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Evaluation of plasma progranulin level in patients with newly diagnosed chronic lymphocytic leukemia and its correlation with clinical stages, hematological parameters, and B2-microglobulin

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Abstract:

BACKGROUND: With a high degree of intratumoral and interpatient heterogeneity, chronic lymphocytic leukemia (CLL) is a malignant lymphoproliferative illness characterized by the accumulation of defective B lymphocytes in the blood and lymphoid tissues. A multifunctional glycoprotein released by the body, progranulin (PGRN) is linked to inflammation, repair, development, and carcinogenesis. As a prognostic indicator, PGRN was discovered to be elevated in a large number of solid tumors and a small number of hematological malignancies.

OBJECTIVES: The objective of the study was to assess the plasma PGRN level in newly diagnosed CLL patients in comparison to healthy controls and to establish a correlation between it with plasma beta-2 microglobulin (β2M), hematological parameters, and disease stage.

PATIENTS, MATERIALS, AND METHODS: From October 1, 2022, to March 1, 2023, 50 newly diagnosed CLL patients visited the Baghdad Teaching Hospital on the Medical City campus. This cross-sectional study was conducted. The diagnosis was made using immunophenotyping by flow cytometry and morphology, with a control group of 30 healthy people. PGRN and β2M plasma levels were assessed using an enzyme-linked immunosorbent assay.

RESULTS: A (P = 0.001) indicated a statistically significant difference in plasma PGRN level between the patients' median of 5.62 ng/mL and the control groups' median of 2.37 ng/mL. The Binet staging system revealed that there was a significant difference with regard to absolute lymphocyte count, smudge cell percentage, and plasma β2M (P = 0.01, 0.001, 0.049), respectively, but there was no statistically significant difference between the stages with regard to age and plasma PGRN level (P = 0.35, 0.9). With a P = 0.046, PGRN demonstrated a strong positive correlation with β2M.

CONCLUSIONS: Compared to healthy controls, patients with CLL showed higher levels of PGRN. The other poor prognostic sign, β2M, and the high PGRN levels at baseline correlate well; however, no difference was found when comparing the levels at later stages of the disease.

Keywords:

Chronic lymphocytic leukemia, enzyme-linked immunosorbent assay, plasma progranulin level

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Introduction

A cancer of mature B lymphocytes, chronic lymphocytic leukemia (CLL)

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is characterized by lymphocytosis of the blood and bone marrow. As the tumor grows, different levels of lymphadenopathy, splenomegaly, and blood cytopenia appear.[1]

CLL is the predominant form of leukemia among adults in Western societies, accounting for approximately 25%–30% of all leukemia cases in the United States. The American Cancer Society predicts that there will be an estimated 21,040 new cases of CLL and roughly 4060 fatalities in the year 2020.[2] In Iraq, leukemia accounts for about 6% of all cancer patients, with CLL making up only 15.7% of leukemia cases.[3]

The diagnosis of CLL requires a sustained presence of over 5×10^9/L B lymphocytes in the peripheral blood (PB) for at least 3 months. The clonality of these B lymphocytes must be confirmed by demonstrating immunoglobulin light chain restriction via flow cytometry. Leukemic cells identified through a blood smear are typically small, mature lymphocytes with a narrow cytoplasmic border and a dense nucleus that lacks visible nucleoli and exhibits partially aggregated chromatin.[4]

Due to the well-known variability in the prognosis of CLL, numerous studies have been conducted to identify novel prognostic markers that may be used to distinguish patients with a more passive course from those whose course is more aggressive and for whom an early start to treatment is necessary. The first clinical staging systems to categorize CLL patients into prognostic groups were Rai and Binet. More recent prognostic markers include poor risk cytogenetic changes in CLL cells, elevated serum beta-2 microglobulin (β2M), TK, unmutated IgVH, and increased expression of ZAP-70 or CD38.[5]

