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VALUE OF SERUM INTERLEUKIN-22 IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA AND ITS RELATION TO DISEASE CHARACTERISTICS.

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ABSTRACT:

Background: Chronic lymphocytic leukaemia (CLL) is a common type of leukaemia characterized by progressive accumulation of monoclonal B cells. The survival and proliferation of CLL cells are stimulated by T-helper cytokines and could be correlated with disease progression. The aim of this study is to assess the value of serum interleukin-22 in patients with chronic lymphocytic leukemia and its relation to disease characteristics.

Methods: The study was carried out on 40 CLL patients (newly diagnosed) and 10 healthy controls. Routine hematological and laboratory investigations were performed for all subjects in addition to interleukin-22 (IL-22) level and ZAP-70 expression.

Results: Mean values of IL-22 level among CLL patients were significantly higher than in the control group. IL-22 mean values were significantly higher in patients with positive ZAP-70 expression. Moreover, there was a significant positive correlation between serum IL-22 level and ZAP-70. According to the ROC curve, the area under the curve was statistically significant (AUC=0.809 p=0.001).

Conclusion: IL-22 & ZAP-70 could act in alliance to maintain survival of CLL cells and its proliferation and could be used as diagnostic as well as prognostic markers. Our findings strongly recommend the incorporation of IL-22 level into routine CLL work up.

Keywords: Chronic lymphocytic leukemia, ZAP-70, Interleukin-22 (IL-22), β2-microglobulin, LDH

1. INTRODUCTION

Chronic lymphocytic leukemia (CLL) is defined as a low-grade lymphoproliferative neoplasm with $\geq 5\times10^9$/L clonal B-cells in the peripheral circulation which express CD5, CD19, CD20, and CD23 by the World Health Organization and the International Consensus Classification Clinical Advisory Committee [1, 2]. For evaluation of CLL patients, Rai and Binet are the two staging systems commonly used [3]. They depend on physical evidence (ie, lymphadenopathy, splenomegaly and/or hepatomegaly) and blood parameters (anemia or thrombocytopenia) to assess the degree of tumor burden and predict treatment requirements and survival. However, it cannot determine patients who may develop aggressive disease [4, 5].

ZAP-70 (70-kDa zeta-associated protein) is an intracellular tyrosine kinase that has a major role in T-cell signaling. It is also associated with the B-cell receptor in CLL. ZAP-70 expression (≥20% of B cells) is associated with an increased risk for the development of adverse outcomes in B-CLL. Thus, it is considered a prognostic factor for these patients [6]. Previous studies reported a correlation between ZAP-70 expression in CLL cells and Immunoglobulin Heavy Chain Gene Mutation (IGVH) status. ZAP-70 positive patients have an aggressive course, an immediate treatment requirement, and lower survival rates. If ZAP-70 expression is present, it remains constant all over the clinical course of the patient, and consequently, it is a valid risk marker regardless of the evaluation's timing [7, 8].

IL-22 is a cytokine that plays a crucial role in many physiological processes, ranging from innate immune responses to tissue regeneration. When its activity is impaired, this can lead to chronic inflammation, impaired wound healing, and infections [3, 4]. IL-22 has tumor-promoting properties such as tumor-cell proliferation, anti-apoptosis, and attraction of immunosuppressive immune cells [9], as well as promoting neoangiogenesis and transition from epithelial to mesenchymal, which are hallmarks of cancer [10]. IL-22 is linked to the pathogenesis of cancer colon in both inflammatory and genetic models [11] and also is linked to more aggressive phenotypes in lung, breast, gastric, and skin cancers, indicating the role of IL-22 in cancer progression [12, 13]. IL-22 up-regulation and down-regulation generate many consequences that determine its biological and pathological activities [14, 15]. Its dual role highlights the therapeutic potential of adjusting the cytokine network to

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achieve tumor prevention and treatment [16, 17].

Nevertheless, the detection of the IL-22 levels in CLL, together with their relation to the clinicopathologic characteristics of the disease need to be explored. The aim of the present work was to study the value of serum interleukin-22 in patients with chronic lymphocytic leukemia and its relation to disease characteristics

Subjects & Methods

Subjects

This study included 40 CLL patients diagnosed according to the International Workshop on CLL Diagnosis and NCCN Guidelines [18, 19] in the Hematology Department, Medical Research Institute, Alexandria University. In addition to 10 age and sex-matched normal subjects as controls.

