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Immunophenotypic Characterization of the Leukemic B-cells from Iranian Patients with Chronic Lymphocytic Leukemia: Association between CD38 Expression and Disease Progression

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ABSTRACT

Background: Patients with B-cell chronic lymphocytic leukemia (B-CLL) have heterogeneous clinical courses, thus several biological parameters need to be added to the current clinical staging systems to predict disease outcome. Recent immunophenotypic studies performed mainly in Western populations have demonstrated the prognostic value of CD38 and ZAP-70 expression in B-CLL. **Objectives:** To investigate the expression pattern of a variety of membrane antigens on leukemic cells from Iranian patients with CLL and to find out if there are any differences in the expression of these markers between indolent and progressive groups. **Methods:** In the present study, peripheral blood samples from 87 Iranian patients with B-CLL were analysed by flow cytometry. **Results:** In all cases, the neoplastic cells displayed B-CLL phenotype ($CD5^+/CD19^+/sIg^+$). The vast majority of the cases expressed CD23, but failed to stain for CD3 or CD14. The leukemic cells of most patients expressed CD27 (84/87, 95.4%) and CD45RO (74/87, 83.9%) molecules, suggesting a memory B-cell phenotype. Comparison between the indolent (n=42) and progressive (n=37) patients revealed significantly higher frequency and intensity of CD38 expression in progressive group (40.5%) compared to indolent (11.9%) patients ($p<0.05$). None of the other membrane antigens were differentially expressed in these two groups of patients. **Conclusion:** Our results obtained in an Asian ethnic population confirm and extend previous findings obtained from Western populations regarding the association of CD38 expression and disease progression in B-CLL.

Keywords: Immunophenotyping, B-CLL, Indolent, Progressive, CD38

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INTRODUCTION

B-cell chronic lymphocytic leukemia (B-CLL) is the most common type of leukemia in the Western societies (1). The malignant B-cells are derived from clonal expansion and gradual accumulation of $CD5^+/CD19^+/sIg^{low}$ lymphocytes in the bone marrow, blood and lymphoid tissues. These cells appear to be phenotypically mature, but are functionally compromised (2).

Differential diagnosis of CLL from other mature B cell lymphoid leukemias is largely based on clinical and laboratory findings. A variety of laboratory methods such as blood cell counting and morphology, immunophenotyping, histopathological and chromosomal analyses are being used to identify the leukemic cells, the type of malignancy and to some extent disease severity and prognosis (3).

There is a compelling need for novel prognostic parameters in addition to the current prognostic features including the Rai or Binet staging system, lymphocyte doubling time (LDT), serum levels of B2-microglobulin, soluble CD23 (4), bone marrow infiltration patterns and cytogenetic abnormalities (5). In recent years a number of biological prognostic factors have been developed (6). Among them, analysis of the mutational status of the expressed immunoglobulin heavy chain variable genes (IgVH) has allowed to identify two clinical subgroups within CLL with different progression patterns. Patients with unmutated leukemic cells have more aggressive clinical course, whereas, patients with mutated malignant cells have frequently indolent disease (7). Unfortunately, VH mutation analysis is complex, expensive, time consuming and not routinely undertaken in most laboratories. Accordingly, many attempts have been made to identify a marker (or markers) that could be as useful as IgVH mutational state in the prognostic assessment of patients with CLL. Membrane expression of CD38 molecule was the first marker that was found to correlate with IgVH mutation (8). Intracytoplasmic detection of ZAP-70 enzyme was subsequently revealed to be correlated with IgVH mutation and B-CLL prognosis (9). Patients with progressive disease are more likely to express both molecules, whereas the leukemic cells from indolent patients tend to be negative for both markers (8). Association between disease progression and immunophenotypic profile of leukemic cells has not been widely studied in B-CLL patients from other ethnic populations. The prevalence of B-CLL has previously been shown to be lower in Asian populations (10). Interestingly, the frequency of VH gene family expression has also been demonstrated to be different in B-CLL patients from a variety of Western populations, compared to Iranian and Japanese patients (11). These findings suggest that there might be some molecular differences between different groups of B-CLL patients, depending on their ethnic background. The present study is the first immunophenotypic investigation conducted in Iranian progressive and indolent B-CLL patients.

