The Role of Immunophenotyping in Differential Diagnosis of Chronic Lymphocytic Leukemia

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SUMMARY

Introduction Accurate diagnosis of chronic lymphocytic leukemia (CLL) acquires immunophenotyping by flow cytometry in order to facilitate differential diagnosis between CLL and other mature B-cell neoplasms (MBCN).

Objective The aim of this study was to define immunological profile of CLL cells.

Methods Immunophenotyping by flow cytometry was performed on peripheral blood specimens at diagnosis in the group of 211 patients with *de novo* MBCN.

Results Absolute count of B-cells was significantly increased in all MBCN patients comparing to healthy control group ($p<0.05$). B-cell monoclonality was detected in 96% of all MBCN patients, by using surface immunoglobulin (sIg) light chain restriction. B-cell antigens, CD19, CD20, CD22, were expressed with very high frequency in CLL and other MBCN. In comparison with other MBCN, in CLL group, the frequency of expression was higher for CD5 and CD23 ($p<0.0001$), though lower for FMC7 antigen ($p<0.0001$). CLL patients were characterized by lower expression patterns of CD20, CD22, CD79b, and sIg ($p<0.0001$) as well as higher expression pattern of CD5 antigen ($p<0.05$). Correlation between the final diagnosis of MBCN and values of CLL scoring system showed that the majority of CLL patients (97%) had higher values (5 or 4) whereas the majority of other MBCN patients (96%) had lower score values (0-3).

Conclusion Our results have shown that characteristic immunophenotype which differentiates CLL from other MBCN is defined by following marker combination - $CD19^{+}$ $CD20^{+low}$ $CD22^{+low}$ $CD5^{+high}$ $CD23^{+}$ $FMC7^{-}$ $CD79b^{+low}$ sIg^{+low}. CLL score values of 5 or 4 points are highly suggestive for diagnosis of CLL.

Keywords: chronic lymphocytic leukemia; immunophenotyping; flow cytometry

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a clonal B-cell neoplasm of mature cells whose diagnosis is based on clinical manifestations, cell morphology and immunophenotyping [1, 2, 3]. Furthermore, absolute count of monoclonal B-cells, demonstrated by immunophenotyping and flow cytometry, should be ≥ 5000 cells/μl, suggesting the diagnosis of B-cell disorder [1, 2].

The most reliable methodology for the diagnosis of CLL is immunophenotyping by flow cytometry (IFC) [4]. IFC is usually performed in all patients with lymphocytosis, in order to confirm the diagnosis of CLL, suspected by morphology and clinical data. Application of immunological markers primary determines B-cells by showing surface immunoglobulin (sIg) light chain restriction [5]. It is also known that there is no single marker exclusively expressed on CLL cells, but a composite immunophenotype which integrates many different B-cell markers and helps distinguishing CLL from other mature B-cell neoplasms (MBCN). The expression profile of these leukocyte antigens is included in the specialized CLL scoring system [6, 7], that was created in order to distinguish typical CLL (4-5 points) from other MBCN (0-3 points) (Table 1). It has also been suggested that CLL cells have immunophenotypic signature similar to that of activated follicular marginal zone B1 cell, which normally express CD5, and coexpress membrane IgM and IgD [8].

Distinguishing CLL from other MBCN, based only on cytomorphology and clinical presentation, may be difficult. Considering the mature B-cell leukemias, CLL needs to be distinguished from prolymphocytic leukemia (PLL) and hairy cell leukemia (HCL), while in the case of B-cell non-Hodgkin lymphomas (B-NHL) in leukemic phase, problems of differential diagnosis arise between CLL and mantle cell lymphoma (MCL), splenic marginal zone lymphoma without/with villous lymphocytes (SMZL/SLVL) and follicular lymphoma (FL) [5]. The precise distinction of CLL from these related diseases is important in terms of the accurate diagnosis, prognosis and adequate treatment of patients.

OBJECTIVE

The aim of this single center retrospective study was to determine the value of each explored membrane leukocyte antigen in differential diagnosis of CLL from other MBCN, and to define immunological profile of CLL.

