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Chronic B-Lymphocytic Leukaemia: Immunoregulatory Molecules, Antigen-Experienced B-Cells and Nurse-Like Cells

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ABSTRACT

Introduction: Chronic lymphocytic leukemia (CLL) is characterized by accumulation of a single clone of $CD5^+$ B cells in the peripheral blood and bone marrow. B-CLL cells are usually arrested in the G0/G1 phase of the cell cycle and therefore their accumulation in vivo appears to result from the inhibition of apoptosis rather than increased proliferation.

Patients and Methods: The study included 40 patients of newly diagnosed CLL as well as 10 age- and sex-matched normal volunteers were taken as controls. Both patients and controls were evaluated for the expression of CD40, CD80, CD86, CD27, CD25, CD14, CD25 and CXCR4. Correlation of these markers with clinical features known to influence prognosis in CLL and their impact on response to chemotherapy were evaluated.

Results: Our results revealed that CD40 was positive in 39/40 (97.5%) of the CLL group and was comparable to the control. CD80 was positive in 6/40 (15%) of the CLL patients with no difference in its expression between the CLL and the control group. CD86 was detected in none of our CLL patients. CD86 was significantly lower in CLL group than in the control group ($p<0.001$). By Roc curve analysis we found that CD86 could be used to predict favourable response to treatment in CLL patients at a cut off value of 3.5%, ($p=0.008$). Thirty eight of 40 patients (95%) were positive for the memory cell marker CD27, implying that all B-CLL cells resemble antigen-experienced and "memory" B lymphocyte. Twenty two of 40 patients (52.5%) were positive for the lymphocyte activation marker CD25 with a significantly higher expression among CLL compared to control (p.value <0.001). CD25 significantly correlated with favourable response to chemotherapy (p.value <0.001), and higher overall survival ($p=0.007$). Thirty six of 40 patients (90%) were positive for CXCR4, with a range of 14-98% and a mean of 53.8±22.5% and significantly four-fold greater than control group ($p<0.001$). CXCR4 showed a positive correlation with BM lymphocyte % ($p=0.003$ $r=0.459$) No correlation was found between over expression of CXCR4 and response to chemotherapy. CD14 (a marker of nurse-like cells which are a subset of monocytes) was negative in all CLL patients at a cut off value of 20% with no significant difference among CLL patients and the control group. Our results revealed that CD14 at a statistical cut off value of 6% could be used to predict poor response to treatment, with 94% sensitivity and 100% specificity (p.value <0.001) and prognosis of CLL patients with short over all survival for patients with high CD14 expression (p.value <0.001).

In conclusion: CD86 is essential for the function of any APC so B-CLL cells are similar to anergic B cells that have a reduced ability to process and present antigen to the T-helper cells. This defective may be one of the pathogenic mechanisms of CLL and a major cause of why the body fails to clear the B-CLL cells via immunological means. Over expression of CXCR4 on CLL cells may increase the avidity of malignant B cells to hematopoietic and possibly also to lymphoid tissue.

Key Words: *B-CLL - Antigen-experienced B-Cells - Nurse-like cells.*

INTRODUCTION

Chronic lymphocytic leukemia (CLL) usually presents as a slowly progressive lymphoproliferative disease characterized by accumulation of a clonal $CD5^+$ B cells in the peripheral blood and bone marrow with frequent involvement of lymph nodes and spleen **[1]**.

B-CLL cells are usually arrested in the G0/G1 phase of the cell cycle and therefore their accumulation in vivo appears to result from the inhibition of apoptosis rather than increased proliferation **[2]**.

Resistance to apoptosis is not an intrinsic property of B-CLL cells. In fact, when they are cultured in vitro, they usually die after a short term incubation, suggesting the existence, in vivo, of a survival-promoting microenvinment [3].

B-CLL cells over express CXCR4, the receptor for stromal derived factor-1 (SDF-1), which contributes to their tropism to bone marrow and lymphoid system where interaction with bone marrow stromal cells and nurse-like cells (NLCs) protect them from spontaneous apoptosis [4].