MATERIALS AND METHODS

Patients. Twenty milliliters of heparinized peripheral blood was collected from 87 CLL patients attending the Oncology Clinics of Imam Khomeini and Firozgar Hospitals, affiliated to Tehran University of Medical Sciences and Iran University of Medical Sciences, respectively. A consent letter was taken from all patients and the study was ap-

proved by the Ethical Committee of Tehran University of Medical Sciences. The diagnosis was based on immunophenotypic analysis, cell blood count, cell morphology and clinical symptoms. The age range of patients was 39–84 years with a median of 63 years. Disease staging was accomplished by Rai staging system. Fifty four patients were male and 33 were female. The patients were classified into indolent (n=42), progressive (n=37) and newly-diagnosed (n=8) groups. Disease progression was identified on the basis of either of the following criteria: lymphocyte count doubling time of less than 1 year; progression to a more advanced Rai stage; development of systemic symptoms; development of Richter's syndrome; downward trend of hemoglobin (Hb) concentration or platelet count to below the normal range (Hb < 13.5 g/dl for males and < 11.5 g/dl for females; platelet count $<150\times10^9$/l) even when not meeting the criteria for stage III or IV disease. Possession of one of these characteristics was sufficient to qualify as progressive disease (12). Twenty one patients, mostly at stage 3 or 4, were under chemotherapy at sampling time. The major clinical characteristics of our patients are summarized in Table 1.

Cell Preparation. Peripheral blood mononuclear cells (PBMCs) were separated using Histopaque (Sigma, St Louis, MO, USA) density-gradient centrifugation. Isolated cells were washed twice with RPMI-1640 medium (Invitrogen, Paisley, Scotland) and counted.

Immunophenotyping. Cell surface antigens were analysed by flow cytometry as described (11). Isolated PBMCs were stained with appropriate dilutions of monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) specific for human: myeloid lineage [CD14 (clone TUK4)], B cell lineage [CD19 (clone HD37), CD20 (clone B-Ly1) and CD23 (clone MHM6)], T cell lineage [CD3 (clone UCHT1) and CD5 (clone DK23)], CD38 (clone AT 13/5), CD27 (clone M-T271), CD45 (clone T29/33), CD45RA (clone 4KB5), CD45RO (clone UCHL1), HLA-DR (clone AB3), and polyclonal FITC- conjugated rabbit F(ab′)2 anti-kappa, lambda light chains and Mu heavy chain (IgM). Fluorochrome conjugated isotype control antibodies of irrelevant specificity served as negative controls (Cytomation; Dako Corp, Glostrup, Denmark). Detection of CD5 and CD19 was performed by double staining. For surface staining, cells were washed with RPMI-1640 and then incubated with 10 µl of conjugated antibodies in phosphate-buffered saline (PBS) containing 0.1% NaN3 for 30 min at 4ºC. Cells were then washed twice with washing solution (PBS, 0.2% bovine serum albumin, 0.1% NaN3 and 0.5 mM ethylenediaminetetraacetic acid) and fixed with 1% paraformaldehyde in cold PBS. Cells were finally examined by Partec PAS II flow cytometer (Partec, Munster, Germany). The criterion for surface marker positivity was expression by at least 20% (30% for CD38) of the leukemic cells after subtraction of background staining with isotype-matched control antibodies.

Statistical Analysis. The analysis was performed using the SPSS statistical package. Comparison of the expression of various antigens in indolent and progressive groups and their association to clinical characteristics of patients was performed using the Mann-Whitney U test, t test and Pearson Chi-Square, as appropriate. Logistic regression was used to predict progression status on the basis of surface antigen expression. Forward stepwise (likelihood ratio) tests were carried out, with p=0.05 entry and 0.10 removal. Statistical significance was assigned when P values were less than 0.05.

