Research Article

Significance of Interleukin-22 and CD38 in Chronic Lymphocytic Leukemia

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Abstract
Chronic lymphocytic leukaemia (CLL) is a common type of leukaemia characterised by an abnormal increase in T-cell count. T-helper cytokines were reported to stimulate the survival and proliferation of CLL cells and could be correlated with disease progression. This study aims to study the possible inter-regulation and correlation between IL-22 and CD38 in CLL patients. It was carried out on newly diagnosed B-CLL patients and healthy controls. Routine laboratory investigations were carried out for all subjects. Haematological laboratory investigations were performed for all subjects, these included CD19, CD38 and interleukin-22 (IL-22) absolute and percentage counts. CLL patients showed a significant ($P<0.05$) increase in CD19, CD38 and IL- 22 absolute and percentage counts by comparison to the healthy control subjects. 25% of CLL patients showed positive CD38 expression. Interestingly, IL-22 percentages showed an association with CD38 expression. IL-22 percentage showed an increase in patients with high CD38 expression and a decrease in patients with low CD38 expression. Moreover, there was a significant ($P<0.05$) positive correlation between CD38 and IL-22 with Pearson's correlation coefficient ($r=0.621$). Thus, CD38 and IL-22 could act in synergy to maintain CLL cell survival and proliferation and could be used as diagnostic and prognostic markers.

Keywords: Chronic lymphocytic leukaemia, Interleukin-22 (IL-22) and CD38

Introduction
Chronic lymphocytic leukaemia (CLL) is one of the most common types of leukaemia in adults and is characterized by genetic and clinical heterogeneity[1]. CLL is B-cell neoplasia associated with the immune system and microenvironment disorders[3]. Pathogenesis of CLL multifactorial including genetic susceptibility, tumor cell modulation, epigenomic tumor cell reprogramming, and microenvironment interactions[2]. Transformation and progression of CLL cells are controlled by many factors as the interactions with tumour microenvironment favours malignant B cell clones' survival and delays their apoptosis. Indeed, this would contribute to the pathogenesis and progression of the disease[4].

There are several prognostic markers for CLL, currently used in clinical practices including CD38 level, ZAP-70 expression, abnormalities of interphase fluorescence in situ hybridization (iFISH) and the heavy-chain variable region (IgVH)[5].
The percentage of CD38 positive cells was reported to be a prognostic factor in CLL, The higher the level of CD38 in CLL, the poorer the prognosis and the response to therapy becomes[6]. One of the characteristic features of CLL is the presence of complex immune disorders that have an important role in disease pathogenesis[4]. Elevated number of CD4 positive T-helper cell subset has been reported in CLL patients[3].

T-helper (Th) cell subsets secrete distinct cytokines which exert specific immunelogical functions and have a strong correlation with the progression of CLL [Matrai, 2005 #14]. They induce the antiapoptotic proteins which maintain CLL cell survival

and proliferation [7][8]. Cytokines produced by Th-17 and Th-22 cell subsets were found to have an impact on the patho-genesis of different auto-immune diseases and solid tumours[9].

Dysregulation of IL-22 expression and signalling was found in patients with skin, liver, and lung cancers[10]. Moreover, IL-22 is implicated in the pathogenesis of some leukemic disorders such as acute myeloid leukaemia (AML)[11], acute lymphocytic leukaemia ALL[12] and myelodysplastic syndrome (MDS)[13]. **This study** aims to assess the possible co-regulation and correlation between IL-22 levels and CD38 expression in CLL.

Subjects and Methods

The present cross-sectional study was carried out at Minia University, Faculty of Medicine, Department of Clinical Pathology.

The study included two cohorts:

Cohort I (**healthy cohort):** Included 35 healthy individuals, 65% males and 35%females, with age 45-68.

Cohort II (CLL patients' cohort): It included 35 patients, recently diagnosed with B-CLL. All samples were collected before the onset of any anti-cancer therapies such as chemotherapy or anti-inflammatory drugs. 63 % males and 37 % females.