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Table 1. Scoring system for the differential diagnosis of chronic lymphocytic leukemia (CLL) [5]

Marker	Points	
	1	0
CD5	Positive	Negative
CD23	Positive	Negative
FMC7	Negative	Positive
sIg	Low	Medium/high
CD22/CD79b	Low/negative	Medium/high

sIg – surface membrane immunoglobulin

METHODS

Patients

We analyzed 211 consecutive untreated patients (pts) with suspected diagnosis of *de novo* MBCN, referred for standard diagnosis to the Laboratory for immunophenotyping and flow cytometry, Clinic of Hematology, Clinical Center of Serbia, in the time period November 2002- October 2005. Final diagnosis of CLL was confirmed in 183 patients. The diagnosis of other MBCN was confirmed in 28 patients with the following diagnosis: MCL (8 pts), SMZL (8 pts), FL (4 pts), lymphoplasmocytic lymphoma (2 pts), B-NHL, not otherwise specified (5 pts), and HCL (1 pt). Diagnosis was established according to the standard criteria [2, 4]. Other than immunophenotypic characteristics, demographic (sex and age) and hematological data (hemoglobin, platelet count, leukocyte count, percentage of lymphocytes in peripheral blood and bone marrow) were analyzed. This study also included peripheral blood specimens of 20 healthy volunteers as a control group.

Flow cytometric immunophenotyping

Immunophenotyping was performed by using direct immunofluorescent technique and flow cytometry [9] on peripheral blood specimens anticoagulated by heparin (40 I.U./ml; 5000 I.U./ml, ICN Galenika). Mononuclear cells were isolated by density gradient centrifugation (Histopaque-1077, SIGMA-ALDRRICH, USA) and washed two times with 10ml of 2% of fetal calf serum in phosphate buffered saline (FCS/PBS), pH 7.4. Cell concentration was adjusted to 10×10^6/ml, and 100 µl aliquots of cell suspension were stained with optimal concentrations of commercial monoclonal antibodies (BD Biosciences, USA) organized in two-color panels: CD2-FITC (clone S5.2), CD3-PE (clone SK7), CD5-PE (clone UCHT2; clone L17F12), CD10-PE (clone HI10a), CD19-PE (clone 4G7), CD20-FITC (clone L27), CD22-PE (clone S-HCL-1), CD23-PE (clone EBVCS-5), CD38-FITC (clone HIT2; clone HB7), CD79b-PE (clone CB3.1), CD103-PE (clone Ber-ACT8), FMC7-FITC (clone FMC7), anti-κ-FITC (polyclonal), anti-λ-FITC (polyclonal). After incubation for 20 minutes at +4°C in the dark, cells were washed with 2ml of 2% FCS/PBS, pH 7.4 solution and fixed with 0.5ml of 1% paraformaldehyde/PBS, pH 7.4 solution. The flow cytometric analysis was performed on a FACSCalibur (BD Biosciences, USA), using the software CellQuest Pro (version 4.0.2). Analysis was performed on lymphocyte population gated according to forward and side scatter characteristics (FSC/SSC). A minimum of 10,000 gated events was acquired for each aliquot per tube. The results were given as a percentage of lymphocytes reactive with antibodies. An antigen was considered positive when at least 30% of gated lymphocytes expressed explored antigen above the isotypic control [6]. The patterns of antigen expression were estimated according to the relative position of a peak of fluorescent emission on the log scale in the fluorescence axis as follows: low (Ag^{+low}), medium ($Ag^{+medium}$), high (Ag^{+high}) expression [6] (Figure 1). Detection of the monoclonal B-cell population in the peripheral blood was done according to overall sIgκ:sIgλ ratio (>3 for sIgκ monoclonality or <0.3 for sIgλ monoclonality) [10].

Absolute counts of peripheral blood B-cells and T-cells were calculated by using double platform methodology [11]. Proportions of peripheral blood B-cells (percentage of $CD19^+$ cells/ lymphocytes) and T-cells (percentage of $CD3^+$ cells/lymphocytes), detected by flow cytometry, were combined with the absolute leukocyte count and lymphocyte differential.

