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Expression Pattern of Chemokine Receptors CXCR3, CXCR4 and CCR7 in B-Cell Lymphoproliferative Disorders

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ABSTRACT

Background: Chemokine receptors are expressed by lymphoid cells and function to mediate cell trafficking under both physiological and pathological conditions. In lymphoproliferative disorders they may play a role in the dissemination of malignant cells.

Aim: To identify the expression of chemokine receptors CXCR3, CXCR4 and CCR7 in malignant B lymphoproliferative disorder and to evaluate their prognostic impact.

Patients and Methods: A total of 60 newly diagnosed cases of B-Lymphoproliferative disorders (30 CLL/SLL, 17 B-ALL/LBL, and 13 B-NHL) and 12 controls were enrolled in our study. The expression of CXCR3, CXCR4 and CCR7 was detected by Flow Cytometry.

Results: Chemokine receptor expression in CLL patients was 90% for both CXCR3 and CCR7, and 73% for CXCR4 that showed a significant correlation with Bone Marrow (BM) lymphocytes, and between CCR7% and Absolute Lymphocyte Count (ALC). In Non-Hodgkin's Lymphoma (NHL), chemokine receptor expression was 53.8%, 69.2% and 61.5% for CXCR3, CXCR4% and CCR7%, respectively; with significant decrease of CXCR4 Mean Florescence Intensity (MFI) in patients with splenomegaly and hepatomegaly and CCR7% and MFI in patients with splenomegaly. A significant positive correlation was found between CXCR3 expression and Total Leukocytic Count (TLC) and ALC. A significant higher overall survival was detected in cases with higher CXCR3 MFI and CCR7. Chemokine receptor expression in B-ALL/LBL group was 64.7%, 82.4% and 23.5% for CXCR3, CXCR4 and CCR7, respectively. There was a significant positive correlation between CXCR3 expression and TLC, and between CXCR4 expression and Hemoglobin (Hb) level.

Conclusions: The expression of some chemokine receptors is heterogenous in lymphoproliferative disorders with a significant higher expression in CLL/SLL.

Key Words: *Chemokine receptors – CXCR3 – CXCR4 – CCR7 – Lymphproliferative disorders.*

INTRODUCTION

Chemokines are a large family of structurally homologus cytokines that stimulate leukocyte movements and regulate the migration of leukocytes from blood to tissues. They were first characterized as chemo-attractants that mediate cell trafficking and localization, and have subsequently been shown to have many functions in homeostasis and pathophysiology **[1]**. Studies have identified 50 human chemokines and 20 chemokine receptors **[2]**.

Chemokines regulate their activity through interaction with their specific G-proteins coupled receptors superfamily. They were shown to play a critical physiological role to establish the complex architecture of secondary lymphoid organs **[2]**, as well as pathological conditions including tumor metastasis, growth, survival and angiogenesis **[3]**. In addition, they were found to regulate antitumor immunity **[4]**.

A given cell can express multiple chemokine receptors. Many of chemokine receptors can bind more than one ligand allowing extensive overlap and redundancy of chemokine functions. They are expressed by many cells, including lymphoid cells. Normal B cells have been reported to express CXCR3, CXCR4, CXCR5, CCR6, and CCR7 **[5]**.

B Cell Lymphoproliferative Disorders (BCLPDs) often represent a defined stage in the normal lymphoid differentiation pathway at which the neoplastic transformation takes place. Neoblastic cell infiltration of extramedullary organs is a character of many BCLPDs

and variation in their chemokine receptor expression could partly explain this behavior **[5]**. Migration and homing to Bone Marrow (BM) and lymphoid tissue may be critical for growth support and rescue from apoptosis that the microenvironment provides for these malignant B cells **[6]**.

The identification of the critical role of chemokine receptors in tumor development and metastasis could lead to the development of new and potent anticancer drugs that target those receptors **[4]**.

In this work we studied the expression pattern of chemokine receptors (CXCR3, CXCR4 and CCR7) in B-CLPDs and their clinical relevance. Also, the changes in the expression of chemokine receptors during early stages of B cells development (ALL) and the more mature ones (CLL) were evaluated.