Table 1. Clinical and laboratory characteristics of the Iranian B-CLL patients

No	Pa-tients	Age	Sex	WBC $\times 10^3$	Lym (%)	Rai stage	Treat-ment	Disease Pro-gression	Disease Dura-tion (yr)	No	Pa-tients	Age	Sex	WBC $\times 10^3$	Lym (%)	Rai stage	Treat-ment	Disease Pro-gression	Disease Dura-tion (yr)
1	CLL1	60	M	35.6	87	0	-	Indolent	2	46	CLL46	40	M	116	94	II	-	Progressive	1
2	CLL2	57	M	45.3	92	II	-	Indolent	4	47	CLL47	53	F	607	80	I	-	Indolent	1
3	CLL3	72	M	19.6	77	0	-	Indolent	2	48	CLL48	67	F	57.3	75	0	-	Indolent	1
4	CLL4	52	M	111	94	I	-	Progressive	3	49	CLL49	70	F	17.3	85	0	-	ND	-
5	CLL5	55	M	21.9	75	0	-	Indolent	2	50	CLL50	69	F	40	91	0	-	Indolent	1
6	CLL6	73	M	23.6	74	0	-	Indolent	2	51	CLL51	66	F	46	83	0	-	Indolent	2
7	CLL7	79	M	14.6	75	0	-	Indolent	4	52	CLL52	65	M	20	90	0	-	Progressive	2
8	CLL8	58	F	116.2	96	0	-	Progressive	5	53	CLL53	67	M	20	85	0	-	Indolent	1
9	CLL9	59	M	8.7	79	IV	Chlo+Flu	Progressive	2	54	CLL54	50	M	17.7	74	0	-	Progressive	1
10	CLL10	47	M	38.7	85	0	-	Indolent	13	55	CLL55	82	M	170	89	II	-	Progressive	1
11	CLL11	61	M	28.5	62	0	-	Indolent	5	56	CLL56	76	F	36.7	84	II	-	ND	-
12	CLL12	71	F	29.7	85	II	-	Indolent	7	57	CLL57	67	F	103	92	0	-	Progressive	6
13	CLL13	67	F	57.1	87	0	-	Indolent	2	58	CLL58	56	M	39.2	89	0	-	Indolent	9
14	CLL14	58	M	39.4	80	0	-	Indolent	2	59	CLL59	70	F	28	80	0	-	Indolent	4
15	CLL15	78	M	16.3	81	III	Pred+Chlo	Indolent	12	60	CLL60	71	M	38.3	86	0	-	Indolent	1
16	CLL16	65	M	72.4	89	IV	Pred	Indolent	10	61	CLL61	50	F	32	76	0	-	Indolent	1
17	CLL17	64	M	17	64	I	-	Indolent	5	62	CLL62	50	M	53.7	87	0	-	Progressive	1
18	CLL18	61	M	34.1	83	III	-	Indolent	5	63	CLL63	45	M	890	95	II	-	Progressive	1
19	CLL19	63	M	32	83	0	-	Indolent	2	64	CLL64	65	M	355	87	II	-	Progressive	1
20	CLL20	62	M	36.2	78	0	-	Indolent	7	65	CLL66	67	F	75.5	82	0	-	Indolent	1
21	CLL21	65	F	13.6	80	0	-	Indolent	1	66	CLL68	55	M	19.8	80	0	-	Indolent	1
22	CLL22	70	M	48.7	90	0	-	Indolent	7	67	CLL69	61	M	98.4	96	IV	Pred+Chlo	Progressive	2
23	CLL23	58	M	65	82	0	-	ND	-	68	CLL70	60	F	21.2	75	II	Pred	Progressive	7
24	CLL24	60	F	22.3	86	0	-	Indolent	1	69	CLL71	58	F	3.2	70	0	Pred+Chlo	Indolent	4
25	CLL25	73	M	103	95	II	-	Progressive	2	70	CLL72	79	M	76.4	93	IV	Pred+Chlo	Progressive	2
26	CLL26	64	M	50	94	I	-	Progressive	4	71	CLL73	75	F	18.4	84	NI	Pred+Chlo	Indolent	3
27	CLL27	73	F	21.2	74	0	-	Indolent	2	72	CLL74	55	M	46.9	85	IV	Pred+Chlo	Progressive	2
28	CLL28	53	M	26.1	89	0	-	Progressive	2	73	CLL75	59	F	87.7	95	II	-	Indolent	14
29	CLL29	76	M	30.6	75	0	-	Progressive	3	74	CLL76	47	M	2.9	43	IV	Flud	Progressive	11
30	CLL30	62	M	13.2	86	0	-	Progressive	2	75	CLL77	50	M	168.4	91	IV	Chlo	Progressive	4
31	CLL31	51	M	27.1	75	0	-	ND	-	76	CLL78	45	F	27.7	81	II	-	Progressive	2
32	CLL32	73	M	30.4	82	0	-	Indolent	4	77	CLL79	83	M	23.7	87	IV	Chlo	Progressive	2
33	CLL33	71	F	41.4	87	0	-	Progressive	2	78	CLL80	65	M	139.8	96	III	-	Progressive	1
34	CLL34	53	M	255	82	II	-	Indolent	5	79	CLL81	67	M	172	80	IV	Flud	Progressive	8
35	CLL35	64	F	22.5	79	0	-	Indolent	5	80	CLL82	50	M	74.1	78	III	Chlo	Progressive	1
36	CLL36	60	F	31.1	69	0	-	ND	-	81	CLL83	64	F	64	94	III	Pred+Chlo	ND	-
37	CLL37	52	M	24.7	80	0	-	Progressive	7	82	CLL84	70	F	50	90	0	Pred+Chlo	Indolent	2
38	CLL38	51	F	37.7	85	II	-	Progressive	11	83	CLL85	55	M	33.1	86	II	Chlo	Progressive	5
39	CLL39	39	F	23.2	74	0	-	Indolent	4	84	CLL86	80	F	150	97	II	Pred+Chlo	Progressive	1
40	CLL40	58	F	39	89	0	-	ND	-	85	CLL87	81	M	181	87	IV	Pred+Chlo	Progressive	2
41	CLL41	69	F	18.5	70	0	-	Indolent	1	86	CLL87	82	M	234	94	II	Chlo+Alo	Progressive	1
42	CLL42	73	M	36.9	69	II	-	ND	-	87	CLL89	75	M	29	89	IV	Flud	Progressive	7
43	CLL43	67	F	41.8	84	0	-	Progressive	2										
44	CLL44	70	M	16.3	85	0	-	Indolent	5										
45	CLL45	58	F	25	60	I	-	Progressive	1										

