 10% of adults over 65 without hematologic illnesses. CHIP is associated with a slightly elevated risk of hematologic malignancies and increased all-cause mortality, largely due to cardiovascular complications [11]. The lower frequency of DNMT3A mutations in CML compared to clonal hematopoiesis of undetermined potential (CHIP), along with their presence in younger CML patients (median age <60 years) and increased prevalence in children and young adults, indicates that these mutations are likely acquired in association with the development of CML, rather than simply reflecting the effects of aging [12].

A significant age difference was observed between CML patients and controls (p = 0.0441; Table 4). Furthermore, a highly significant difference in age distribution was noted between the two groups when categorized into age ranges (>40, 40-60, <60; p = 0.0001; Table 2). As shown in Table 9, age had a statistically significant impact on the levels of IL-18 (p = 0.048), PLT (p = 0.033), and PCT (p = 0.029). A preliminary investigation into gender differences in leukemia outcomes revealed that women tended to fare better than men. However, this finding was not statistically significant after controlling for age and disease stage. Consistent with this trend, a review of our first Medical Research Council leukemia trial showed that women had a higher overall survival rate, regardless of age or tumor stage, although the difference was not statistically significant [13]. Given the absence of sex differences in target cells at younger ages, AML target cells may exhibit a greater degree of lineage commitment compared to their CML counterparts. The subsequent appearance of sex-related disparities in AML at older ages further suggests a progression of AML target cells towards a phenotype more characteristic of HSC reserve cells [14]. As indicated in Table 8, significant differences were observed between males and females in terms of WBC count (WBC; p = 0.026) and age (p = 0.03).

Platelet distribution width (PDW) is an indicator of the heterogeneity in circulating platelet size. Previous investigations into the relationship between PDW and mortality have been limited by inadequate sample sizes, thus precluding robust conclusions regarding its prognostic value [15]. In the management of acute myeloid leukemia (AML), platelet count (PLT) is a key factor in predicting long-term outcomes after treatment. Moreover, a low PLT-to-lymphocyte ratio, increased mean platelet volume (MPV), and elevated platelet distribution width (PDW), potentially serving as a novel independent prognostic marker, may identify patients at higher risk for adverse outcomes [16]. In some individuals, the platelet count remains normal or is only slightly elevated, with thrombocytosis as the primary finding. Hemoglobin levels usually exceed 10 g/dL (100 g/L) [17]. A significant difference in platelet distribution width (PDW) levels was observed between CML patients and controls (p = 0.0001; Table 7). In contrast, there was no discernible difference in PDW levels based on gender (p = 0.887; Table 8) or age (categorized as <40, 40-60, and >60 years; p = 0.978; Table 9).

Chronic myelogenous leukemia (CML) is classified as a myeloproliferative neoplasm, affecting both the bone marrow and peripheral blood [18]. Leukemogenic fusion proteins can arise from a variety of chromosomal translocations, highlighting a common mechanism in leukemia development [19]. The presence of this oncogene in the blood of healthy individuals without leukemia or lymphoma raises questions about its role in normal hematopoiesis and potential pre-leukemic states [20]. Our study revealed a highly significant difference in red blood cell (RBC) levels between CML patients and controls (p = 0.0001; Table 4).

In contrast, no significant differences in RBC levels were observed based on gender (p = 0.079; Table 8) or age group (<40, 40-60, and >60 years; p = 0.840; Table 9). An MCH level of less than 27 pg/cell is considered low in adults, with anemia being the most common cause. Hemolysis, or the breakdown of red blood cells, can lead to a low MCH. While hemolysis is a normal physiological process, excessive hemolysis can result in anemia [21].

Intracellular dehydration in hemolytic anemia (HA) leads to the formation of dense erythrocytes or spherocytes, observable on peripheral blood smears. These morphological changes are frequently accompanied by increased mean corpuscular hemoglobin concentration (MCHC) and a disrupted pattern of erythrocyte distribution [22]. Membrane abnormalities in sickle cell disease (SCD) and hereditary spherocytosis (HS) result in the shedding of microparticles from red blood cells (RBCs). Consequently, the RBC surface-to-volume ratio decreases, leading to an increase in the mean corpuscular hemoglobin concentration (MCHC) [23].

A statistically significant difference in red blood cell (RBC) levels was found between CML patients and controls (p = 0.0001; Table 7). However, there were no significant differences in RBC levels between males and females (p = 0.169; Table 8) or across the different age groups (<40, 40-60, and >60 years; p = 0.692; Table 9). Red blood cell distribution width (RDW) is used in investigating the etiology of anemia. While an RDW value above 15.0% has been used to predict prognosis in some contexts, its utility may be limited in newly diagnosed CML patients at initial hospitalization. Novel RDW cutoff values are being explored based on clinical outcomes in patients with myelodysplastic syndromes [24]. Lower RDW at diagnosis was significantly associated with the achievement of 3-month early molecular response (3M-EMR) and 6-month complete cytogenetic response (6M-CCyR), and RDW levels predicted treatment responses at 3 and 6 months, but not at 12 months. Patients who failed to achieve 12-month major molecular response (12M-MMR) did not exhibit significant differences in RDW.