Although the Binet and Rai clinical staging systems are still commonly used to guide the management of CLL, they have proven inadequate in differentiating between prognostic subgroups with advancements in CLL therapy. The most relevant prognostic score in CLL is the CLL International Prognostic Index (IPI), which combines clinical, biological, and genetic information.[6]

Progranulin (PGRN) is a multifunctional protein that is alternatively referred to as acrogranin, granulin (GRN) epithelin precursor, proepithelin, PC cell-derived growth factor, and 88-kDa glycoprotein. The GRN gene, responsible for encoding PGRN, is located on chromosome 17q21.31. It is expressed in various organs including marrow stromal cells, endothelial cells, neurons, and both the innate and acquired immune system. PGRN, a growth factor, has a prominent function in cancer by stimulating cell division, migration, invasion, angiogenesis, malignant transformation, resistance to chemotherapy, and evasion of the immune system. Elevated levels of PGRN have been observed in both tumor tissue and PB samples from individuals with several types of tumors, including breast cancer, ovarian cancer, and glioblastoma.[7,8]

Researchers have identified the significance of PGRN in acute myeloid leukemia, a type of blood cancer, and its potential as a therapeutic target in hematological neoplasms.[9] Elevated blood PGRN levels were seen in certain patients with CLL and malignant lymphoma. High plasma levels of PGRN were found to be strongly linked with adverse risk factors in CLL patients, such as unmutated immunoglobulin heavy-chain variable region (IGHV) status, expression of CD38 and ZAP-70, and poor-risk cytogenetics (11q–, 17p–). This suggests that PGRN is a novel, robust, and independent prognostic marker in CLL.[10] In addition, high serum PGRN levels were associated with poor prognosis in patients with diffuse large B-cell lymphoma.[11] This increase in PGRN levels is linked to reduced overall survival, disease-free survival, relapse-free survival, and progression-free survival. Thus, PGRN has the potential to serve as a valuable standalone prognostic indicator in those disorders.[12]

β2M is produced by all nucleated cells and constitutes the light chain subunit of the major histocompatibility complex Class I antigen. Higher serum levels of β2M are linked to poorer prognostic factors, including advanced CLL stage and high tumor burden. Elevated serum β2M levels are associated with shorter progression-free survival, lower rates of complete remission, and reduced failure-free and overall survival.[13,14] This study aims to assess the plasma PGRN levels in newly diagnosed patients with CLL in comparison to healthy controls and its correlation with disease stage, hematological parameters, and plasma β2M.

Patients, Materials, and Methods

This study recruited 50 recently diagnosed patients with CLL who were receiving treatment at Baghdad Teaching Hospital, Medical City campus from October 1, 2022, to March 1, 2023. The diagnosis was determined by analyzing the morphology and immunophenotyping of the cells using an advanced eight-color flow cytometer (BD FACS Canto™ II Flow Cytometer, USA). Obtaining ethical permission to conduct the research was accomplished by obtaining consent from the hospitals involved and all participants in the study. Information was gathered for each patient by extracting data from their medical records using a standardized questionnaire.

The Binet staging system was employed for the purpose of clinical staging. For every participant in this research, a blood sample was taken from

a vein using ethylenediaminetetraacetic acid for comprehensive blood analysis, including a blood smear and measurement of reticulocytes. The percentage of stem cells (SC%) was determined using the formula: SC% = SC number/200 (lymphocytes + SC) ×100.[15] The leftover blood, which had been treated with anticoagulant, was subjected to centrifugation for 10 min at a speed of 3000 revolutions per minute. The resulting plasma was then stored in an Eppendorf tube at a temperature of − 80°C. This plasma was intended for use in the PGRN and β2M enzyme-linked immunosorbent assay (ELISA) Kit from My BioSource/USA.

This study contained a control group consisting of 30 healthy adults. Blood samples were taken from each participant in the control group for complete blood count and plasma PGRN quantification using an ELISA-based approach.