WBC: white blood cell count; Lym: lymphocytes; Hb: hemoglobin; PLT: platelet; LAP: lymphadenopathy; SPM: splenomegaly; HPM: hepatomegaly; M: male; F: female; Chlo: chlorambucil; Flud: fludarabine; Pred: prednisolone; Alo: allopurinol; ND: newly diagnosed; NI: not identified

RESULTS

General Immunophenotypic Features of Leukemic Cells. The expression of 15 surface membrane molecules (CD3, CD5, CD14, CD19, CD20, CD23, CD27, CD38, CD45, CD45RA, CD45RO, HLA-DR, κ and λ light chains and μ heavy chain) was determined in leukemic cells from 87 Iranian CLL patients. Representative results are illustrated in Figure 1 and cumulative results are depicted in Figure 2. In all cases the neoplastic cells displayed B-CLL phenotype ($CD5^+/CD19^+/sIg^+$) with high expression of CD45 and HLA-DR. Leukemic cells from the vast majority of samples expressed CD20 and CD23 but failed to express CD3 or CD14. Peripheral mononuclear cells from most patients expressed CD45RA (85.1%) and CD45RO (83.9%), these two molecules were co-expressed in 70 % of patients. There was no significant difference in the expression of CD45 isoforms between indolent and progressive patients (Table 2). The mean fluorescence intensity (MFI) of CD45 isoforms was also similar in these two groups of patients (data not presented). The expression pattern of CD27 was similar to that of the CD45 and its isoforms, with no significant difference between indolent (97.6%) and progressive (95.4 %) patients (Table 2).

Table 2. Comparison of CD20, CD23, CD27, CD38, CD45RA and CD45RO expression with clinical characteristics of Iranian patients with CLL

	No (%) of patients	CD20+	CD23+	CD27+	CD38+	CD45RA+	CD45RO+
All patients	87	73 (83.9)	77 (88.5)	83 (95.4)	24 (27.6)	74 (85.1)	73 (83.9)
Indolent	42 (48.3)†	32 (76.2)	38 (90.5)	41 (97.6)	5 (11.9)*	33 (78.6)	33 (78.6)
Progressive	37 (42.5)	35 (94.6)	33 (89.2)	35 (94.6)	15 (40.5)	33 (89.2)	33 (89.2)
Treated	21 (24.1)	18 (85.7)	17 (81)	18 (85.7)	7 (33.3)	17 (81)	18 (85.7)
Untreated	66 (75.9)	55 (83.3)	60 (90.9)	65 (98.5)	17 (25.8)	57 (86.4)	55 (83.3)
‡ Rai stage: I	48 (55.2)	43 (89.6)	42 (87.5)	47 (97.9)	11 (22.9)	40 (83.3)	40 (83.3)
II	22 (25.3)	16 (72.7)	21 (95.5)	21 (95.5)	8 (36.4)	20 (90.9)	16 (72.7)
III	16 (18.4)	13 (81.3)	14 (87.5)	14 (87.5)	5 (31.3)	13 (81.3)	16 (100)
Gender: Male	54 (62.1)	44 (81.5)	48 (88.9)	51 (94.4)	15 (27.8)	46 (85.2)	45 (83.3)
Female	33 (37.9)	29 (87.9)	29 (87.9)	32 (97)	9 (27.3)	28 (84.8)	28 (84.8)
LAP	19 (21.8)	16 (84.2)	18 (94.7)	17 (89.5)	6 (31.6)	16 (84.2)	16 (84.2)
SPM	27 (31)	21 (77.8)	23 (85.2)	24 (88.9)	9 (33.3)	22 (81.5)	21 (77.8)
HPM	4 (4.6)	2 (50)	3 (75)	4 (100)	0	3 (75)	4 (100)
Anemia	11 (12.6)	10 (90.9)	10 (90.9)	9 (81.8)	5 (45.5)	9 (81.8)	11 (100)
Thrombocytopenia	11 (12.6)	8 (81.8)	10 (90.9)	10 (90.9)	5 (45.5)	11 (100)	11 (100)