The study was approved by the local ethics committee. Informed written consents were obtained from patients and controls.

Methods

All patients were subjected to: history taking, clinical examination, hematological investigations, including a complete blood count (CBC), reticulocyte count, coomb's test [20], and bone marrow examination if needed [21]. Immunophenotyping for CLL panel (to confirm diagnosis) [22]. Chemical investigations: lactate dehydrogenase enzyme (LDH) [23], Beta-2-microglobulin, C-reactive protein [24], liver and kidney function tests [25, 26]. Radiological investigations: plain chest X-rays, abdomino-pelvic ultrasound. Estimation of ZAP-70 expression, which was measured by three-color flow cytometric analysis [27]. Estimation of serum levels of IL-22 by a quantitative enzyme immunoassay technique using the Human IL-22 Platinum ELISA BMS2047 Kit (Affymetrix eBioscience) [28].

Statistical analysis was done using IBM SPSS software package version 23.0 (Armonk, NY: IBM Corp.). The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Data were not normally distributed, and accordingly, non-parametric tests were used. Qualitative data were tested by Chi-square test. Quantitative data were tested by Mann Whitney and Kruskal-Wallis tests. The Spearman correlation coefficient (r) tests were conducted for the correlation of hematological and biochemical markers to IL-22 in CLL patients. The significance level was set at $p \leq 0.05$. The receiver operating characteristic (ROC) curve was used for the detection of IL-22 as a sensitive predictor of ZAP-70 positivity.

Results

The study included 40 CLL patients: 22 males (55.0%) and 18 females (45.0%). Their age ranged from 44 years to 81 years, with a mean of 63.55 ± 9.8 years. In addition to 10 healthy controls (5 males and 5 females) with ages ranging from 37 to 70 years and a mean of 54.50 ±10.0 years. Table 1 shows the main hematologic parameters in CLL patients and controls. As regards clinical parameters, lymphadenopathy was detected in 82.5%, splenomegaly in 85.0%, and hepatomegaly in 32.5% of CLL patients.

Table 1: Main hematologic parameters in CLL patients and controls

Parameter	CLL patients (n=40)		Control (n=10)		*p*-value [a]
	Mean ± SD	Min. - Max.	Mean ± SD	Min. - Max.	
Hemoglobin concentration (g/dl)	10.81 ± 2.0	8.0 – 14.8	13.63 ± 1.14	12.0 – 15.7	0.001*
Total WBCs count ($\times 10^9$/L)	99.21 ± 134.2	5.20 – 487	7.93 ± 1.60	5.40 – 9.70	0.038*
Absolute lymphocytic count ($\times 10^9$/L)	86.32 ± 122.0	2.38 - 441.6	2.53 ± 0.58	1.55 – 3.39	0.036*
Platelets count ($\times 10^9$/L)	164.6 ± 88.5	18.0 – 382	247.9 ± 56.13	161 – 348	0.007*

WBCs: white blood cells
SD: standard deviation
[a] Mann Whitney test
* statistically significant

ZAP-70 expression and disease staging

ZAP-70 expression was positive in 26 (65%) patients and negative in 14 (35%) CLL patients. According to Rai staging, a significantly higher percentage of positive ZAP-70 was among stage III (57.7%), and a higher percentage of negative ZAP-70 was among stage II (57.1%) [p = 0.002*]. As regards Binet staging, 16 patients (61.5%) with positive ZAP-70 were in stage C, and 5 patients (35.7%) with negative ZAP-70 were in stage B and similarly in stage C. However, there was no significant difference between these percentages [p = 0.235] (Table 2).

Also, there is a significantly higher median level of β2-microglobulin among positive ZAP-70 compared to negative ZAP-70-expressing patients (4.15 vs. 3.25 mg/L) (p = 0.011). Serum LDH was also higher in positive ZAP-70 than in negative-expressing patients but was not statistically significant (p >0.05). CRP was similar in CLL patients with positive or negative ZAP-70 (p >0.05).