CD40 is a molecuole of the family of tumour necrosis factor receptor (TNFR), which is expressed throughtout B-cell development and is implicated in cell survival and differentiation. Its physiological ligand is, CD40L (CD154) a member of TNF family. CD40/CD40L interaction stimulates B-cells, dendritic cells and monocytes to proliferate, differentiate, up-regulate co-stimulatory molecules and increase antigen presentation [5].

Finally, the CD40 on CLL cells can downregulate CD40 on activated T-cells protecting the cells from their cytolytic effect, and this may contribute to the T-cell dysfunction seen in this disease [5].

CD80 and CD86 molecules deliver costimulatory signals to T-cells and serve as counter-receptors that transduce distinct signal to the antigen presenting cell (APC) upon engagement by CD28 or CTLA-4 [5].

B-CLL cells from all cases express the classical activation markers CD23, CD25, CD69 and CD71. Furthermore, the increased density of expression of CD5 and CD27 (which is typically a marker of memory B-cells) is in line with the activation hypothesis, since the level of both of these markers can be up-regulated upon cellular activation [5].

The expression of costimulatory and immunoregulatory molecules in CLL patients potentially influences the clinical progression and drug response in these patients [5].

The nurse-like cells (NLCs), which are a subset of peripheral blood CD14^{+} mononuclear cells that develop into large round, adherent cells when grown in the presence of CLL cells, produce survival factors, including SDF-1, for the leukemia cells [6]. These NLCs protect CLL cells from undergoing spontaneous or drug-induced apoptosis by enhancing the expression of antiapoptotic proteins of the bcl-2 family [7].

Patients with CLL may have greater numbers of circulating NLC progenitor cells. CLL cells may elaborate factors, such as transforming growth factor-beta (TGF-ß) that could support survival of NLC in vitro [8] and a potent differentiation factor for stromal cells [9].

The freshly isolated CD14^{+} splenocytes from patients with CLL expressed significantly higher levels of CD68^{+} than did the CD14^{+} splenocytes from patients without lymphoproliferative disease. Moreover, when the mononuclear splenocytes of CLL patients were cultured, after 1 to 2 days of culture, cells that had a morphology similar to that of the NLCs and that differentiated from CD14^{+} blood cells after 11 to 14 days in vitro were identified [7].

Therefore, the relative number and activity of such stromal elements and NLCs might be a limiting factor governing tumor progression particularly during early stages of disease when the interdependency of leukemia cells with accessory cells seems most apparent [7].

The knowledge that B-CLL is the outcome of many different molecular defects may allow the development of chemotherapies, immunotherapies and gene therapies targeting that specific defect [10].

Aim of the work:

The aim of this study is to asses the expression of CD40, CD80, CD86, CD27, CD25, CD14 and CXCR4 and their correlation with clinical features known to influence progression in CLL patients (age, Hb level, WBCs, PB lymlphocyte, absolute lymphocyte count, platelet count and BM lymphocytes) as well as their impact on response to chemotherapy.

PATIENTS AND METHODS

A- Patients:

The present study was carried out on forty patients with de novo chronic lymphocytic leukemia (CLL), who had not received any treatment attending the outpatient clinic of the medical oncology department National Cancer

Institute, Cairo University during the period between December 2002 and May 2005. The patients were 22 males (55%) and 18 females (45%) with a male: female ratio of 1.2: 1.0. Their ages ranged from 36 to 71 years with a mean ± SD of 57.6±8.35 and a median of 56.5.

In addition, 10 age- and sex-matched normal volunteers were taken as controls. They were 6 males and 4 females, with a male: female ratio 1.5: 1.0 and an age range of 40-70 years with a mean ± SD of 55.7±8.3 and a median of 56.0.

Patients were subjected to thorough history taking, full clinical examination (particularly for hepatomegaly, splenomegaly and lymphadenopathy), morphology and immunophenotyping.

Patients as well as the controls were evaluated for the expression of CD40, CD80, CD86, CD27, CD25, CD14 and CXCR4. These were CD80 FITC, CD40 PE, CD86 PE, CD27 PE, CD25 PE, and CXCR4 PE. All monoclonal antibodies used were purchased from Dako A/S Denmark. All marker studies were done on flow cytometry (Partec III) and results were expressed as the percentage of cells showing positive expression.