Ethical consideration

Ethical permission to conduct the research was obtained from these hospitals and from all participants in this study and from each patient verbal consent was obtained for accepting to take PB samples, and the study was approved by the Iraqi Board for Medical Specializations.

Criteria of inclusion

A new diagnosis of CLL was established by morphology and immunophenotyping with 4 or 5 Matutes score.

Criteria of exclusion

Patients on chemotherapy or other treatment modalities for CLL, patients with other hematological neoplasms, and patients with other solid neoplasms were excluded from this study.

Statistical analyses

The statistical analysis was conducted using two software applications: the Statistical Package for the Social Sciences (IBM SPSS Statistics V. 25, USA) and Microsoft Office Excel 2019. The data are displayed as the average and middle values for quantitative variables and as the count and proportion for qualitative variables. The Kruskal–Wallis and Mann–Whitney tests The *U*-tests were employed to assess the disparity between several groups in cases when the data had nonnormal distribution. The Pearson rank correlation test was employed to examine the associations among various variables. A $P < 0.05$ was deemed to be statistically significant.

Results

Patient's characteristics

The demographic and hematological findings for the patient group are summarized in Tables 1 and 2 and Figures 1 and 2. The age group with the highest percentage of patients (32%) is between 60 and 69 years old. CLL was more prevalent in males compared to females, with a male-to-female ratio of 2.3:1.

Table 1: Age distribution of patients group

Age group	*n* (%)
<50	5 (10)
50–59	14 (28)
60–69	16 (32)
≥70	15 (30)
Total	50 (100)

Table 2: Hematological parameters of chronic lymphocytic leukemia patients

Hematological parameters	Mean	SD	Median	Minimum	Maximum	IQR
WBC (×10⁹/L)	65.65	47.41	52.02	10.30	228.50	50.76
ALC (×10⁹/L)	57.02	43.13	41.75	6.50	192.00	51.58
Hb (g/dL)	12.49	2.45	12.91	5.40	16.63	3.29
PLT (×10⁹/L)	197.47	115.49	166.95	49.60	661.00	92

WBC=White blood cell, ALC=Absolute lymphocyte count, Hb=Hemoglobin, SD=Standard deviation, IQR=Interquartile range, PLT=Platelet

Plasma progranulin in chronic lymphocytic leukemia patients compared to control group

A statistically significant difference in plasma PGRN levels was observed between the patients and control groups, with a $P = 0.001$, as shown in Table 3 and Figure 3.

Comparison of different variables according to stage

The Binet staging system did not show any significant statistical difference in age and plasma PGRN level among patients at different stages, with $P = 0.35$ and 0.9, respectively. However, a significant difference was observed in absolute lymphocyte count (ALC), smudge cell %, and plasma β2M, with $P = 0.01$, 0.001, and 0.049, respectively. Patients with Stage C exhibited the greatest levels of Plasma β2M and ALC, surpassing those with Stage B and A. Conversely, the highest level of smear cell % was observed in patients with Stage A [Figure 4 and Table 4].

Correlation between progranulin and beta-2 microglobulin

The Pearson rank correlation test was employed to investigate the potential link between plasma PGRN and β2M in recently diagnosed CLL patients. The analysis revealed a notable positive association between PGRN and β2M, with a $P = 0.046$ [Figure 5].

Correlation between progranulin and hematological parameters

The Spearman rank correlation test was employed to investigate the potential link between plasma PGRN and other factors in recently diagnosed CLL patients.