Table 2: ZAP-70 expression in relation to staging of CLL patients

Parameter	Zap-70 expression				*p*-value [a]
	ZAP-70 positive (n=26) (65%)		ZAP-70 negative (n=14) (35%)		
	№	%	№	%	
- Rai staging:					
I (n= 1) (2.5%)	0	0.0	1	7.1	**0.002***
II (n= 10) (25.0%)	2	7.7	8	57.1	
III (n= 18) (45.0%)	15	57.7	3	21.4	
IV (n= 11) (27.5%)	9	34.6	2	14.3	
- Binet staging:					
A (n= 7) (17.5%)	3	11.5	4	28.6	0.235
B (n= 12) (30.0%)	7	26.9	5	35.7	
C (n= 21) (52.5%)	16	61.5	5	35.7	

**p*-values are statistically significant
[a] Chi-square test

Serum IL-22

Mean serum concentration of IL-22 showed significantly higher values among CLL patients compared to the control group (47.01 vs. 10.64 pg/ml) (p =0.0001). Table 3 shows the mean values of serum IL-22 according to the staging of CLL patients. There were significant differences in mean serum IL-22 regarding Rai and Binet staging of CLL patients (p = 0.0001,p=0.018,respectively).

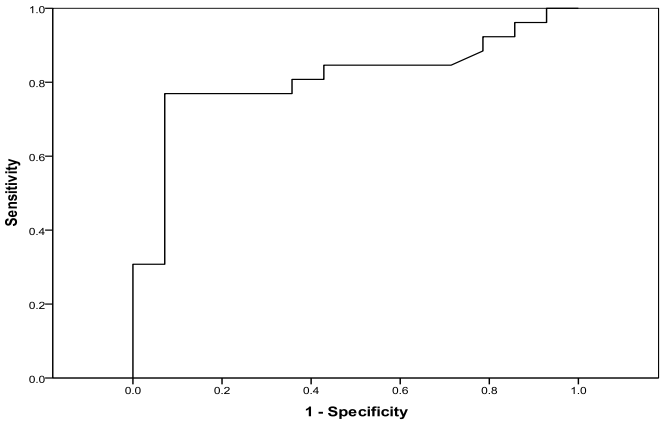
Table 3: Serum IL-22 according to staging of CLL patients

Parameter	IL-22 (pg/ml)		*p*-value [a]
	Median	Mean ± SD (Min. – Max.)	
- Rai staging:			
I (n= 1)	31.59	31.59	**0.0001***
II (n= 10)	37.19	37.36 ± 8.75 (26.68 - 54.42)	
III (n= 18)	46.85	44.85 ± 11.90 (20.68 - 62.09)	
IV (n= 11)	59.70	60.70 ± 10.37 (42.87 - 75.83)	
- Binet staging:			
A (n= 7)	31.59	38.68 ± 11.78 (26.68 - 54.42)	**0.018***
B (n= 12)	38.86	41.96 ± 10.54 (28.14 - 62.09)	
C (n= 21)	53.87	52.66 ± 14.08 (20.68 - 75.83)	

**p*-values are statistically significant [a] Kruskal-Wallis test
SD: standard deviation

IL-22 &ZAP-70

Significantly higher mean values of serum IL-22 were observed among ZAP-70 positive compared to ZAP-70 negative patients (51.94 vs. 37.83 pg/ml) (p =0.001*). The IL-22 ideal cut-off value was established at 41.22 by the ROC curve to evaluate IL-22 as a sensitive predictor of ZAP-70 positivity. In addition, 84.62% was the sensitivity, 71.43% was the specificity, PPV was 84.6%, and NPV was 71.4%. The area under the curve was statistically significant (AUC = 0.809, 95% CI=0.669 - 0.949, p =0.001). (Figure 1) (Table 4)



Diagonal segments are produced by ties.
Figure1: ROC curve for detection of IL-22 as a sensitive predictor of ZAP-70 positivity

Table 4: Prognostic performance for detection of IL-22 as a sensitive predictor of ZAP-70 positivity

	AUC	p	95% C.I	Cut off#	Sensitivity	Specificity	PPV	NPV
IL – 22	0.809*	0.001*	0.669 – 0.949	**>41.22**	84.62	71.43	84.6	71.4

AUC: Area Under a Curve p value: Probability value CI: Confidence Intervals
NPV: Negative predictive value PPV: Positive predictive value
*: Statistically significant at $p \leq 0.05$

IL-22 level showed a significant positive correlation to β2-microglobulin (r=0.448), LDH (r=0.463), CRP (r= 0.462) and ZAP-70 (r= 0.459), while other hematological parameters and serum markers did not show significant correlation. (Table 5, Figure2).