The erythrocyte lysing reagent was FACS lysing solution B D Bioscience Cat. No. 349202.

B- Methods:

Procedure of the techinque for immunophenotyping:

1- Fifty UL of the anticoagulated EDTA blood containing 10^4 cells were transferred to each of test tubes.

2- Five UL of each monoclonal antibody were added to its corresponding tube and mixed gently.

3- The first tube was a control one where no monoclonal antibody was added and with which the machine was adjusted to obtain the basic histogram showing the main cell populations and to adjust the auto fluorescence region.

4- Tubes were incubated in the dark for 30 minutes.

5- Two ml of erythrocyte lysing reagent were added to each tubes and mixed gently and left for 5 minutes.

6- Tubes were then centrifuged at 1500rpm for 5 minutes and supernatant aspirated.

7- Three ml of 0.01mol/L PBS were added to each tube and vortexed gently and step 6 was repeated.

8- The cell button was resupended in 0.5ml 0.01mol/L PBS and analyzed on the flowcytometer.

Flowcytometric analysis and immunophenotyping:

After warming up the argon laser (488nm) for 30 minutes, the full alignment procedures were performed using the standared immunocheck aligment flurospheres for adjusting forward scatter, side scatter and photomultiplier tubes for an orange and red adjustements.

The proper protocol for each monoclonal was loaded and used for interpretation of each.

The control sample was the first tube to be introduced to the machine, to show the cell populations in the basic histogram and to adjust autoflurescence region. Then, the other tubes were introduced sequentially.

Five thousand events (cells) were passed in front of the laser for each case for each mononclonal antibody. Lymphocytes were then selectively gated for the expression of our studied markers. Cells having high both forward angle light scatter (FSC) and side angle light scatter (SCC) corresponding to nurse-like cells (NLCs) were selected and analyzed for the expression of CD14 [11].

Interpretation of results:

The number of cells expressing the receptor will emit fluoresce signals which will be summated and multiplied in the photomultiplier tubes. These data will be shown as single and double coloured frequency histograms.

For each sample, the expression of each of the studied markers was defined as positive when it is present on more than or equal to 20% of cells [3].

Statistical methods:

SPSS (Statistical Package for Social Sciences) version 12.0 was used for data analysis. Mean and standard deviation are descriptive

values for quantitative data. Student *t* test was used for comparing means of two independent groups and Kruskal Wallis ANOVA (analysis of variance) for comparing means of more than 2 independents groups. Chi-square and Fisher-exact tests compared independent proportions.

ROC analysis helped to choose the best cut-off points for quantitative parameters to be used either as diagnostic or prognostic variables. Kaplan-Meier method estimated probability of overall and disease free survival. Long rank test was used for comparing survival curves. *p* value is significant at 0.05 level.

RESULTS

The present study included fourty patients with newly diagnosed CLL, who presented to the National Cancer Institute, Cairo University during the period between Dec. 2002 and May 2005. Ten age-and sex-matched normal volunteers were taken as a control gruoup.

All patients were of the B-cell type and all showed weak positivity for surface immunoglobulin (SIg) of either kappa or lambda.

Expression of the studied markers:

Thirty nine patients (97.5%) were positive for CD40 with a range of 18-87% showing no statistically significant difference as compared to the control. Six patients (15%) were positive for CD80 with a range of 0-74% showing no statistically significant difference as compared to the control. All patients were positive for CD86 with a range of 0-10 showing a statistically significant lower expression in the CLL group as compared to the control ($p<0.001$). Thirty eight patients (95%) were positive for CD27 with a range of 7-82 showing no statistically significant difference as compared to the control group. Twenty one patients (52.5%) were positive for CD25 with a range of 0-86 showing a statistically significant higher expression as compared to the control group ($p<0.001$). CXCR4 was positive in 36 patients (90.0%) with a range of 14-98 showing a statistically significant higher expression as compared to the control group ($p<0.001$). Finally, CD14 was expressed in none of the CLL patients with a range of 0-18 showing no statistically significant difference as compared to the control group (Table 1).

Table (1): The studied markers in 40 CLL patients before treatment and controls.