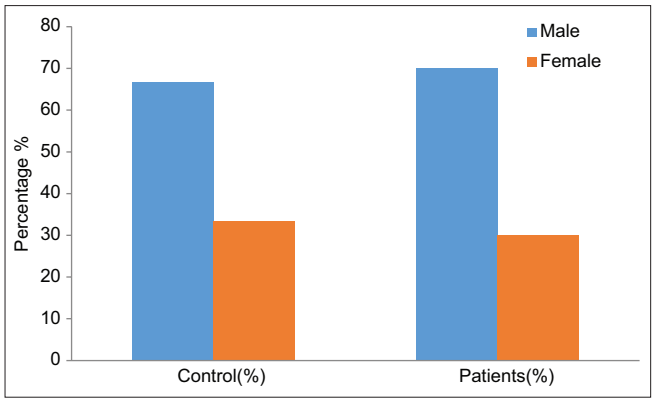

Figure 1: Distribution of the patients and control according to their sex

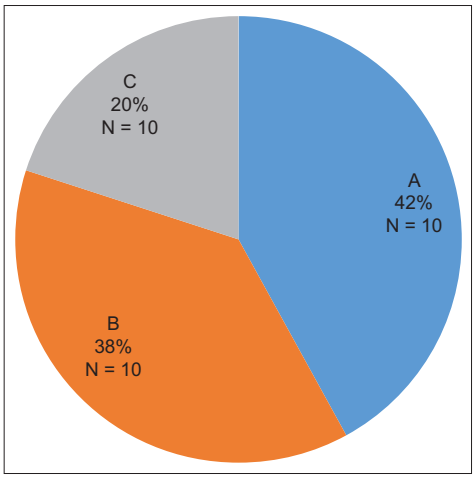

Figure 2: Distribution of chronic lymphocytic leukemia patients according to the Binet staging

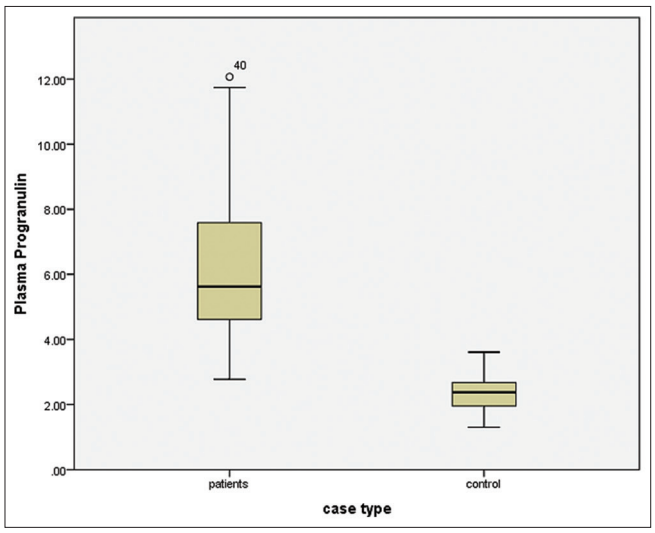

Figure 3: Box plot for plasma progranulin between patients and control $P = 0.001$

A positive but statistically insignificant association was seen between plasma PGRN level and white blood cell count, hemoglobin, and platelet. Conversely, a negative but statistically insignificant correlation was found between PGRN and ALC, SC%, and age [Table 5].

Table 3: Comparison of plasma progranulin levels between patient's group and control group

Parameters	Control (n=30)	Patients (n=50)	P
PGRN (ng/mL)			
Median	2.37	5.62	0.001*
IQR	0.76	3.00	
Range	1.30–3.61	2.77–12.07	
Mean rank	15.87	55.38	
Sum of ranks	476.00	2864.00	

*There was statistically significant difference for plasma progranulin level between patients and control groups with a P-value of 0.001. Mann–Whitney test. IQR=Interquartile range, PGRN=Progranulin

Discussion

CLL has significant heterogeneity. To accomplish this, it is crucial to evaluate risk factors and prognosis signs while diagnosing and determining optimal treatment options. These risk variables and prognostic markers are useful for predicting both survival and illness progression, as well as therapy response.[16]