Patients were diagnosed in accordance with the guidelines outlined by the International Workshop on CLL Diagnosis (Hallek et al.,[14]). They were subjected to full assessment of the following:

- History taking, considering age, sex, fever, bleeding tendency, easy fatigability and history of haemolytic attacks.
- Clinical examination, including pallor, purpura, hepatomegaly, splenomegaly and lymph nodes enlargement.
- Routine laboratory tests, including complete blood count, erythrocyte sedimentation rate, renal and liver functions tests, lactate dehydrogenase enzyme, C-reactive protein, bone marrow (BM) aspirations.
- Specialised haematological tests by flowcytometry **(**BD-FACS FLOW, Argon laser, USA). These testes included counting CD19, IL22 and CD38 expressing cells in peripheral blood and working out the percentages and the absolute counts. Data were processed by XL software.

Statistical analysis

SPSS program (Statistical Package for Social Sciences) software version 25 was used for data analysis. The parametric quantitative data were analysed using descriptive statistics by mean ± SD, and an independent-sample T test was utilized to analyse the minimum & maximum of the range. As for the non-parametric quantitative data, they were analysed by median using the Mann-Whitney test. Pearson's correlation coefficient was used for the correlation between markers and laboratory data. Data were expressed as mean ± SD. The significance level was taken at (P value < 0.05).

Results

1. Increase CD19 positive cell counts in CLL:

CD19 percentage and absolute count showed a significant (P<**0.001**) upregu-lation in the CLL (34.3± 13.4) and (16.4±17.8) respectively by comparison to the healthy control cohort (9.3± 3.2) and (0.23±0.11) respectively as summa-rised in (table 1).

2. Increase in CD38 positive cell counts in CLL:

CD38 percentage and absolute counts showed a significant (P<**0.019 and P<0.001 respectively)** upregulation in CLL (12.6±13.9) and (6.03±10.14) respectively by comparison to the healthy control (5.1±2.7) and (0.1±0.06) respectively as summarised in (table 1).

3. Increase in IL-22 expressing cells in CLL:

IL-22 showed a significant (P<**0.001**) increase in both the percentage and absolute counts in CLL (4.6±2.7 and 2.43±3.3 respectively) by comparison to the healthy control cohort (1.0±0.6 and 0.022 ±0.015 respectively) as summarised in (table 1).

4. Interrelation between IL-22 and CD38 percentages in CLL patients:

CLL patients with high CD38 expression (≥20%) showed increase in IL-22 percentage (4.1 -11.4%). Whereas CLL with low CD38 expression (<20%) showed decrease in IL-22 percentage (0.5 -8.3%). Thus, was a statistically significant increase in the percentage of IL-22 in patients with high CD38 expression when compared with patients with low CD38 expression (7.5±2.4 versus 3.8±2.3) respectively (P=0.003) Data summarised in (table 2). Pearson's correlation coefficient statistical analysis was performed in order to find out the significancy of this data and if there are any correlations.

5. Correlation between CD38 and IL-22 percentage in CLL:

There was a significant (P<0.001) positive correlation (r=0.621) between CD38 percentage and IL-22 percentage in CLL patients. However, there were no correlations between IL-22 percentage and other assessed haematological parameters. Data are summarised in (table 2).

6. correlation between absolute CD38 and absolute IL22 expressing cell count

There was a significant (P< 0.001) positive correlation between absolute CD38 count and absolute IL 22 count (r= 0.717). Data summarised in (table 4).

Table (1): CD19, CD38 and IL-22 (percentage and absolute positive cell count) in both groups.

		CLL patients	**Control**	**P-value**
Parameters	Descriptive data	N=35	N=35	
CD19%	Range Mean ± SD Median	(23-71) 34.3±13.4 30	(2-15) 9.3±3.2 10	**<0.001****
Absolute CD19 x10^3/µl	Range Mean ± SD Median	(2.02-100.8) 16.4±17.8 12.8	(0.07-0.49) 0.23±0.11 0.2	**<0.001****
CD38%	Range Mean ± SD Median	(1.6-64) 12.6±13.9 6.2	(1.1-11) 5.1±2.7 4.7	**0.019***
Absolute CD38 x10^3/µl	Range Mean ± SD Median	(0.21-53.9) 6.03±10.14 1.91	(0.02-0.24) 0.1±0.06 0.1	**<0.001****
IL-22%	Range Mean ± SD Median	(0.5-11.4) 4.6±2.7 4.1	(0.3-2.2) 1±0.6 0.9	**<0.001****
Absolute IL-22 x10^3/µl	Range Mean ± SD Median	(0.06-14.5) 2.43±3.3 1.2	(0.004-0.06) 0.022 ±0.015 0.02	**<0.001****

: Significant difference at P- value < 0.05

****: Significant difference at P- value < 0.001***

Table (2): Coregulation between IL-22 and CD38 percentages in CLL patients.