Table 5a: Correlation of hematological and biochemical markers to IL-22 in CLL patients

Variables	IL-22 (pg/ml)	
	r	*P*
Hemoglobin concentration (g/dl)	- 0.307	0.054
Total leukocytic count ($\times 10^9$/L)	- 0.156	0.337
Absolute lymphocytes count ($\times 10^9$/L)	- 0.166	0.305
Platelets count ($\times 10^9$/L)	- 0.227	0.159
Reticulocytes count (%)	0.078	0.632
β2-microglobulin (mg/L)	0.448*	0.004*
Lactate dehydrogenase (U/L)	0.463*	0.003*
C-reactive protein (mg/dl)	0.462*	0.003*
SGPT(ALT) (U/L)	- 0.051	0.756
SGOT(AST) (U/L)	0.094	0.564
Serum urea (mg/dl)	- 0.167	0.302
Serum creatinine (mg/dl)	- 0.055	0.738
ZAP-70 expression (%)	0.459*	0.003*

r: Spearman correlation coefficient * Significant statistical correlation

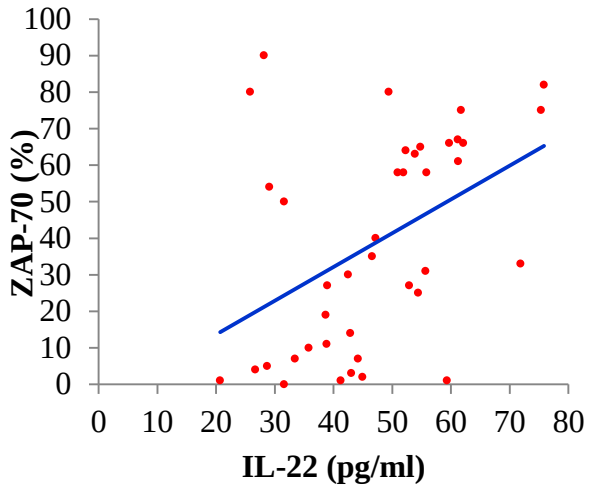


Figure 2: Positive correlation between IL-22 and ZAP-70

Discussion

CLL is characterized by clonal proliferation and accumulation of mature CD5-positive B-cells in blood, bone marrow, lymph nodes, and spleen. Apparently, the development of clonal B cells occurs at the hematopoietic stem cell (HSC) stage, raising the possibility that multipotent HSCs are involved in the primary leukemogenic state in CLL [5].

The diagnosis of CLL requires the presence of ≥5000 B-lymphocytes/μL in the peripheral blood (not less than 3 months). The clonality of the circulating B-lymphocytes is confirmed by flow cytometry. The blood smears of CLL patients show mature lymphocytes that are small in size with a narrow border of cytoplasm and a dense nucleus lacking discernible nucleoli and having partially aggregated chromatin [29].

Clinical progression of CLL patients varies largely, as some patients show rapid progression while others don't require treatment and live for years. Most patients are diagnosed in early stages; thus, Rai and Binet staging systems do not identify patients with poor prognosis [30]. Immunoglobulin heavy-chain gene (IGHV) mutation status, cytogenetic abnormalities, and expression of certain proteins have emerged as important prognostic indicators for CLL. For many clinical laboratories, routine analysis is difficult and expensive [31]. As a result, readily accessible surrogate markers are needed.

ZAP-70 expression in CLL is linked to IGHV mutational status, survival, and disease progression. Previous studies demonstrated that aberrant B-cell receptor (BCR) signalling, proliferation, and migration towards tumor microenvironment are all influenced by the abnormal expression of the ZAP-70 protein in CLL cells [30, 32].

In the current study, 65.0% of CLL patients have positive ZAP-70 expression. This was in accordance with the percentage reported by Singleterry et al. [33], but Zeeshan et al. [34] found that ZAP-70 was positive in 12 (13.5%) patients, while 77 (86.5%) were negative. Previous studies by Liu et al. [30] concluded that ZAP-70 is overexpressed in IGHV-unmutated CLL but not in normal "B" lymphocyte cells and showed a similar clinical value to IGHV mutational status in terms of disease progression and survival.