In recent years, numerous newly emerging prognostic markers have been investigated with the goal of determining their potential influence on treatment decisions. The clinical staging system developed by Rai and Benet *et al*. was among the initial factors identified to have a significant impact on treatment choices (reference for staging). B2-microglobulin is another well-established factor, and both clinical stage and B2-microglobulin are included in the IPI for CLL.[6]

Hence, the objective of our present study was to investigate the prognostic importance of plasma PGRN in relation to widely recognized prognostic markers. Our findings revealed that the plasma concentration of PGRN in patients with CLL was significantly elevated compared to that in individuals without the disease (P = 0.001), which aligns with the results of a previous study conducted in Australia and Germany.[17,18] PGRN has been shown to function as a regulator of carcinogenesis by promoting many processes including cell proliferation, migration, invasion, angiogenesis, malignant transformation, resistance to anticancer treatments, and immunological evasion.[18]

When comparing plasma PRGN levels among different Binet stages of CLL, no statistically significant difference was seen. These findings are inconsistent with previous research.[17,18] It is plausible that these findings can be attributed to variations in sample sizes. In the previous investigations, the levels of GRN were measured in the serum of 249 patients who had reached an advanced stage of the disease (Binet B and C).[18] This investigation served as a supplement and expansion of an independent study conducted by Göbel *et al*.,[17] which primarily focused on patients with CLL at an early clinical stage (Binet A). Curiously, the study found that adding recombinant