PATIENTS AND METHODS

Patients:

Sixty newly diagnosed cases of B-CLPD were enrolled from the out patient clinic at the National Cancer Institute (NCI), Cairo University. Twelve healthy, hematological free, age and sex matched subjects were included as normal controls.

Patients were divided into three groups: Group I: Thirty patients with Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL). Diagnosis of CLL was based on morphological and phenotypic scoring system; all cases showed score ≥3 with dim expression of surface κ (17 cases) or λ (13 cases). Group II: Thirteen patients diagnosed by lymph node and BM biopsy and immunohistochemistry as stage IV B-Non Hodgkin Lymphoma (B-NHL). Group III: Seventeen patients with B-Acute Lymphoblastic Leukemia/Lymphoblastic Lymphoma (B-ALL/LBL); they were diagnosed by BM morphology, cytochemistry and immunophenotyping by Flowc cytometry, 11/17 were C-ALL (64.7%) and 6 Pre B ALL (35.3%).

The existence of hepatomegaly, splenomegaly, lymphadenopathy, infiltration to other organs, and the overall survival were checked to determine extent of disease and relation to the studied markers. This study was approved by the Institutional Review Board (IRB) of the NCI, Cairo University.

Methods:

Direct surface staining for chemokine receptors:

Fresh bone marrow or peripheral blood EDTA samples were used. The leucocytic count was adjusted to 5-10 X 10^3 cell/μl. Cells were washed by PBS three times before adding the monoclonal. Monoclonal antibodies (Mo Abs) labeled with Phycoerythrien (PE) against human CXCR3 (FAB160p), CXCR4 (FAB170p) and CCR7 (FAB197p) and appropriate isotype control supplied by R and D systems Inc (Minneapolis, Minnesota, USA) were used. Fifty μl of the washed sample were dispensed and 10μl of each monoclonal antibody were added to appropriately labeled tubes, properly mixed and incubated at 2-8ºC for 30 minutes in the dark. The RBCs were lysed by adding 2ml of erythrocyte lysing solution (BD. 349202), mixed gently and incubated for 5 minutes at room temperature, centrifuged at 1500g. Cells were washed twice with 3ml PBS and re-suspended in 200-400ml of PBS buffer for immediate analysis.

Flow cytometric analysis:

Immunophenotypic expression was measured by (FAC Vantage): Becton Dickinson, San Diego, USA) using the Cell Quest software program (Becton Dickinson). Analytical gates were set on the clone of malignant lymphoid cells based on forward and side scatters combined with exclusion of normal cells using a CD45 gating protocol. In short, we first gated on the CD45 dim population (G1) then reflected those cells (G1) on the FSC/SCC dot plot to get gate 2 (G2) that is fixed in all tubes and used for analysis of all markers.

Marker expression was determined as percent positivity of stained cells within the lymphoid population as well as Mean Florescent Intensity (MFI) obtained by dividing the channel number of the specific marker by that of the isotype control **[3]**.

Statistical analysis:

Data was analyzed using SPSS statistical package version 16. Numerical data were expressed as mean ± standard deviation, median, and range. Qualitative data were expressed as frequency and percentage. Chi-square test was used to examine the relation between qualitative variables.

For quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric *t*-test) because the variables are not normally distributed. Comparison between 3 groups was done using Kruskal-Wallis test (non-parametric ANOVA test) followed by post-Hoc comparison test "Schefe test" on the rank of variables was used to compare pairs of groups.

Survival analysis was done using Kaplan-Meier method. Comparison between two survival curves was done using Log-rank test. Relation between different numerical variables was tested using Spearman's rho correlation. Correlation coefficient (*r*) of 0.5 was considered fair correlation, if more than 0.5 to 0.75, it was considered good correlation, and if more than 0.75, it was considered as very good correlation.

Receiver Operating Characteristic (ROC) curves were plotted for identification of the cut off values for CXCR4, CXCR3, CCR7 both % and MFI.

Probability (*p*-value) <0.05 was considered significant and less than 0.001 considered as highly significant.