Parameter	CLL Frequency	CLL Range	CLL Percentage	CLL Mean ± SD	Control Mean ± SD	*p*. value
CD40	39	18-87	97.5	55.9±17.4	61.2±24.0	0.44
CD80	6	0-74	15	9.9±16.7	10.9±6.1	0.08
CD86	0	0-10	0	3.9±2.4	41.6±20.8	<0.001**
CD27	38	7-82	95	54.6±18.7	49.7±20.7	0.47
CD25	21	0-68	52.5	19.5±13.4	4.4±1.4	<0.001**
CXCR4	36	14-98	90	53.8±22.5	12.6±6.6	<0.001**
CD14	0	0-18	0	6.0±4.9	7.6±2.5	0.14

** Highly statistically significant.

Relation between the studied markers and response to chemotherapy:

There was no statistically significant difference in expression of CD40, CD80, CD27 and CXCR4 and response to chemotherapy. However, there was a statistically significant higher expression of both CD86 and CD25 for patients who entered complete remission (CR) as compared to those with either partial remission (PR), or stable disease (SD)/progressive disease (PD) ($p= 0.02$, <0.001 respectively). Also, there was a statistically significant lower expression of CD14 for patients who entered CR as compared to those with either PR or SD/PD ($p= <0.001$) (Table 2).

Table (2): Relation between the studied markers and response to chemotherapy.

		CR (22)	PR (11)	SD/PD (7)	*p.* value
CD40	<20	1	–	–	
	≥20	21	11	7	0.66
CD80	<20	19	8	7	
	≥20	3	3	–	0.28
CD86	<3.5	6	8	5	0.02*
	≥3.5	16	3	2	
CD27	<20	–	2	–	
	≥20	22	9	7	0.10
CD25	<20	2	10	7	
	≥20	20	1	–	<0.001**
CXCR4	<20	4	–	–	
	≥20	18	11	7	0.19
CD14	<6	22	2	–	
	≥6	–	9	7	<0.001**

* Statistically significant. ** Highly statistically significant.

Studied markers as predictive factors:

By Roc curve analysis we found that CD86 could be used as a prognostic factor to predict response of the patients; as at a value of 3.5% the sensitivity was 72.7% and the specificity was 72.2% with the area under the curve is equal to 0.745 and *p*.value 0.008 (Fig. 1).

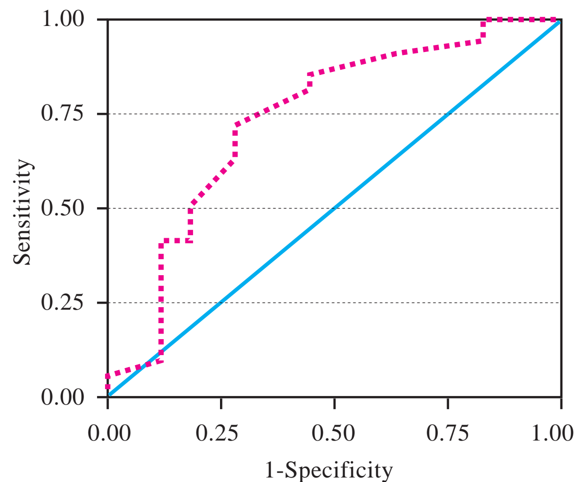

Fig. (1): Roc curve analysis for CD86 in CLL patients before treatment.
Area: 0.745 & *p*. value 0.008

CD25 could be used as a prognostic factors to predict response of the patients; as at a value of 20%, the sensitivity was 90% and the specificity was 95% with the area under the curve is equal to 0.956 and *p*.value <0.001 (Fig. 2).