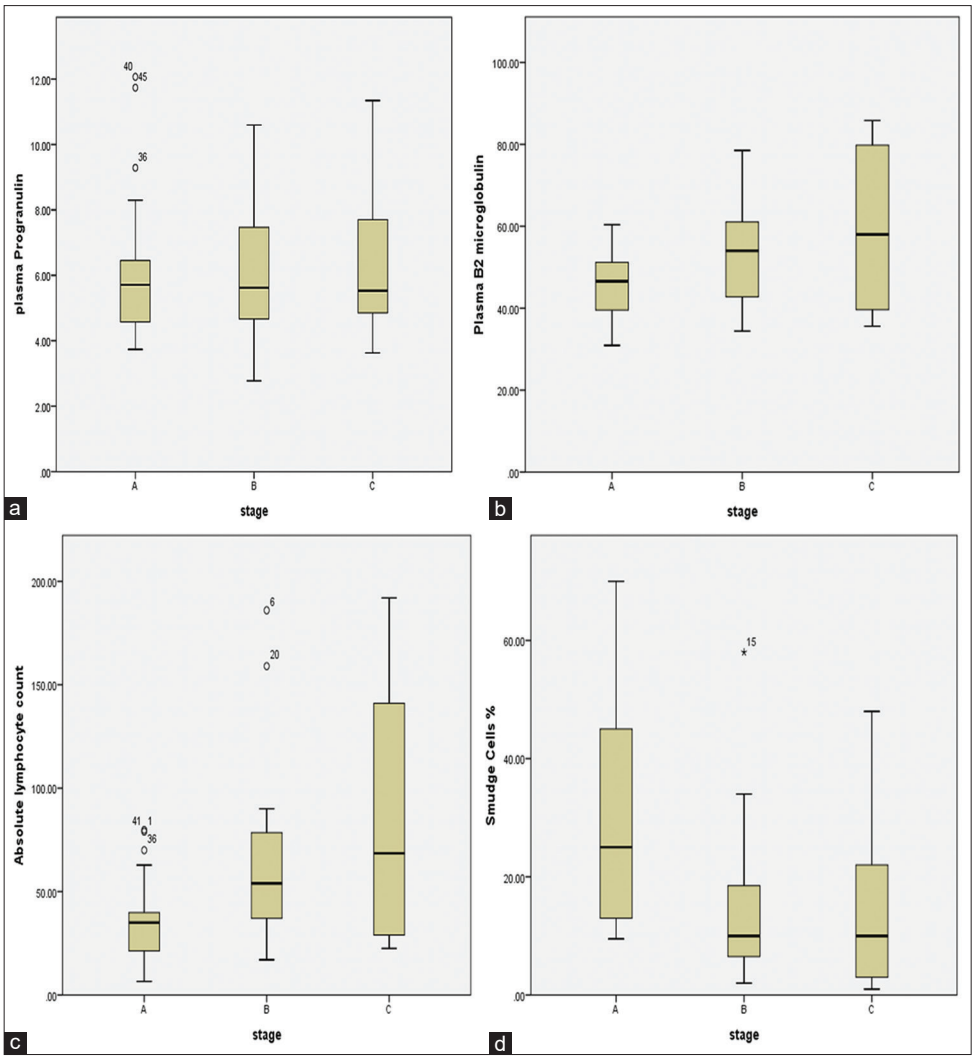

Figure 4: Comparison of different variables according to stage. According to the Binet staging system, (a) no significant statistical difference was seen between different stages of patients with respect to plasma progranulin level with a (P = 0.9), the highest level of Plasma beta-2 microglobulin and absolute lymphocyte count was seen in patients with Stage C, compared to Stage B and A (b and c), while for smudge cell % the highest level was seen in Stage A (d)

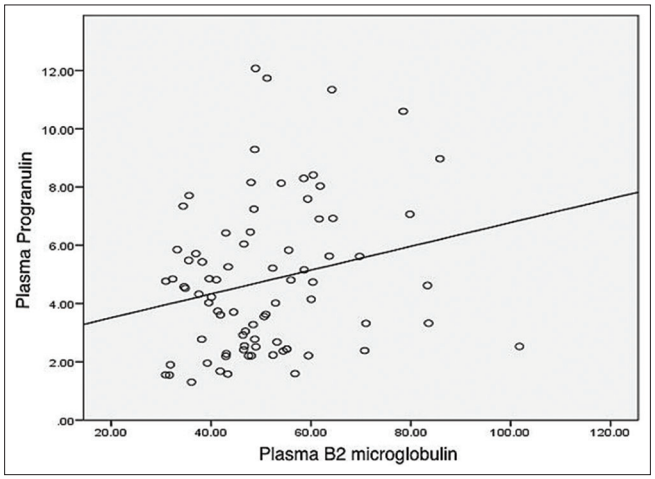

Figure 5: Correlation between plasma progranulin and beta-2 microglobulin
P = 0.046

PGRN to CLL cell culture *in vitro* did not affect the viability of the cells or the proportion of cells that were proliferating. In addition, in animal models, the removal of GRN in hematopoietic cells did not have an impact on the development of CLL *in vivo*. Therefore, the study concluded that there is no evidence suggesting that PGRN drives the disease *in vitro*. Another possible explanation for this outcome is that PGRN functions as an adipokine, and various studies have demonstrated its connections with physical activity and body mass index in older individuals. Specifically, higher levels of physical activity were linked to lower concentrations of PGRN, whereas individuals with obesity exhibited the opposite pattern. The levels of PGRN are directly associated with body fat percentage and waist circumference, as indicated by studies.[19,20] The prevailing pattern in Iraq is characterized by a high prevalence of obesity and a lack of physical exercise, which could be an additional element influencing this situation.

The present study demonstrated that β2M levels were elevated in Stages B and C compared to Stage A, consistent

Table 4: Comparison of different variables according to stage

Laboratory parameter	Stage A	Stage B	Stage C	*P*
ALC (10^9/L)				
Median (IQR)	35.00 (21.39)	54.00 (45.5)	68.500 (114.9)	0.01*
Mean rank	18.43	29.71	32.35	
SC%				
Median (IQR)	25.0 (35)	10.0 (16)	10.00 (21.3)	0.001*
Mean rank	34.26	19.08	19.3	
Plasma PGRN (ng/mL)				
Median (IQR)	5.71 (2.91)	5.61 (3.06)	5.52 (3.23)	0.919*
Mean rank	24.9	25.26	27.2	
Plasma β2M (ng/mL)				
Median (IQR)	46.57 (13.79)	54.02 (20.57)	57.99 (41.4)	0.049*
Mean rank	19.98	27.97	32.4	
Age (years)				
Median (IQR)	62.00 (17)	60.00 (14.00)	67.00 (21)	0.35*
Mean rank	26.69	21.95	29.75	