RESULTS

Clinical parameters of the studied patient's groups are shown in (Table 1).

Chemokine receptor expression in CLL patients:

Table (2) shows the expression of Chemokine receptors in CLL patients.

Splenomegaly and/or hepatomegaly had no impact on the expression of CXCR3, CXCR4 or CCR7 (p=>0.05). Neither did the performance state (p=0.09, 0.85, 0.29, respectively) or the modified Rai stage (p=0.18, 0.87, 0.65, respectively).

Also, expression of κ (17 patients, 57%) or λ (13 patients, 43%) had no significant effect on CXCR3, CXCR4 or CCR7 expression (p= 0.6, 0.14, 0.13, respectively) neither CD38 positive expression (5 patients, 17%) (p=0.19, 0.9, 0.27, respectively).

CXCR3 expression was comparable to control group while both CXCR4 and CCR7 were significantly higher in CLL compared to control group. No significant differences from other groups were encountered except for CCR7 which was higher than ALL and NHL significantly in both % expression and MFI for ALL (p=<0.001) and in MFI for NHL (p=0.05) (Table 3).

Correlation between chemokine receptors expression and hematological data is presented in (Table 4). Significant positive correlation was encountered between CXCR4 and BM Lymphocytes (p=0.02) as well as between CCR7% and absolute lymphocyte count (p=0.04).

The follow-up period of CLL/SLL patients ranged between 1 and 91 with a median of 19 months. The Cumulative Overall Survival (CS) was 72.7%. Chemokine receptors expression had no impact on and overall survival, Fig. (1).

As regards Disease Free Survival (DFS), 14/30 patients (47%) achieved Complete Remission (CR) after initial chemotherapy. Time to Disease Progression (TDP) ranged from 1 to 32 with a median of 16 months with no impact of chemokine receptors expression (p>0.05), Fig. (2).

Chemokine receptor expression in NHL patients:

Chemokine receptor expression in NHL patients is shown in (Table 2).

Patients with splenomegaly (11/13, 85%) showed significantly lower values of CXCR4 MFI (6.6±6.7), CCR7% (27.8±30.2) and CCR7 MFI (4.2±4.9) when compared to patients without splenomegaly (CXCR4 MFI, 23.3±13.4, CCR7% 87.2±4.5 and CCR7 MFI 30.2±13.1); p-values were 0.04, 0.04 and p=0.03, respectively.

The mean ± SD of CXCR4 MFI expression was 4.4±4.6 in NHL patients with hepatomegaly (8/13, 62%) which was significantly lower than patients without hepatomegaly (mean ± SD 16.7±11.1) (p=0.02).

There was no significant association of the chemokine receptor expression with other parameters (p>0.05).

CXCR4% and CXCR4 MFI were significantly higher in patients with performance state 0, I (4/13, 31%) compared to (9/13, 69%) in patients with performance state (II, III). The CXCR4% and MFI were 61.9±19.7 and 14.6± 10.2, respectively in patients with performance

state 0, I, compared to 21±25 and 2.8±2.4 in patients with performance state (II, III) (p=0.02 and 0.006, respectively). No association of CXCR3 or CCR7 expression and performance status was encountered (p=0.75 and 0.1, respectively).

Significant positive correlation was encountered between CXCR3 expression and TLC (r=0.6), ALC (r=0.65) and peripheral blood lymphocytes % (r=0.54) (Table 4).

The follow-up period of B-NHL patients ranged between 1 and 64 with a median of 14 months. The cumulative overall survival (CS) was 39% with a mean ± SE (13.7±5.1).

Significant higher overall survival was associated with higher CXCR3 MFI (p=0.005) and with higher CCR7 positive cases (p=0.011). There was no statistically significant association of CXCR4 expression and overall survival in NHL patients (Table 5), Figs. (3,4).

In B-NHL patients, only 3/13 cases responded to the initial chemotherapy and entered in Complete Remission (CR) but relapsed after that. The follow-up period of DFS for those three B-NHL patients ranged (16-26), with a median follow-up of 16.2 months and the mean ± SD was 19.4±5.7. The CS at 16 months was 67.7% with a median ± SE 16.1±0.0. No significant relation with chemokine receptors expression could be estimated.