-†Data are presented as number (%), *P value<0.05, determined by Pearson Chi-Square test, ‡ Modified Rai staging system, LAP: lymphadenopathy, SPM: splenomegaly, HPM: hepatomegaly

Of 87 CLL patients, 62 (71.3%) cases expressed κ immunoglobulin light chain and 25 (28.7%) expressed λ light chain (ratio 2.5:1). The κ/λ ratio was lower in indolent (2:1) compared to progressive (4:1) patients, though the difference did not reach statistical significance.

Expression Profile of CD38 Molecule in CLL Subgroups. A given leukemic population was considered positive for CD38 when ≥30% of the B-CLL cells expressed the

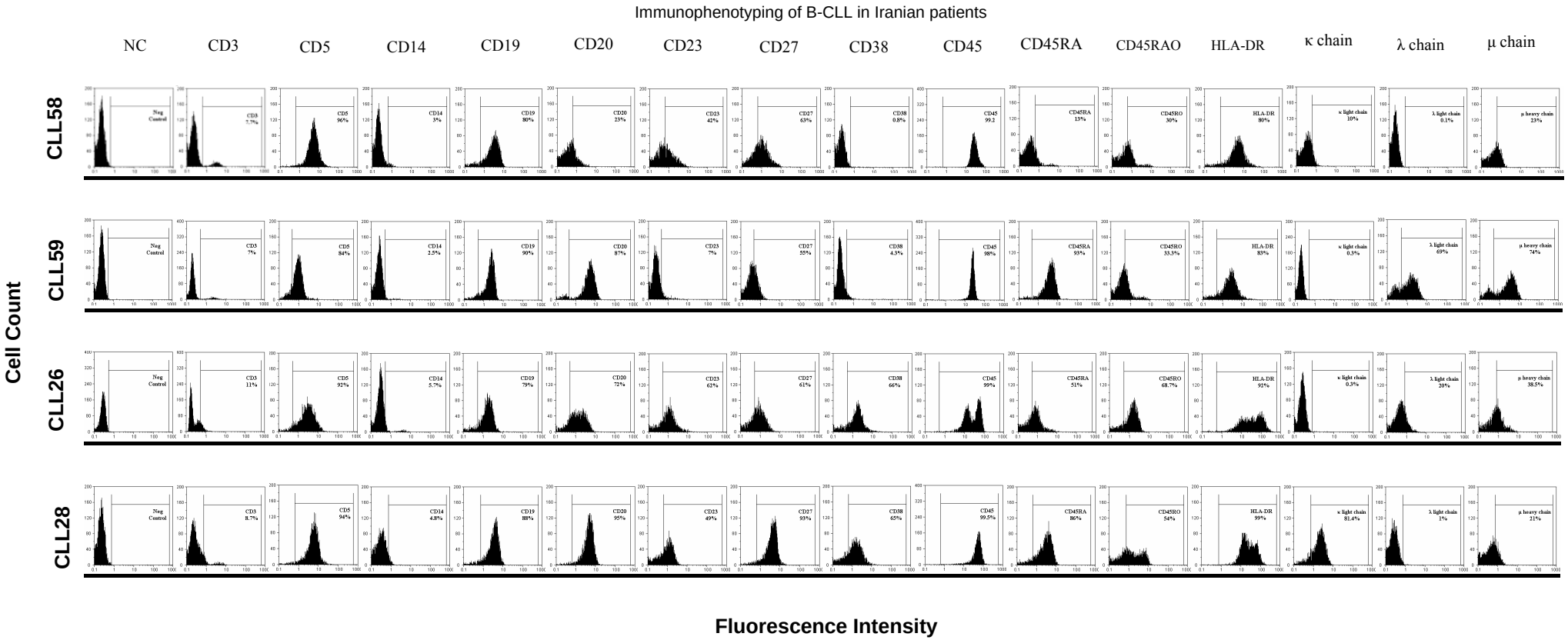

Figure 1. Representative flow cytometry results for 4 patients with B-CLL
The samples belong to patients with indolent (CLL58 and CLL59) or progressive (CLL26 and CLL28) disease. Clinical and laboratory characteristics of the patients are given in Table 1.