Statistics

Statistical analyses were performed using the Statistica® (version 7.1, StatSoft®, Inc. Tulsa USA, 2005) licensed sta-

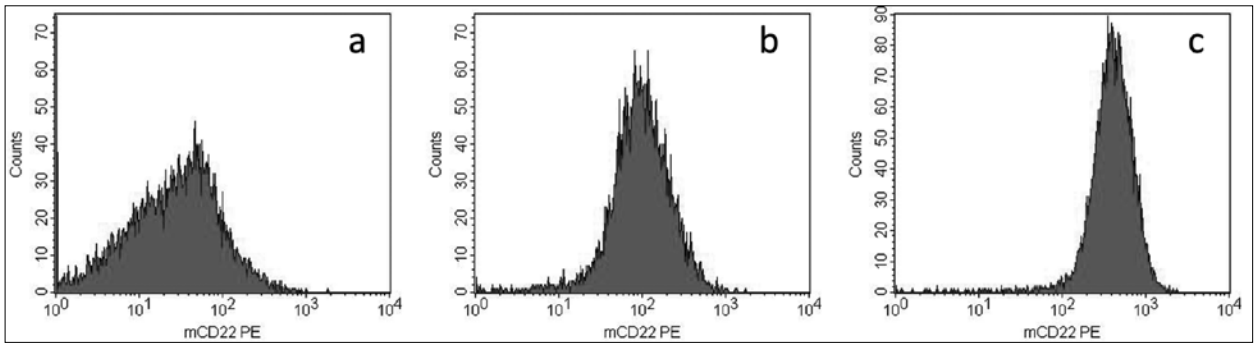


Figure 1. The patterns of antigen (Ag) expression were estimated according to the relative peak position of phycoerythrin (PE) fluorescent emission on the log scale in the fluorescence axis, as follows: a) low (Ag^{+low}), when the peak of fluorescent emission was within the first log percentile; b) medium ($Ag^{+medium}$), when the peak of fluorescent emission was within the second log percentile; c) high (Ag^{+high}), when the peak of fluorescent emission was within or more than three log percentiles.

doi: 10.2298/SARH1404197D

Table 2. Demographic and hematological characteristics of MBCN patients

Characteristics		Groups of patients			p value
		MBCN (N=211)	CLL (N=183)	Other MBCN (N=28)	
Sex (n)	Male	147	127	19	NS
	Female	64	56	9	
	Ratio	2.3/1	2.3/1	2.1/1	
Age (years)	Mean±SD	62±11	62±11	62±10	NS
	Median	64	64	63	
	Range	32–83	32–83	37–75	
Hemoglobin (g/l)	Mean±SD	127±21	129±19	112±25	0.0001
	Median	130	132	118	
	Range	54–160	61–160	54–147	
Platelets ($\times10^9$/l)	Mean±SD	197±97	207±94	137±100	0.0005
	Median	185	197	103	
	Range	3–751	3–751	15–455	
WBC count ($\times10^9$/l)	Mean±SD	61±93	61±96	63±74	NS
	Median	34	35	25	
	Range	5–910	6–910	5–350	
Peripheral blood Ly (%)	Mean±SD	77±13	77±12	77±15	NS
	Median	80	79	83	
	Range	39–100	39–100	49–100	
Bone marrow Ly (%)	Mean±SD	65±22	64±22	75±19	NS
	Median	72	70	79	
	Range	5–98	5–98	28–96	

n – number of patients with the specific characteristics; WBC – white blood cells; Ly – lymphocytes; MBCN – mature B-cell neoplasm; N – total number of the analyzed patients; CLL – chronic lymphocytic leukemia; p value – CLL *vs.* other MBCN; NS – not significant

tistical analysis software package. Data were expressed as mean and median values and interquartile range. Comparison between the groups was performed using Chi-square test and Student's t-test. Statistical significance was defined as p value <0.05.

RESULTS

Patient characteristics

The main demographic and hematological characteristics of CLL and other MBCN patients were shown in Table 2. Population of males dominated in both groups with the mean age of 62 years. For the most of studied characteristics no significant differences between CLL and other MBCN patient groups were found. The only significant difference was established regarding the values of hemoglobin and platelets. The patients with CLL had significantly higher mean values of hemoglobin and platelets in comparison to other MBCN patients (p<0.05). At presentation, anemia (Hb<110 g/L) was present in 16% and thrombocytopenia (Tr<100×10^9/L) in 6% of CLL patients.