Chemokine receptor expression in B-Lymphoblastic leukemia/lymphoblastic lymphoma (B-ALL/LBL) group:

Table (2) shows the expression of Chemokine receptors in ALL patients.

In ALL, there was no impact of splenomegaly or hepatomegaly on the expression of CXCR3, CXCR4 or CCR7 (p=0.4, 0.2, 0.2 for splenomegaly and 0.18, 0.87, 0.65 for hepatomegaly respectively).

Significant positive correlation was encountered between CXCR3% and TLC (r=0.5, p= 0.04) and significant negative correlation between CXCR4% and Hb (r=–0.61, p=0.009) (Table 4).

Follow-up period of B-ALL/LBL ranged from 6 to 40 with a median of 37.6 months. The CS at 37.5m was 70.6% with no impact of chemokine receptor expression (Table 5).

Follow-up period for Disease Free Survival (DFS) ranged from 3-39 with a median of 35 months; 15/17 (88%) patients responded to the initial chemotherapy and achieved Complete Remission (CR) with no impact of chemokine receptor expression on DFS.

Table (1): Clinical and laboratory data of the studied groups at diagnosis.

Characteristic	CLL (n=30)	NHL (n=13)	ALL (n=17)
Sex: Male/female	19/11	7/6	13/4
Age*	60.5 (37-75)	58 (36-73)	6 (1.2-17)
TLC X 10^9/L*	80.5 (12-278)	40.1 (5.2-15)	17.8 (2-60)
Hb g/d*	10 (3.1-13.5)	11 (6.9-13.8)	7.4 (3-12.6)
Platelets X 10^9/L*	168 (79-474)	144 (83-379)	40 (6-70)
B cell population in PB%*	88.5 (39-99)	72 (30-89)	60 (20-95)
B cell population in BM%*	78.5 (21-97)	6.5 (0.2-84)	90 (20-97)
Presence of B Symptoms**	2 (7%)	4 (30.8)	8 (41.7)
Lymphadenopathy**	27 (90%)	12 (92.3)	11 (64.7)
Splenomegaly**	25 (83%)	11 (84.6)	9 (52.9)
Hepatomegaly**	17 (57%)	8 (61.5)	9 (52.9)
Number achieved CR**	14 (47%)	3 (23.1%)	15 (88.2%)
*Survival status***:*			
- Alive	28 (93.3%)	8 (61.5)	16 (94.1)
- Dead	2 (6.7%)	5 (38.5)	1 (5.9)

CLL : Chronic Lymphocytic Leukemia.
NHL : Non-Hodgkin's Lymphoma.
CR : Complete Remission.
* : Median (range).
** : No (%).

Table (2): Chemokine receptors expression among the studied groups.

Parameter	CLL 30 cases	NHL 13 cases	ALL 17 cases
CXCR3%*	27 (90%) 16.3 (1-83)	7 (4%) 6.5 (0.2-84)	11 (5%) 4.4 (1-35)
CXCR3 MFI**	7 (23%) 23 (77%)	5 (9%) 8 (2%)	7 (41.2%) 10 (9%)
CXCR4%*	22 (73%) 46.6 (1.2-97)	9 (69.2%) 43 (1.3-80)	14 (82.4%) 167 (2.4-25)
CXCR4 MFI**	8 (27%) 22 (73%)	5 (9%) 8 (2%)	6 (35.3%) 11 (65%)
CCR7%*	27 (90%) 68.8 (1-96)	8 (2%) 27 (1.2-90.4)	4 (4%) 8.1 (3.1-17)
CCR7 MFI**	5 (17%) 25 (83%)	6 (46.2%) 7 (4%)	10 (9%) 7 (41.2%)

CLL : Chronic lymphocytic leukemia. *% expression:* * Frequency (%) *MFI***:* Low: Frequency (%)
NHL: Non-Hodgkin's lymphoma. Median (range) High: Frequency (%)
ALL : Acute lymphoblastic leukemia.