molecule (8). Accordingly, 24 (27.6%) cases were $CD38^+$ of whom 5 (11.9%) and 15 (40.5%) cases displayed indolent and progressive diseases, respectively (p=0.02). Four $CD38^+$ samples belonged to newly diagnosed patients. Overall, the range of CD38 expression in the leukemic cells of CLL patients was from 0.1% to 87%. It is interesting to note that only 10 patients expressed CD38 above the level of 50%, 9 of whom were in progressive group and the last one was from the newly-diagnosed group. Stronger expression of CD38 was also observed in progressive group when mean fluorescent intensity was taken into consideration, but the difference was statistically insignificant (data not shown). No significant differences were found for CD38 expression and the clinical staging of the patients, though $CD38^+$ leukemic B-cells were slightly higher in stage II and III patients compared to those at stage I (Table 2).

According to the forward stepwise logistic regression approach, CD38 was found to be the only significant predictor of progression status of patients, among all other markers tested (p=0.001; with a 95% confidence interval of 1.184-1.566).

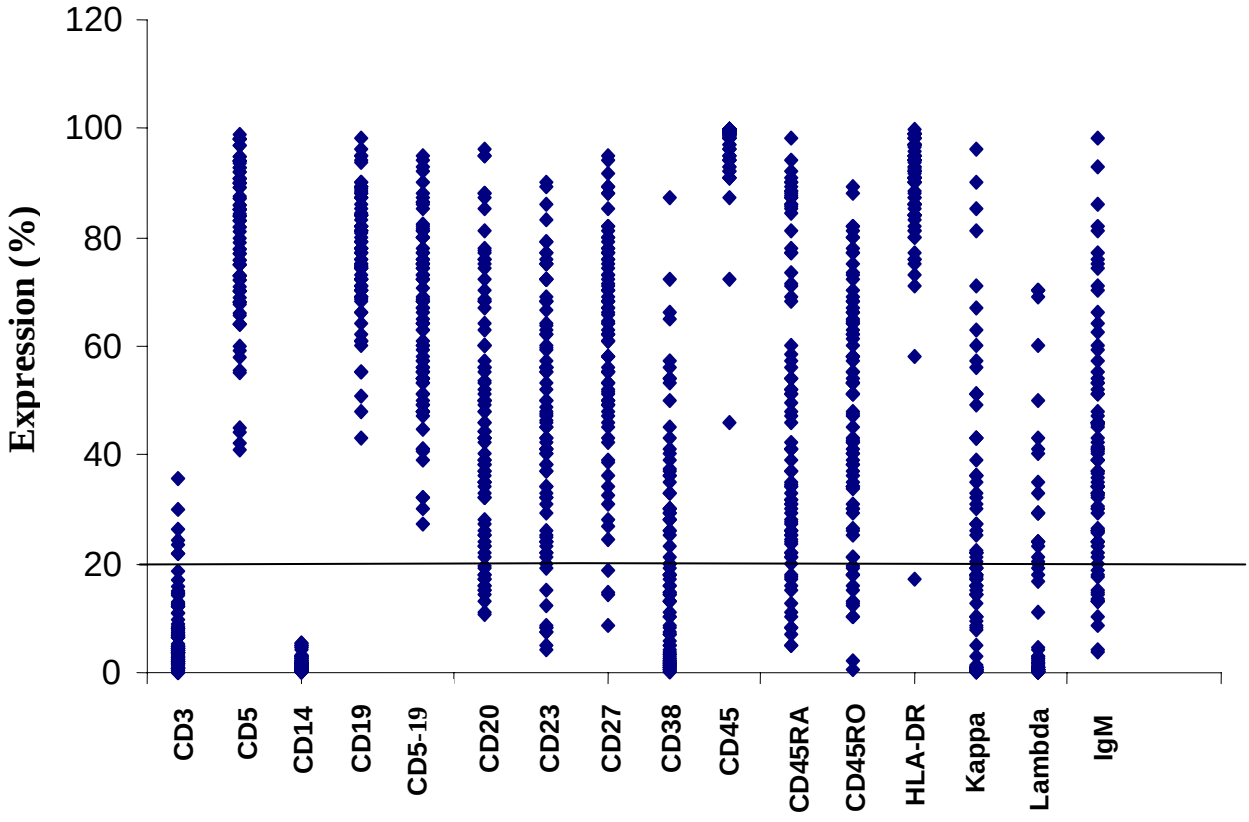

Figure 2. Expression pattern of major immunophenotypic markers in Iranian CLL patients Baseline denotes a cutoff expression value (20%) arbitrary assigned to differentiate positive and negative results.