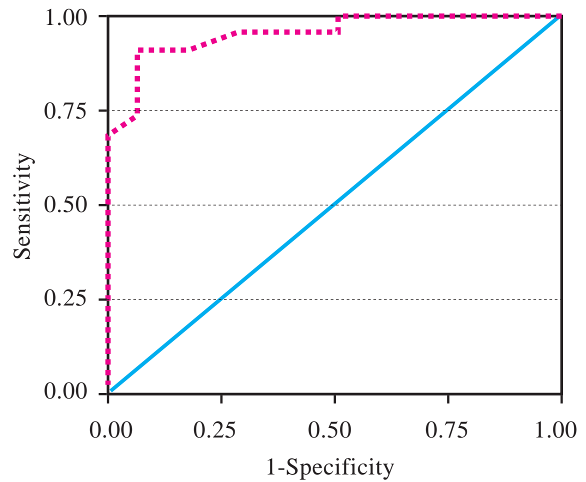

Fig. (2): Roc curve analysis for CD25 in CLL patients before treatment.
Area: 0.956 & *p*. value <0.001.

CD14 could be used as a prognostic factors to predict response of the patients; as at a value of 6%, the sensitivity was 94% and the specificity was 100% with area under the curve is equal to 0.997 and *p*.value <0.001 (Fig. 3).

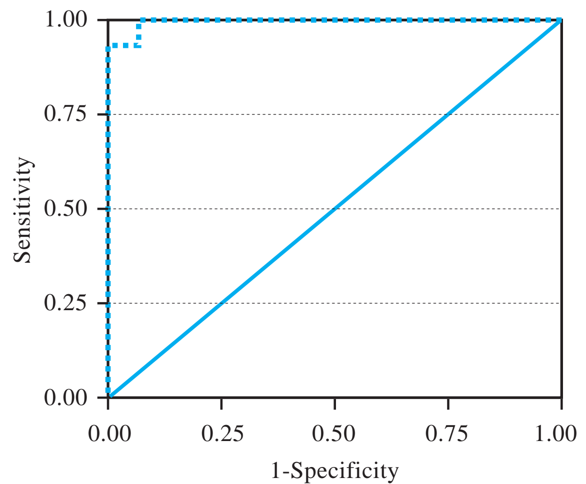

Fig. (3): Roc curve analysis for CD14 in CLL patients before treatment.
Area: 0.997& *p*. value <0.001

Correlation study:

Correlation study revealed no statistically significant correlation for CD40, CD80, CD86, or CD27 with either age, Hb level, TLC, PB lymphocyte, ALC, platelet count, or BM lymphocytes. However, CD25 showed a statistically significant correlation with TLC and ALC (r=–0.417, p=0.007 and r=–0.381, p=0.015

respectively), CXCR4 with BM lymphocytes (r=0.459, p=0.003) and CD14 with Hb level (r=–0.321, p=0.043), TLC (r=0.589, p=0.000), PB lymphocyte (r=0.339, p=0.032), ALC (r=0.589, p=0.000), platelet count (r=–0.327, p=0.040) and BM lymphocytes (r=0.340, p=0.040) (Table 3).

Survival analysis:

Survival analysis revealed a significantly higher overall survival for patients with low CD14 expression (p<0.001) (Fig. 4) as well as a significantly higher overall survival in CLL patients with higher CD25 expression (p 0.007) (Fig. 5).

Table (3): Correlation between the expression of studied markers and prognostic variables of CLL patients.

	CD40	CD80	CD86	CD27	CD25	CXCR4	CD14
Age:							
r	-0.003	–0.004	0.114	–0.183	0.074	–0.097	0.213
p	0.987	0.982	0.484	0.258	0.651	0.553	0.187
Hb level:							
r	–0.188	0.041	0.249	0.159	0.094	0.173	–0.321*
p	0.245	0.802	0.122	0.327	0.564	0.285	0.043
TLC:							
r	0.101	0.023	–0.262	0.075	–0.417**	0.171	0.589**
p	0.533	0.890	0.102	0.644	0.007	0.292	0.000
PB Lymph:							
r	0.159	0.011	0.067	-0.106	0.089	0.068	0.339*
p	0.326	0.944	0.680	0.515	0.586	0.678	0.032
ALC:							
r	0.116	0.006	–0.252	0.061	–0.381*	0.171	0.589**
p	0.477	0.973	0.117	0.709	0.015	0.292	0.000
Plt:							
r	-0.048	–0.142	0.191	0.186	0.231	–0.159	–0.327*
p	0.769	0.382	0.237	0.250	0.151	0.326	0.040
BM Lymph:							
r	0.205	–0.103	-0.234	0.014	–0.296	0.459**	0.340*
p	0.204	0.527	0.146	0.931	0.064	0.003	0.040

* Correlation is significant at 0.05 level (2-tailed).
** Correlation is highly significant at the 0.01 level (2-tailed).