Table (3): Comparison between the expression of chemokine receptors in the different studied groups and control.

Chemokine / Groups	CXCR3 (%)	CXCR3 (MFI)	CXCR4 (%)	CXCR4 (MFI)	CCR7 (%)	CCR7 (MFI)
CLL vs. control	0.14	0.28	0.02	0.01	<0.001	0.009
NHL vs. control	0.73	0.59	0.14	0.24	0.24	0.9
ALL vs. control	0.51	0.95	0.12	0.79	0.9	0.5
CLL vs. NHL	0.75	0.98	0.98	0.78	0.11	0.05
CLL vs. ALL	0.8	0.51	0.96	0.09	<0.001	<0.001
ALL vs. NHL	0.9	0.83	1.0	0.69	0.5	0.18

CLL : Chronic lymphocytic leukemia. NHL: Non-Hodgkin's lymphoma. ALL : Acute lymphoblastic leukemia.

Table (4): Correlations between the expression of chemokine receptors and some prognostic hematological parameters in the different studied groups.

Parameter	CXCR3%		CXCR4%		CCR7%	
	r	*p*	*r*	*p*	*r*	*p*
Chronic lymphocytic leukemia:						
TLC ($X10^9$/L)	0.3	0.11	0.13	0.49	0.27	0.15
HB (g/dL)	–0.19	0.33	0.001	0.99	0.13	0.49
PLT ($X10^9$/L)	–0.14	0.45	–0.16	0.39	–0.35	0.057
ALC	0.28	0.13	0.17	0.35	0.36	0.04*
BM lymphocyte (%)	0.19	0.9	0.5	0.02*	0.28	0.13
Non-Hodgkin's Lymphoma:						
TLC ($X10^9$/L)	0.6	0.04*	0.04	0.89	0.43	0.14
HB (g/dL)	–0.25	0.41	0.52	0.86	–0.09	0.77
PLT ($X10^9$/L)	–0.2	0.5	–0.02	0.96	–0.27	0.37
ALC ($X10^9$/L)	0.65	0.02*	0.15	0.62	0.5	0.1
PB lymphocyte %	0.54	0.048*	0.49	0.08	0.21	0.49
BM lymphocyte %	0.23	0.45	–0.36	0.22	0.19	0.52
Acute lymphoblastic leukemia:						
TLC ($X10^9$/L)	0.5	0.04*	0.17	0.5	0.35	0.17
HB (g/dL)	–0.44	0.08	–0.61	0.009*	–0.45	0.07
PLT ($X10^9$/L)	–0.28	0.27	–0.08	0.77	–0.26	0.32
PB Blasts %	0.37	0.15	0.18	0.49	0.23	0.38
BM Blasts %	0.12	0.65	0.36	0.15	0.02	0.94

*significant (p=<0.05).