DISCUSSION

The incidence of B-CLL in Asian populations has been reported to be lower than in Western people, where it constitutes the most abundant type of leukemias (1). Due to its limited abundance, the immunophenotypic features of the B-CLL tumor cells have not been widely investigated in Asian populations. Recent results mainly reported in Western B-CLL patients have demonstrated association between CD38 expression and progressive disease (13). The results of this study conducted for the first time in an Iranian population, confirm and extend previous findings.

Light chain expression was demonstrated in all of our cases. Of 87 CLL patients, 71.3% and 28.7% expressed κ and λ light chains on their leukemic B cells (ratio 2.5:1), respectively. This is in agreement with the results of Stamatopoulos et al. (14), in which from 276 CLL patients 64.9% expressed κ and 35.1% were expressing λ light chains but slightly different from the study of Hulkkonen et al. (15), in which from 35 CLL cases, 54% and 46% of the cases expressed κ and λ light chains. In the report of Lewis et al. (16), of 23 CLL cases expressing surface Ig, 65.2 and 34.8% were κ and λ light chain positive, respectively.

We confirmed that CD38 expression is a clinically relevant marker (≥30% expression on leukemic B-CLL cells), for predicting disease progression. This finding is in agreement with the study of Hayat et al (13).

CD38 was initially proposed as a surrogate marker for the absence of IgVH gene mutation in CLL patients by Damle et al (8). Although a subsequent study by Hamblin et al. (17) failed to establish a clear cut correlation with the absence of mutations in IgVH genes, it has been confirmed by many studies that CD38 expression can be considered as an independent and reliable negative prognostic marker in CLL (18). CD38 protein is a type II single-chain transmembrane molecule displaying a canonical molecular weight of approximately 45 kDa (19). It has been suggested that CD38 performs as a receptor in B-CLL cells upon translocation into the lipid raft and physical association with the BCR/CD19 complex. Environmental conditions were found to be crucial in modulating CD38 surface expression and functions: IL-2 was identified as a surrogate indicator of the relevance of soluble factor(s), working in synergy to determine the final biologic effects. The signals delivered by CD38 and IL-2 induce proliferation and prolonged survival of a subpopulation of B-CLL cells (20). Also it has been shown by Deaglio et al. (21) that $CD38^+$ B-CLL cells bind to murine fibroblasts transfected with the CD31 ligand with resulting increased growth and survival. Further this work shows that CD38/CD31 crosstalk is part of an intricate network of communication between neoplastic cells and bystander non neoplastic cells. Indeed, CD38/CD31 interactions lead to increased B-CLL proliferation and survival by means of a direct cooperation with CD100, a cell surface receptor member of the semaphorin family, which interact with CD72 (a low affinity ligand, co expressed by B-CLL cells), and with plexin-B1 (high-affinity ligand, expressed by stromal and endothelial cells)(22, 23).

CD38 mediated signals are followed by increased expression of cell surface CD100 and simultaneous down-modulation of CD72, which is the prototype of negative regulation of B cells (24). It has been shown by Burger et al (25) that nurse like cells as professional supporters of B-CLL cells, express high levels of CD31 and plexin-B1. This supports the role of CD38 in which $CD38^+$ cells can interact with nurse like cells with the final result of a significant improvement in their growth potential. Collectively, it can be suggested that CD38 is not merely a marker in B-CLL, but it is a receptor that plays an

important role in the pathogenesis of B-CLL, controlling the proliferation and survival of malignant B-CLL cells.

Our findings showed that most of our CLL cases (95.4%) displayed a strong expression of CD27 on their leukemic B-CLL cells. This is in agreement with the finding of Vilpo and coworkers (26), in which all of 22 CLL cases expressed CD27 on their leukemic B cells. Also other studies confirmed the expression of CD27 in their CLL cases. CD27 is accepted as a memory cell marker, as previously proposed (27). CD27 is a transmembrane disulfide-linked homodimer belonging to nerve growth factor receptor (NGFr) superfamily, a group of homologous molecules involving in the processes of lymphocyte differentiation and selection that include the two tumor necrosis factor (TNF) receptors, the B cell antigen CD40 and the lymphocyte activation antigen CD30, as well as APO-1/Fas (CD95), a molecule which controls lymphocyte survival (28). Expression of CD27 had been considered previously as a peculiar finding of leukemic $CD5^+$ B-CLL cells; as a matter of fact normal follicular $CD5^+$ mantle zone cells, which represent the non neoplastic counterpart of B-CLL cells, are CD27 negative (29). Cross linking of the CD27 antigen on T cells provides a costimulatory signals that, in concert with T-cell receptor cross linking, can induce T-cell proliferation and cellular immune activation (30). The expression of CD27 and also its ligand (CD70) on B-CLL leukemic cells may have the same function as T cells on the proliferation and to some extent survival of these leukemic cells.