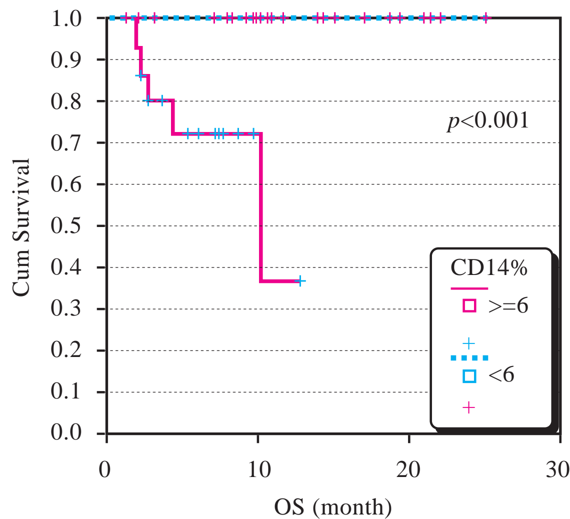

Fig. (4): Overall survival of CLL patients regarding the expression of CD14.

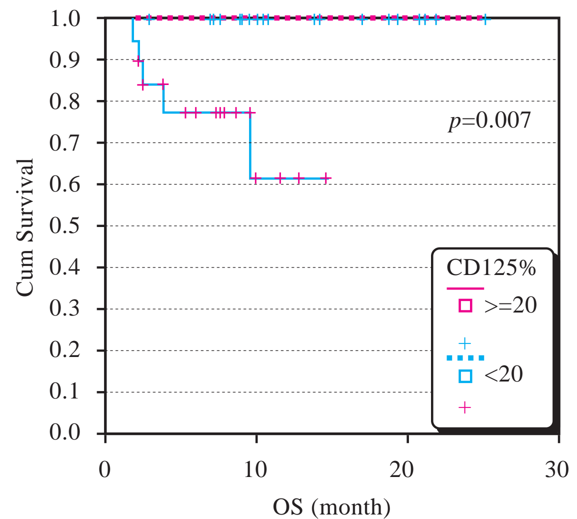

Fig. (5): Overall survival of CLL patients regarding the expression of CD25.

DISCUSSION

Chronic lymphocytic leukemia (CLL) is a unique lymphoproliferative disorder characterized by accumulation of a single clone of $CD5^+$ B cells which did not retain the capacity to differentiate into functionally mature cells, and accumulate in the peripheral blood and bone marrow with frequent involvement of lymph nodes and spleen [1].

There is currently much evidence demonstrating that both pharmacological and cellular mechanisms contribute to whether a patient with CLL is cured or not and leukemic cells are rarely eradicated with chemotherapy. Even when an initial response is observed secondary progression is the rule [12].

The study included 40 patients of newly diagnosed CLL and 10 age- and sex-matched controls. Both patients and control were studied for the expression of CD40, CD80, CD86, CD27, CD25 and CXCR4. Correlation of these markers with clinical features known to influence prognosis in CLL as well as their impact on response to therapy were also evaluated.

In our work, thirty nine of 40 patients (97.5%) were CD40 postive. This was in agreement with both Hulkkonen et al. [5] and Younes et al. [13].

Similarily, Orsini et al. [14] found higher levels of CD40 on the surface of CLL cells leading to down-regulation of CD40L on activated T cells, thus protecting the leukemic cells from their cytotoxic effect and this may contribute to T-cell dysfunction seen in this disease.

Also, Romano et al. [15] reported that CLL cells express CD40 and release CD40L leading to its elevated level in the plasma of these patients. The released CD40L may induce growth, survival and proliferation of neoplastic B-CLL cells and stimulate non-malignant B cells to produce autoantibodies. This may be the mechanism for the autoimmune phenomenon in CLL.

Six of our forty CLL patients (15%) were positive for CD80. This was in agreement with both Hulkkonen et al. [5] and Tsukado et al. [16] who showed a heterogeneous pattern of expression of CD80 in B-cell cells with most cases being negative.