Peripheral blood B-cell counting

Detection and estimation of peripheral blood B-cell and T-cell populations were performed by IFC in the whole MBCN patient group. The analysis of relative distribution of B-cells and T-cells in lymphocyte population showed significantly higher mean proportion of B-cells (82% *vs.* 7.5%) as well as significantly lower mean proportion of T-cells (13% *vs.* 75%), compared to the healthy controls (p<0.05). Besides, the mean absolute count of peripheral blood B-cells in the MBCN group was significantly higher compared to the healthy control group (42267 *vs.* 196 cells/μl) (p<0.05). It was also found that the vast majority of CLL patients (95%) had absolute count of peripheral B-cells above 5000 cells/μl.

Immunophenotypic characteristics of MBCN patients

To define immunological diagnosis, immunophenotyping data for each patient with MBCN were analyzed regarding CLL scoring system by calculating score value [5]. The patients were divided into two groups according to immunological diagnosis: immuno dg CLL (score 5 and 4) (180/211 pts) and immuno dg non-CLL (score ≤3) (31/211 pts). Basic immunophenotypic characteristics of these groups were shown in Tables 3 and 4.

It was recorded that CD19 antigen was expressed in all analyzed MBCN patients. Considering differences in antigens expression between the groups of patients with different immuno diagnosis, it was found that CD5 antigen was expressed with higher frequency in CLL group comparing to non-CLL group (99% vs. 74%, p<0.0001). The similar result was found for CD23 antigen, where it was expressed in majority of CLL comparing to non-CLL (98% vs. 32%, p<0.0001) patients. The analysis of expression patterns of CD5 antigen revealed that pattern $CD5^{+high}$ predominated in the CLL group (p=0.0489). In case of CD23 antigen, CLL group was characterized by predomination of higher expression patterns ($CD23^{+medium}$ and $CD23^{+high}$) comparing to non-CLL group characterized mainly with $CD23^{+low}$ expression pattern (p>0.05). Regarding FMC7 antigen expression, we found very low frequency of expression in CLL group comparing to non-CLL group (8% vs. 100%, p<0.0001).

Expression of CD20 and CD22 antigens was detected with very high frequency in CLL group (99% and 94%,

Table 3. The frequency of antigen expression in MBCN patients, group with CLL (score 4-5) and non-CLL (score ≤3)

Antigen	% (n/N)			p value
	MBCN	CLL (score 4-5)	Non-CLL (score ≤3)	
CD19	100 (211/211)	100 (180/180)	100 (31/31)	/
CD5	95 (201/211)	99 (178/180)	74 (23/31)	<0.0001
CD23	88 (186/211)	98 (176/180)	32 (10/31)	<0.0001
FMC7	21 (45/211)	8 (14/180)	100 (31/31)	<0.0001
CD20	99 (209/211)	99 (178/180)	100 (31/31)	NS
CD22	95 (199/210)	94 (169/180)	100 (30/30)	NS
CD79b	63 (103/163)	57 (80/139)	96 (23/24)	0.001
sIgκ	57 (120/211)	57 (103/180)	52 (16/31)	NS
sIgλ	39 (83/211)	38 (68/180)	48 (15/31)	NS
CD38	35 (63/182)	32 (50/156)	50 (13/26)	NS

n – number of patients that are positive for the analyzed antigen; N – total number of the analyzed patients; p value – CLL vs. non-CLL

Table 4. The frequency of the antigen expression patterns in group with CLL (score 4-5) and non-CLL (score ≤3)

Pattern of antigen expression		% (n/N)		p value
		CLL (score 4-5)	Non-CLL (score ≤3)	
CD20	Low	70 (124/178)	0 (0/31)	<0.0001
	Medium	21 (37/178)	0 (0/31)	
	High	9 (17/178)	100 (31/31)	
CD22	Low	74 (125/168)	23 (7/30)	<0.0001
	Medium	21 (36/168)	20 (6/30)	
	High	4 (7/168)	57 (17/30)	
CD79b	Low	99 (79/80)	30 (7/23)	<0.0001
	Medium	1 (1/80)	30 (7/23)	
	High	0 (0/80)	40 (9/23)	
sIg	Low	97 (165/171)	22 (7/32)	<0.0001
	Medium	2 (4/171)	41 (13/32)	
	High	1 (2/171)	37 (12/32)	
CD5	Low	12 (12/104)	30 (6/20)	0.049
	Medium	23 (24/104)	30 (6/20)	
	High	65 (68/104)	40 (8/20)	
CD23	Low	42 (74/176)	90 (9/10)	NS
	Medium	31 (54/176)	10 (1/10)	
	High	27 (48/176)	0 (0/10)	
FMC7	Low	93 (13/14)	61 (19/31)	NS
	Medium	7 (1/14)	39 (12/31)	
	High	0 (0/14)	0 (0/31)	