A study by Iriyama et al. demonstrated dynamic changes in RDW during tyrosine kinase inhibitor (TKI) therapy, with a transient increase at one month, followed by a decrease at three and six months, and a return to baseline by 12 months [25]. Research indicates that elevated red blood cell distribution width (RDW) is associated with poorer prognosis and survival in patients with CML. Multivariate analysis in previous studies has demonstrated a correlation between advanced disease phase and RDW levels at the time of diagnosis. Monitoring the dynamic changes in RDW values during treatment may provide valuable information for healthcare providers to improve patient care and management [26].

A statistically significant difference in red blood cell (RBC) levels was observed between CML patients and controls (p = 0.0001; Table 7). However, there were no significant differences in RBC levels between males and females (p = 0.468; Table 8) or among the different age groups (<40, 40-60, and >60 years; p = 0.779; Table 9). Evidence suggests that a low mean platelet volume (MPV) may be associated with worse outcomes in cancer patients. Conversely, a retrospective investigation indicated that cancer patients with elevated MPV had a higher risk of venous thromboembolism (VTE) compared to those with lower MPV [27].

While mean platelet volume (MPV) can provide valuable information, it is not without limitations as a clinical metric. Given its susceptibility to laboratory variance, abnormal MPV results should always be validated by examining a peripheral blood smear. Furthermore, due

to the contradictory nature of MPV's prognostic value in various cancer contexts, its predictive role remains under investigation, and additional research is required to establish its suitability for therapeutic purposes [28]. Our study demonstrated a significant difference in mean platelet volume (MPV) between CML patients and controls ($p = 0.0006$; Table 7). However, no significant differences in MPV were found based on gender ($p = 0.961$; Table 8) or age group (<40, 40-60, and >60 years; $p = 0.872$; Table 9). Leukoerythroblastosis, characterized by the presence of immature WBCs, can occur in critically ill patients and is often observed in myelophthisic anemias, where hematopoietic cells in the bone marrow are displaced by fibrosis, tumors, or other space-occupying lesions.

In rare instances, sickle cell hemoglobinopathies can lead to leukoerythroblastosis, a condition that may be associated with fat embolism syndrome and bone marrow necrosis [29].

Our study investigated the association between various hematological parameters, IL-18 levels, and CML. We observed no significant difference in WBC levels between CML patients and controls overall ($p = 0.662$), which is inconsistent with literature indicating differences in the WBC levels. The significant impact of gender on WBC levels ($p = 0.026$), with gender levels being elevated, warrants further exploration to clarify the relationship between gender-specific factors, potentially hormonal influences, and CML pathogenesis.

In contrast, our findings revealed highly significant differences in MCHC and RDW-CV between CML patients and controls ($p < 0.0001$ for both), as well as a significant difference in RDW-SD ($p = 0.0498$). This suggests that CML is associated with alterations in hemoglobin concentration within red blood cells and variations in the size distribution of red blood cells which may be used for diagnosis. It is interesting that the increase of red cells is significantly lower in patients with CML.

Regarding PLT and IL-18 levels, we found no significant differences between CML patients and controls ($p = 0.216$ and $p = 0.259$, respectively). This finding contrasts previous studies, suggesting that the role of CML may be more nuanced than initially understood. It is important to note that the levels of these compounds don't vary much with CML.

In addition, we found significant differences in MCH, HCT, and PCT levels between the CML group and the controls. Lastly, we noted that age had a significant effect on MCH, HCT, and PCT levels between the CML group and the controls ($p = 0.048$). This suggests the importance of including age as a variable in future studies.

5. Conclusion

In conclusion, our study highlights the complex interplay between IL-18 and various blood components in CML patients. We observed no significant differences in PLT and IL-18 levels between CML patients and controls. However, significant differences in MCH, HCT, and PCT levels were found, alongside age. These findings contribute to a deeper understanding of the pathophysiology of CML and may inform future diagnostic and therapeutic strategies. Further research is warranted to elucidate the precise mechanisms underlying these associations and to explore the potential of these parameters as biomarkers for disease progression and treatment response.

Abbreviations

CML: Chronic Myeloid Leukemia; **IL-18:** Interleukin-18; **PLT:** Platelet; **WBC:** White Blood Cell; **MCH:** Mean Corpuscular Hemoglobin; **HCT:** Hematocrit; **PCT:** Plateletcrit; **PDW:** Platelet Distribution Width; **RBC:** Red Blood Cell; **MCHC:** Mean Corpuscular Hemoglobin Concentration; **RDW-CV:** Red Cell Distribution Width-Coefficient of Variation; **MCV:** Mean Corpuscular Volume; **MPV:** Mean Platelet Volume; **RDW-SD:** Red Cell Distribution Width-Standard Deviation; **ELISA:** Enzyme-Linked Immunosorbent Assay; **TKI:** Tyrosine Kinase Inhibitor.

Data availability

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Conflicts of interest

The authors report no conflicts of interest in this work.

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