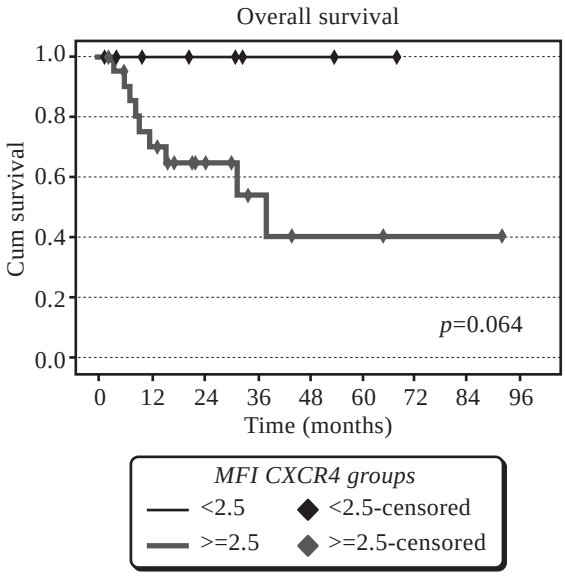

Fig. (1): Impact of CXCR4 MFI on overall survival in CLL/SLL patients

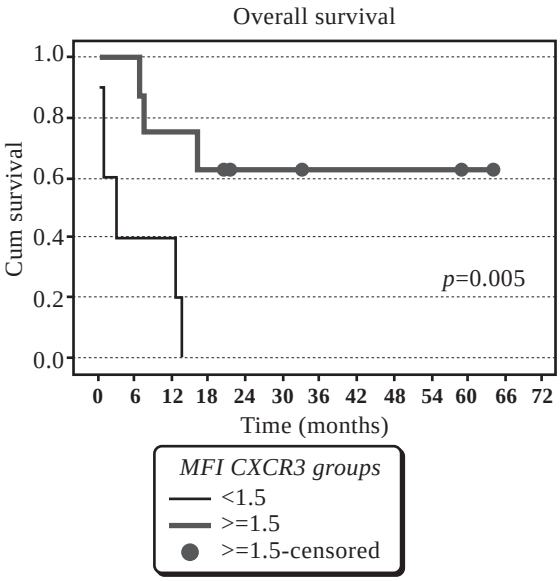

Fig. (3): Impact of CXCR3 MFI on overall survival in B-NHL patients.

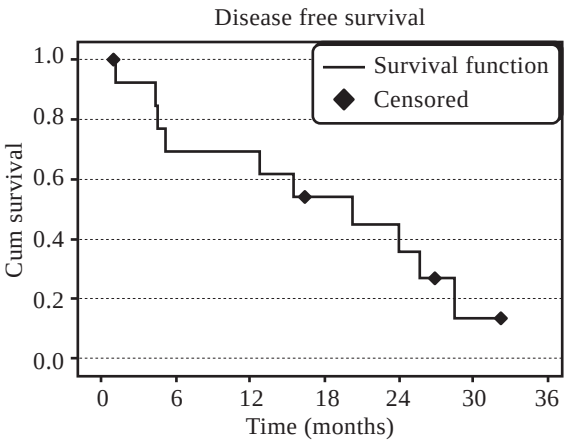

Fig. (2): Disease free survival of CLL/SLL patients.

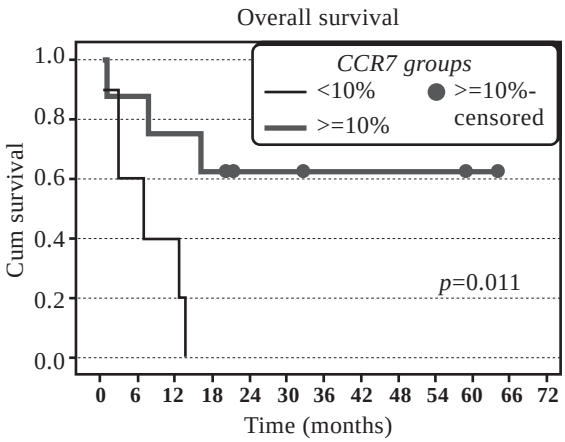

Fig. (4): Impact of CCR7% of expression on overall survival of B-NHL patients.

Table (5): Relation between chemokine receptors expression and survival in B-NHL and B-ALL/LBL patients.

Parameter		NHL			B-ALL/LBL		
		No	Overall survival %	*p*-value	No	Cumulative survival %	*p*-value
Overall group		13	38.5%		17	70.6%	
CXCR3 %:	Negative	6	16.7%	0.098	6	66.7%	0.854
	Positive	7	57%		11	72.7%	
CXCR3 MFI:	Low	5	0	0.005**	7	71.4%	0.899
	High	8	62.5%		10	70%	
CXCR4 %:	Negative	4	0%	*	3	100%	*
	Positive	9	55.6%		14	64.3%	
CXCR4 MFI:	Low	5	20%	0.169	6	66.7%	0.854
	High	8	50%		11	72.7%	
CCR7 %:	Negative	5	0%	0.011**	13	69.2%	*
	Positive	8	62.5%		4	75%	
CCR7 MFI:	Low	6	17%	0.114	10	70%	1.00
	High	7	57.1%		7	71.4%	

**: Statistically significant: (p<0.05). *: No p-value because of small no of cases within group.

DISCUSSION

Chemokines are a family of peptide hormones that were originally identified as chemoattractant cytokines and later as growth factors for hematopoietic cells and other cell types. Chemokines comprise of a large family of molecules that have been implicated in numerous different functions including the regulation of cellular adhesion, migration, proliferation and survival through their specific G protein coupled receptors **[7]**.