In the present work, ZAP-70 expression showed a highly significant association with Rai staging (p = 0.002) but not with Binet staging. This finding was in accordance with the study of Patel [35], who stated that in Binet staging, there was no significant difference in ZAP-70 expression. Zeeshan R et al. [34] revealed that ZAP-70 protein and Rai stage III disease were found to have a high positive correlation (p=0.004). These factors imply that ZAP-70 expression in CLL may represent a malignant clone in an activated state and be linked to a progressing disease. As a result, a laboratory test for ZAP-70 expression may be a crucial addition to the patient's overall care.

In the present study, patients with positive ZAP-70 expression had a significantly higher median level of β2-microglobulin compared to negative ZAP-70-expressing patients (p = 0.011). Serum LDH was also higher in positive ZAP-70 than in negative-expressing patients but was not statistically significant. The study of Assem et al. [36] reported a significant rise in LDH and β2M serum levels in ZAP-70 positive groups when compared to negative groups and concluded that ZAP-70 is predictive of disease progression. Amaya-Chanaga et al. [37] declared that, while choosing the ideal plan of treatment and prognosis for CLL patients, physicians must take ZAP-70 expression into account as one of the independent prognostic tools.

IL-22 is a glycoprotein released by several types of CD4-positive cells, including Th-22 cells. According to reports, it contributes to the development and progression of malignancies. The IL-22/IL-22 receptor-1 signalling cascade is another route by which cancer stem cells can endure and multiply. Additionally, it is clear that IL-22 levels are elevated in many types of cancers [38].

The mean level of IL-22 in our study was significantly higher in CLL patients than in the control group. This is in concordance with Heiba et al. [38] and Abd El-Hamed et al. [39], who revealed an elevation in IL-22 plasma levels in CLL patients compared to healthy subjects. Lim et al. [10] and Voigta et al. [9] reported that IL-22 is a cancer-promoting cytokine.

Our work demonstrates a highly significant difference between the level of serum IL-22 according to Rai staging (p = 0.0001) and Binet staging of CLL patients (p = 0.018). However, in contrast to our results, Gangemi et al. [40] reported that throughout the various stages of CLL, there were no statistically significant changes in the levels of IL-22. Also, Kouzegaran et al. [41] found that levels of IL-22 were not significantly associated with the different stages of disease (Rai stages). Our results were partly concordant with those of Atreya et al. [42], who showed a direct association between staging of colorectal cancer and IL-22, which confirms the involvement of IL-22 in the progression of carcinogenesis.

Our results indicate that CLL patients presenting with positive expression of ZAP-70 had significantly higher IL-22 levels in comparison with those negative ZaP-70. This finding is in accordance with Heiba et al. [38], who found

that patients with positive ZAP-70 expression had higher plasma levels of IL-22 than healthy individuals. Abd El-Hamed et al. [39] suggested that a high level of IL-22 and positive ZAP-70 expression have a synergistic action in inhibiting apoptosis and triggering proliferative responses.

Finally, this study found a highly significant correlation between IL-22 and β2-microglobulin, LDH, CRP, and ZAP-70, while other hematological parameters and serum markers did not show a statistically significant correlation. In accordance with our result, Kouzegaran et al. [41] found that CLL patients with positive CD38 and ZAP-70 had higher plasma levels of IL-22 as compared to healthy individuals. On the contrary, Gangemi et al. [43] reported that levels of ZAP-70, LDH, and β2-microglobulin did not significantly correlate with plasma IL-22 levels. Protopsaltis et al. [44] demonstrated that IL-22 can induce angiogenesis by directly acting on endothelial cells. Blockage of IL-22 inhibits tumor growth and reduces tumor angiogenesis, thus providing a possible cancer treatment through the development of anti-IL-22 therapies.

Conclusion

According to these findings, which denote the sensitivity of serum IL-22 in predicting ZAP-70 positivity among CLL patients, serum IL-22 could be used as a surrogate and reliable sole marker for ZAP-70 expression. In addition, IL-22 may be used as a novel goal for CLL immunotherapy approaches.

Recommendations

Assessment of IL-22 should be included in the CLL work-up.

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