	CD38		**P-value**
	<20%	**≥20%**	
	26	**9**	
IL-22			***0.003****
Range	(0.5-8.3)	(4.1-11.4)	
Mean ± SD	3.8±2.3	7.5±2.4	
Median	3.5	7.8	

**** Significant difference at P- value < 0.005**

Table (3): correlations between IL-22% and haematological parameters (HB, Platelets, Lymphocyte%, absolute lymphocytic count, LDH, CD19% and CD38%) in CLL patients

CLL patients	IL-22%	
	Pearson's correlation coefficient (**R**)	**P value**
HB	-0.275	0.110
Platelets	-0.041	0.817
Lymphocyte %	0.058	0.740
Absolute lymphocytic count	0.088	0.617
LDH	0.167	0.339
CD19%	0.168	0.336
CD38%	**0.621**	**<0.001***

: Significant difference at P- value < 0.005

Table (4): correlation between absolute CD38 and absolute IL22 expressing cell count in CLL group.

	Absolute IL22 ($x10^3$)	
	r	**P value**
Absolute CD38 ($x10^3$)	0.717	P<0.001*

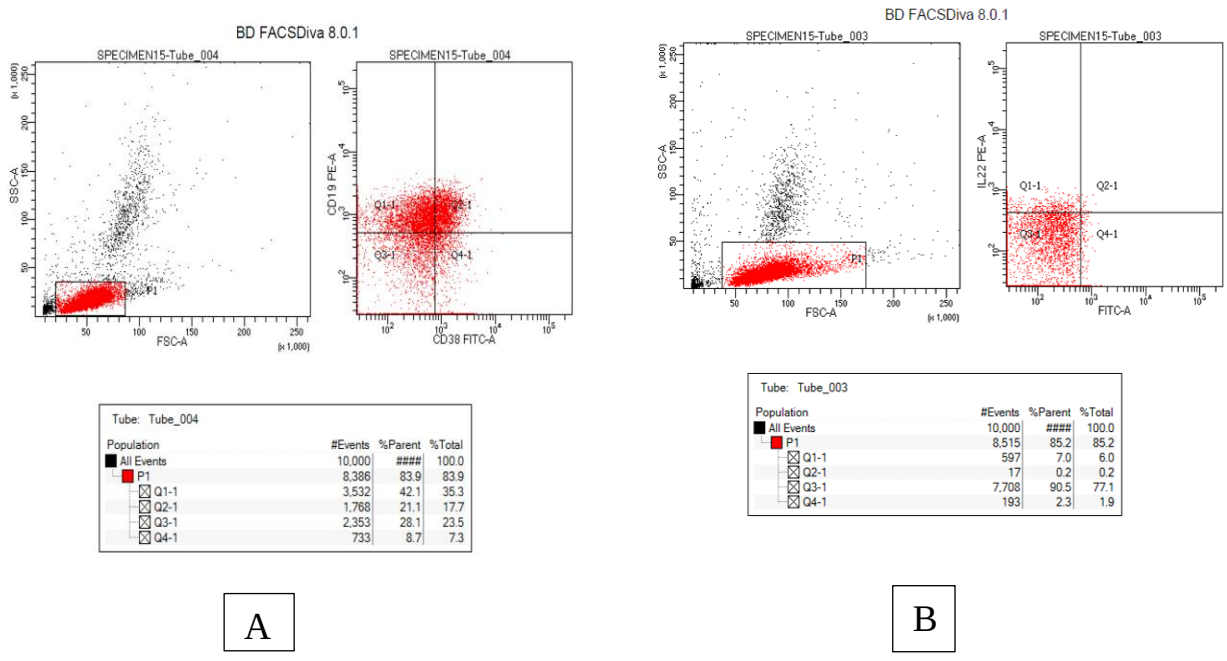

Figure 1: A) percentage of CD38 and CD19 in CLL patients (CD 38 =29.8% and CD 19=63.2%). B) Percentage of IL-22 in CLL patients (IL-22=7.2%)