In our work, no difference was found between B-CLL and normal control group regarding the expression of CD80. This was in agreement with Dai et al. [17] who reported no significant difference between B-CLL group and normal control group either in the B7.1 (CD80) expression or in co-expression of B7.1 and B7.2.

In our study, none of our patients expressed CD86. This was in agreement with Freedman et al. [18], Dozzi et al. [19] and Von Berwelt-Bailden et al. [20] as all found that CLL cells, in common with other B-cell malignancies, fail to express, or express only weakly, adhesion molecules especially CD86. CD86 is essential for the function of any APC and so B-CLL cells are similar to anergic B cells that have a reduced ability to process and present antigen to the T-helper cells. Treatment of both normal and malignant B cells by activation via CD40 results in up regulation of B7 (CD80, CD86) expression and generation of professional autologous APC.

In our work, we found a significantly lower expression of CD86 in the B-CLL group as compared to the normal control group (p value <0.001).This was in agreement with Van den Hove et al. [21]. This defective expression of CD86 may be one of the pathogenic mechanisms of CLL and a major cause of why the body fails to clear the B-CLL cells via immunological mechanisms. T cell unresponsiveness towards autologous B-CLL cells may be, at least in part, attributed to inadequate costimulatory capacity of this tumor.

However, our results regarding expression of CD80 & CD86 on CLL cells were not in agreement with Trentin et al. [22] who found that CD40 was expressed on all B cells (normal and malignant), CD27 & CD70 were expressed on tumor B cells and not on normal B cells, CD80 was distributed on all neoplastic cells at an intermediate density and CD86 was present at a low density. This probably indicates that B CLL cells are equipped with different co stimulatory molecules.

In our study we reported by Roc curve analysis that CD86 could be used to predict response to treatment in CLL patients at a cut off value of 3.5%, with 72.8% sensitivity and 72.2% specificity (p value 0.008), where most patients with high CD86 had a good response to treatment and a favorable prognosis.

In this work, 38 out of 40 patients (95%) were positive for CD27. This was in agreement with Hulkkonen et al. [5] and Klein et al. [23]. This finding of CD27, a memory cell marker, implies that all B-CLL cells resemble antigen-experienced and "memory" B lymphocyte and differ from both $CD5^+$ naive B-cell or follicle center cells. So, the origin of CLL cells appears to be the memory B cell, regardless of whether or not there was mutation of IgV gene.

On the other hand, only one report of Sembries et al. [24], showed a reduced expression of CD27 in CLL along with different costimulatory molecules.

In our work, we found 22 of 40 patients (52.5%) to be positive for CD25. This was in agreement with Hulkkonen et al. [5], and Gattei et al. [25]. The latter divided 123 well characterized B-CLL patients into at least three subgroups, one of them included 62 cases (50.4%) over expressing CD25, CD55, CD62L, CD54 and CD49c and 80% of this subgroup were of mutated IgVH cases.

In our results, we found a significantly higher expression of the lymphocyte activation marker CD25 among CLL compared to control (*p*.value <0.001). Similar results were reported by both Damle et al. [26] and Sellitto et al. [27].

In our study, the expression of CD25 significantly correlated with response to chemotherapy (*p*.value <0.001), with most CD25 positive patients showing good response and achieving complete remission. Our results were in agreement with Damle et al. [26]. Also, CD25 could be used to predict overall survival, with a significantly higher over all survival in patients with higher CD25 expression (*p*.value 0.007). The same results were reported by Gattei et al. [25]. Therefore, CD25 overexpression may be used as an additional prognostic factor to identify good prognosis B-CLL patients.

In this work 36/40 patients (90%) were positive for CXCR4, with a range of 14-98% and a mean of 53.8±22.5% and significantly four-fold greater than control group (*p*.value <0.001). This was in agreement with Möhle et al. [28]. Also, Barretina et al. [29] reported a five-fold over expression of CXCR4 on the cells of their CLL patients as well as lowered plasma level of SDF-1 as compared to control group. These results suggest that the CXCR4/SDF-1 system appears to be important for tissue localization and increased survival of B-CLL cells.