*According to Binet staging system,a significant difference was demonstrated between different stages of patients in respect to ALC, smudge cell% and plasma β2M with *P* value of (0.01, 0.001 and 0.049) respectively, while no significant statistical difference was seen in respect to age and plasma progranulin level with a *P* value (0.35 and 0.9) respectively. Kruskal–Wallis test. IQR=Interquartile range, ALC=Absolute lymphocyte count, PGRN=Progranulin, SC%=Percentage of stem cells, β2M=Beta-2 microglobulin

Table 5: Correlation between progranulin with hematological parameters

Parameter	PGRN	
	r	*P*
WBC ($\times10^9$/L)	0.69	0.63
ALC ($\times10^9$/L)	−0.009	0.95
Hb (g/dL)	0.008	0.95
PLT (g/dL)	0.16	0.25
SC%	−0.074	0.61
Age (years)	−0.052	0.71

WBC=White blood cell, ALC=Absolute lymphocyte count, Hb=Hemoglobin, SC%=Percentage of stem cells, PLT=Platelet, PGRN=Progranulin

with a previous finding suggesting that increased serum β2M levels may be associated with poor prognoses, such as advanced disease stage or high tumor burden in the bone marrow.[21] These findings are in agreement with those reported by Ali *et al*. in 2020.[14]

While the current study did not identify a difference in plasma PGRN level between different illness stages, it did establish a strong positive connection between plasma PGRN level and the well-known prognostic marker β2M. This conclusion is consistent with past studies.[17,18]

Other variables, such as the ALC and the proportion of smear cells, did not show a significant connection with plasma PGRN levels throughout different stages of the disease. Multiple investigations have established a correlation between elevated ALC levels and advanced disease stage as well as unfavorable prognosis.,[22] Calculate the proportion of smudge cells using the Nowakowski method.[15] Some researchers have shown that a straightforward method, known as smudge cell analysis, is associated with illness stage and prognosis. It has been observed that individuals in Stage A of the disease had a higher percentage of smudge cells compared to those in more advanced stages.,[23] The presence of the cytoskeletal protein vimentin in leukemic cells is associated with smudge cell production. Researchers observed that a higher amount of vimentin led to a lower percentage of smudge cells, which in turn was linked to a higher risk of illness.[24]

The precise impact of plasma PGRN on prognosis in patients with CLL requires additional investigation and comparison with recognized prognostic markers such as Immunoglobulin variable heavy-chain (IVH) mutational status, cytogenetic markers, and others. This is necessary to definitively determine its potential function.

Conclusions

The levels of plasma PGRN were notably elevated in patients with CLL at the time of diagnosis compared to the control group. There was a notable statistical disparity observed across various stages of CLL patients regarding ALC, smudge cell percentage, and plasma β2M, but no significant difference was observed in relation to plasma PGRN. The plasma levels of PGRN exhibited a notable positive correlation with β2M.

Recommendations

Further studies on larger numbers of patients and longer periods of follow-up are required to collect information about progression-free survival and overall survival in patients with high PGRN expression. Additionally, it is necessary to investigate the correlation between PGRN level and other biological or clinical prognostic factors, including IGHV gene status, CD49d, ZAP-70, and CD38. Further studies are needed to explore the therapeutic value of targeting PGRN in patients with

CLL, which might provide new insights into treating patients with CLL. Encourage the detection of plasma PGRN level in CLL patients as a marker for disease burden by ELISA and/or other methods such as quantitative polymerase chain reaction.

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Conflicts of interest
There are no conflicts of interest.

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