In the present study 90% of CLL/SLL patients were expressing CXCR3, 73% were expressing CXCR4 and 90% were expressing CCR7. These results are comparable to results obtained by Ghobrial et al., **[3]** who stated that CXCR3, CXCR4 and CCR7 are constitutively expressed by B-CLL/SLL, suggesting that these receptors might represent a hall mark of this disease.

In agreement with Wong and Fulcher **[5]**, there was no significant difference between CXCR3 expression percent or intensity when compared to the control (p>0.05). However, others reported a significant increase in CXCR3 expression in B-CLL/SLL patients when compared with the normal B-lymphocytes of control samples. This difference could be attributed to technical issues or due to the small sample size **[3]**.

We detected a statistical significant increase of both CXCR4% and CXCR4 MFI in CLL/SLL cases when compared with the control group. This result is in agreement with other publications **[8-10]**. Our results and other's suggest that the pathogenesis of B CLL may be linked to an over expression of CXCR4 and the importance of CXCR4-SDF-1 system for tissue localization and increased survival of B-CLL cells.

Also, an over expression of CCR7% and MFI in the CLL/SLL group was detected in our study when compared to the control group. This is in agreement with previous reports **[11-13]**.

As regards B-NHL group, 53.8% of cases expressed CXCR3, 69.2% expressed CXCR4% and 61.5% expressed CCR7%. Comparable results were previously reported **[14-16]**, which may indicate the involvement of chemockine receptors in lymphoma cell trafficking and homing.

In the current study, the expression of CXCR3% and MFI in NHL was comparable to control. Similar results were previously reported **[5,14]**, while significant higher expression of CXCR3 in NHL was reported by another study **[15]**.

Also CXCR4 expression % and MFI were not significantly different from control. This is in concordance with some **[8]** but not others **[15,17]**.

The same for CCR7% and MFI, we did not find any significant difference compared to the control group. This is similar to another report **[18]**.

In the current study, 5% of B-ALL/LBL patients expressed CXCR3, 82.4% expressed CXCR4 and 4% expressed CCR7 with no significant difference from control. Similar data was previously reported **[5,14,19]**. In contrast, other authors reported a significant increase in CXCR4 expression in B-ALL/LBL patients **[5,8]**.

Regarding the relation between CXCR3, CXCR4, and CCR7 expression and presence of hepatomegaly or splenomegay as a sign of extramedullary spread of the disease in our studied patients, no significant relation could be detected in CLL/SLL patients or B-ALL/LBL. This result is in accordance with Lopez-Giral et al., **[18]**. Their data and ours could indicate that CXCR3, CXCR4 and CCR7 chemokine receptors expression on lymphocytes from CLL and blasts of B-ALL/LBL have no role in extramedullary spread of the disease. On the contrary, Crazzolara and Bernhard **[19]** reported higher expression of CXCR4 in B-ALL/LBL patients with extramedullary infiltration.

On the other hand, B-NHL showed significant decrease of CXCR4 MFI in cases with splenomegaly and hepatomegaly and significant decrease of both CCR7% and CCR7 MFI in patients with splenomegaly. These results may indicate that down regulation of CXCR4 and CCR7 could play a role in tumor cell migration outside the LN and metastatic destination in B-NHL.

In the current study, there was no association between CLL/SLL Rai stage and CXCR3, CXCR4 or CCR7 expression. This is in agreement with previous reports **[8,9,20]** regarding CXCR4 expression. On the other hand, Ghobrial et al., **[3]** reported a significant higher expression of CCR7 in patients with advanced Rai stage (III, IV).