Discussion

In the present work, we have investigated the potential role of IL-22 in patients newly diagnosed with B-cell CLL and the correlation between IL-22% and CD38% in malignant cells in those patients.

IL-22 is a glycoprotein, and it is a member of the IL-10 family that was named as IL-10-related T cell-derived inducible factor[15] IL-22 is released by several types of CD4 positive cells including Th-22 cells

and CD8 positive T lymphocytes, natural killer T cells, type 3 ILCs and γδ T lymphocytes[16].

IL-22 binds to the IL-10 receptor 2 and the IL-22 receptor-1 to induce differentiation. It initially binds to the IL-22 receptor-1 subunit that undergoes a conformational change which allows binding of IL-10 receptor 2. This initiates the downstream signalling cascade which promotes pro-survival and anti-apoptotic genes and consequently enhances carcinogenesis[17].

IL-22 is reported to have a role in the initiation and progression of cancers. Additionally, cancer stem cells can survive and proliferate through the IL-22/ IL-22 receptor-1 signalling cascade[18]. Moreover, increased level of IL-22 is evident in several malignancies[9].

In our flowcytometric study, the percentage of IL-22 is found to be significantly higher in patients with B-CLL than healthy individuals. This agrees with[7] and[19] have reported increase in IL-22 plasma levels and mRNA in CLL patients by comparison to the healthy individuals.

Several studies have reported crucial role for IL-22 in the pathogenesis of different haematological malignancies including MDS, ALL, and AML[11], [12] and [13]. ***There are*** several prognostic markers for CLL including lymphocyte count doubling time, Rai and Binet staging, cytogenetics, mutations, expression of zeta-chain-associated protein kinase 70 and the Ig heavy chain V-III region VH26 gene mutation and CD38 expression[20].

CD38 is a type II transmembrane glycoprotein that not only acts as an ectoenzyme or a receptor molecule but also as a signalling factor in lymphocytic cells. It participates in many cellular activities that include cell adhesion, calcium regulation and signal transduction[21]. There are increasing evidence that CD38 is involved in CLL cell trafficking. Higher CD38 levels is correlated with the increased chemotaxis of CLL cells toward chemokines like CXCL12 and CCL21, that locate in lymph nodes and regulate CLL cell accumulation within the lymphoid niches. Furthermore, CD38 expression is associated with poor prognosis in CLL[22]. Moreover, high levels of CD38 in CLL cells are generally associated with advanced disease stages, shorter time to the first treatment and poor response to therapy[6].

Herein, we have shown a significant positive correlation between IL-22 percentage and CD38 percentage on malignant B cells. Interestingly, the percentage of IL-22 in patients with high CD38 expression was higher than in CLL patients with low CD38 expression

This finding follows[23] who reported that patients with high expression of CD38 had significantly higher IL-22 levels than those with low level of CD38. Thus, our study suggests that high expression of IL-22 and high expression of CD38 might have a synergistic effect in the activation of proliferative responses and inhibition of apoptosis. This agrees with [8] and [9] who reported that IL-22 overexpression was coupled with high CD38 percentage and associated with poor prognosis in B-CLL patients. Furthermore, there are no significant association between IL-22 expression and other clinical parameters including age, sex, HB levels, platelet count, absolute lymphocytic count or LDH levels. This finding agrees with [8] who found that these **features do not influence the level of IL-22.**

Conclusions

CD38 expression stands as a good prognostic indicator in patients with CLL. High expression of IL-22 is associated with high expression of CD38. Thus, both parameters could act in synergy to activate proliferative responses and inhibiti of apoptosis.

Recommendations: Further studies with larger cohort are needed to assess the use of IL-22 level as prognostic marker in patients with B-CLL.

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