In our study we did not find any differential expression of CD45 isoforms in our CLL groups (indolent vs. progressive). Our results did not confirm the findings of Vilpo and his coworkers (26) who reported a correlation in the expression of CD45RO with mutated CLL patients (most of indolent patients with a good prognosis). High expression of CD45RO and CD27 in our CLL patients suggests that B-CLL cells may be more mature cells, perhaps of memory type. But it is unclear why these cells also express CD45RA at the same level as CD45RO. Isoforms of CD45 marker are generated by alternative splicing of three exons (exon 4, 5, and 6) in mRNA (31). CD45RO (P180) and CD45RA (P205, 220) have been most intensively studied because CD45RO is widely regarded as a marker of memory lymphocytes (32). The expression pattern of CD45 isoforms is different in various cell types. The CD45RO isoform is expressed in the majority of granulocytes and monocytes. Naïve T cells and the majority of normal B cells express the high molecular isoform of CD45 (CD45RA), and only a few peripheral blood B cells express low levels of CD45RO (32). In the tonsil the majority of T cells express CD45RO and only a few B cells express this marker (33). The molecular studies by Yu and coworkers (34) showed that the transition from CD45RA high phenotype to CD45RA low phenotype in CLL is associated with the generation of the exon 5 splice product of CD45 mRNA. The CD45 isoforms expressed by various B cell malignancies can be assumed to reflect the stage of differentiation of those B cells that are expanded in the neoplastic process. The high concentration of CD45RO isoforms was found among peripheral blood lymphocytes in patients with multiple myeloma and Waldenstrom' s macroglobulinemia, in which the population of B cells are of late B or early pre-plasma cells (35). Another view point may suggest that similar to T cell system, where CD45RO is expressed not only on mature memory T cells, but also on the majority of immature thymus cells, the expression of CD45RO on CLL cells may reflect relative immaturity of B-CLL cells. The distribution of CD45RA and CD45RO in our patients was uniform. Among our patients, 85.5 and 84% expressed CD45RA and CD45RO on their leukemic B cells, respectively. Also 70% of the patients coexpressed

both isoforms. Two other studies showed that about half of the CLL patients coexpressed both CD45 isoforms, while in the remaining patients CLL cells expressed only the CD45RA isoform (27, 32). Of our 87 B-CLL cases 13 (14.8%) and 14 (16%) only expressed CD45RA and CD45RO, respectively. In the study of Yu et al. (34), most CLL cases (91%) expressed CD45RA and only 18% expressed CD45RO (coexpressed with CD45RA). In the study of Vilpo et al. (26) all CLL cases expressed CD45RA uniformly and their patients had differential expression of CD45RO in which about half of the cases expressed CD45RO below the range of 20% of positive cells. The reason for the differences in the expression of CD45 isoforms in different groups of CLL patients is not clear. Also their role in the pathogenesis of the disease is not clearly defined. In a recent study by Matto et al (36), it was suggested that the two isoforms had distinct effects on BCR or cytokine-induced cellular proliferation of B cells. They showed that BCR stimulation significantly increased the proliferation of the follicular lymphoma cell line expressing CD45RO (HF28RO cell line), in contrast to a decreased proliferation of HF28RA cell line (the same cell line expressing CD45RA). Moreover, proliferation of HF28RO cells was significantly increased after the addition of IL-2, 4, 6, 10, 12, 13, 15, IFN-γ and TNF-α, whereas most of these cytokines significantly inhibited the proliferation of HF28RA cells.

In conclusion it seems that some membrane molecules may have important roles on the proliferation and survival of leukemic B-cells in CLL. Among the most important differentially expressed molecules, CD38 may have important role in the progression of CLL. The key role of this molecule in the pathogenesis of B-CLL needs further investigation. Our results obtained in an Asian ethnic population confirm and extend previous findings obtained from Western populations regarding the association of CD38 expression and disease progression in B-CLL.

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