In our work, we found a positive correlation between the expression of CXCR4 on B-CLL cells and BM lymphocyte % (*p*.value 0.003) and this was in accordance with Burger et al. [30] suggesting that over expression of CXCR4 on CLL cells may increase the avidity of malignant B cells to hematopoietic and possibly also to lymphoid tissue, eventually leading to bone marrow infiltration and suppression of hematopoiesis as well as lymph node and spleen enlargement.

Also, Burger et al. [31] found that the growth and survival of B-CLL cells are favored by interaction between CLL cells and non tumoral accessory cells. Marrow stromal cells and NLCs constitutively secret $CXCL_{12}$(SDF-1), the ligand for CXCR4, thereby attracting and resuscitating B-CLL cells from apoptosis in a contact dependent fashion.

In our work, we found no correlation between over expression of CXCR4 and response to chemotherapy. Our results go hand in hand with those of Barretina et al. [29].

In this work, we found all patients to be negative for the expression of CD14 at a cut off value of 20% and no significant difference was found among CLL patients and the control group. Our results were in agreement with those of Polliack et al. [32] and Tassies et al. [33] who stated that CD14 was negative in all CLL patients.

On the other hand, two earlier studies, Molica et al. [34] and Pinto et al. [35] reported a high CD14 antigen frequency in their B-CLL patients than their control group with a direct correlation between CD14 expression and advanced clinical stages.

In our study, we found a significant correlation between the expression of CD14 at a statistical cut off value of 6%, corresponding to NLCs, and poor response to treatment and advanced disease as shown by high TLC, increased ALC, low hemoglobin level, decreased platelet count and high lymphocyte percentage in both bone marrow and peripheral blood (p=0.000, p=0.000, p<0.001, p=0.043, p=0.040, p=0.04, and p=0.032 respectively).

Our results go hand in hand with that of Pinto et al. [35] who reported a direct correlation between CD14 expression and advanced disease, diffuse bone marrow infiltration and bad prognosis.

From our study, we conclude that CD14 at a cut of value of 6% could be used to predict poor response to treatment, with 94% sensitivity and 100% specificity (*p*.value <0.001) and prognosis of CLL patients with short over all survival for patients with high CD14 expression (*p*.value <0.001).

A similar conclusion was drawn by Callea et al. [36], as they detected a cut off of 5% for positivity of CD14 and this was considered as an independent significant factor for the prediction of over all survival. They also suggested the inclusion of CD14 in the B-CLL immunological panel because of its ability to discriminate between groups with different prognoses which may need more appropriate treatments.

Conclusion:

- CD40 was detected in 97.5% of our patients. Its expression on the surface of B-CLL cells may lead to down regulation of CD40L on activated T-cells, thus protecting the leukaemic cells from the cytotoxic effect of T-cells and this may contribute to T-cell dysfunction seen in this disease.
- CD80 was detected in 15% of CLL patients with no difference in its expression between the CLL and the control group.
- B-CLL cells failed to express the adhesion molecule CD86 which is essential for the function of any APC and so B-CLL cells are similar to anergic B lymphocytes that have a reduced capacity to process and present antigens to T- helper lymphocytes. This may be a major cause of why the body fails to clear the CLL cells via immunological mean.
- CD86 could be used to predict the response to treatment in CLL patients at a statistical cut-off value of 3.5%.
- The expression of CD27, a marker of memory B-cells, on the surface of B-CLL cells reflects that B-CLL cells resemble antigen- experienced and memory B lymphocytes and differ from both $CD5^+$ naïve B-cells or follicle center cells. So, the origin of CLL cells appears to be the memory B cell.
- The B-CLL cells express the functional chemokine receptor CXCR4, allowoing B-CLL cells to actively migrate to BM and to lymphoid tissue where interaction of B-CLL cells with stromal cells and Nurse-like cells occurs.
- CD14 could be used at a statistical cut off value of 6% to predict response to treatment, prognosis and overall survival in CLL patients.
- CD25 could be used at a statistical cut-off value of 20% as a prognostic marker to predict good response to chemotherapy and overall survival in B-CLL patients.

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