In the studied B-NHL cases, there was a significant increase of both CXCR4% and MFI in patients with PS (0, I) compared to patients with PS (II, III). This may suggest that down regulation of CXCR4 can play a role in advanced clinical stage and disease progression.

Within the CLL group, no significant relationship could be detected between CD38 expression and chemokine receptors. In agreement with our work, many authors could not find a relation between CXCR4 and CD38 expression **[8,9,20]** or between CCR7 and CD38 expression **[16,18]**. For CXCR3 expression, however, some authors reported similar results to ours **[3,20]** while one author reported association of higher expression of CXCR3 with low CD38 expression **[2]**. In the current study, CLL patients showed no association of CXCR3 expression with the hematological prognostic factors. This is in agreement with previous reports **[2,20]**. While in B-NHL a significant positive correlation was found between CXCR3 (% and MFI) and TLC (r=0.6), ALC (r=0.65) and peripheral blood lymphocytes percentage (r=0.54). Similar results were previously reported **[14,15]**. Also, a significant positive correlation between CXCR3% and TLC (r=0.5) was detected in B-ALL/LBL patients. These results might indicate a role of the expression of CXCR3 on neoplastic cells of B-ALL/LBL and B-NHL in their trafficking to the peripheral blood.

In our studied CLL patients, a significant positive correlation between CXCR4 and BM Lymphocytes (p=0.02, r=0.5) was detected. This result is in agreement with many publications **[3,6,9]**, which reflect the important role of CXCR4/SDF-1 system in the physical interaction between leukemic cells and the microenvironment regulating apoptosis. Also it was found that; it has both chemotactic effect and pro-survival effect on CLL cells, being a crucial mechanism through which stromal cells support CLL cells in vitro **[25]**. Also, a significant positive correlation was detected between CCR7% and peripheral blood lymphocytes (r=0.56) as previously reported **[3]**. This can be explained by the fact that CCR7 plays an important role in migration of lymphocytes from the BM to LN **[26]**.

In the current study, chemokine receptor expression had no impact on survival in CLL/SLL patients. In contrast, shorter survival was reported in patients with positive as compared to those with negative CXCR3 **[2]**.

Survival analysis of B-NHL cases revealed a significant higher overall survival in cases expressing CXCR3 with a higher MFI and in cases expressing CCR7. These results could provide an evidence for the role of these chemokine receptors and may be considered as a good prognostic marker in B-NHL.

In the current study, chemokine receptor expression had no impact on disease free survival in ALL/LBL patients. However, it was previously reported that over expression of CXCR4 in B-ALL/LBL patients is associated with shorter disease free survival **[19]**. Based on this finding, therapeutic use of CXCR4 inhibitors might improve patient's survival. Previous studies have demonstrated that CXCR4 mediates bone marrow microenvironment signalling. Using a xenograft model of ALL with MLL rearrangement, Sisson et al., **[22]** found that targeting leukaemia-stroma interactions with CXCR4 inhibitors may prove useful in this high-risk subtype of pediatric ALL. Along the same line Welschinger et al., **[23]** demonstrated that mobilizing agents, like CXCR4 antagonist Plerixafor (AMD3100), can increase the therapeutic effect of cell cycle dependent chemotherapeutic agents. Similar to the CXCR3/ CXCL10 circuit, another chemokine/receptor couple CXCL12/CXCR4 has been shown to mediate crosstalk between BM stroma and tumor cells in a number of hematologic malignancies **[24]**.

In conclusion, expression of the chemokine receptors; CXCR3, CXCR4 and CCR7 is heterogenous in B-lymphoproliferative disorders with a significant higher expression of CXCR4 and CCR7 in CLL when compared to the control group. SDF-1/CXCR4 pathway is an attractive target for the development of novel therapeutic approaches that can be specifically blocked by

monoclonal antibodies against CXCR4 (CXCR4 antagonists) leading to elimination of residual CLL and ALL cells hiding in stromal niches within the marrow and the lymphatic tissue. Positive CXCR3 and CCR7 expression were associated with higher OS of B-NHL patients. CXCR3 and CCR7 expression may be a good prognostic marker of B-NHL patients.

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