n – number of patients that exhibit specific pattern of the antigen expression; N – total number of the analyzed patients; p value – CLL vs. non-CLL

respectively) as well as in all patients of non-CLL group. The analysis of their expression patterns showed that the majority of CLL patients had $CD20^{+low}$ and $CD22^{+low}$ expression patterns (70% and 74%, respectively) compared to non-CLL patients where predominated higher expression patterns $CD20^{+high}$ and $CD22^{+med/high}$ (100% and 77%, respectively) ($p<0.0001$).

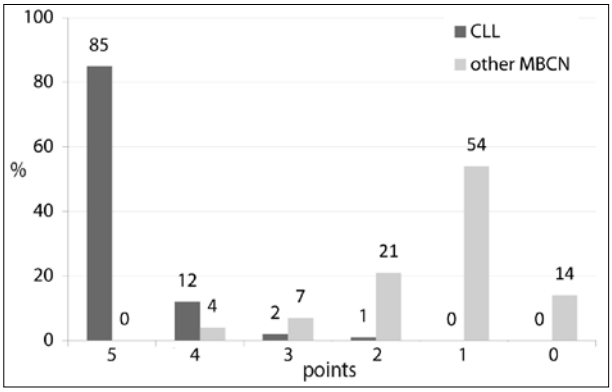
The analysis of the component of B-cell receptor CD79b revealed that it was expressed with lower frequency in CLL group compared to non-CLL group (57% *vs.* 96%, p=0.001) whereas $CD79b^{+low}$ expression pattern was detected in the majority of CLL patients compared to non-CLL ones (99% *vs.* 30%, $p<0.0001$).

Monoclonality of B-cell population, defined according to the presence of sIg light chain restriction, was detected in 95% of CLL patients as well as in all non-CLL patients. Frequency of $sIg\kappa^+$ was higher than $sIg\lambda^+$ cases, in both analyzed groups. The analysis of sIg expression patterns demonstrated that the majority of CLL patients had sIg^{+low} expression pattern compared to non-CLL patients (97% *vs.* 22%) ($p<0.0001$).

Prognostic marker CD38 was expressed with lower frequency in CLL group comparing to non-CLL group of patients (32% vs. 50%, $p>0.05$).

Correlation of CLL score with final diagnosis of MBCN

The final diagnosis of MBCN was correlated with CLL score and the obtained results were shown in Graph 1. The great majority of CLL patients had score of 5 or 4 points



Graph 1. Comparison of frequency of the chronic lymphocytic leukemia (CLL) score points in patients with the final diagnosis of CLL and other mature B-cell neoplasms (MBCN)

$p<0.0001$

(97%). CLL patients with score ≤3 points were distributed as follows: 3 points (2%), and 2 points (1%). There were no CLL patients with score of 1 or 0 points. In the group of other MBCN, the majority of patients (96%) had lower scores (≤3 points). Only 1 patient had score of 4 points and there were no patient with score of 5 points in the group of other MBCN.

DISCUSSION

Immunophenotyping by flow cytometry is essential for the diagnosis and subclassification of MBCN, particularly CLL [1, 5]. Because the clinical evolution and response to treatment differ between CLL and other MBCN, an accurate diagnosis is very important. Although CLL has a relatively specific immunophenotypic profile comparing to other MBCN, there is some overlapping regarding the expression of membrane markers. The CLL scoring system was created in order to make distinction between CLL and other MBCN entities easier [6, 7].

This study retrospectively analyzed immunophenotypic features of 211 patients with *de novo* MBCN, including 183 patients with final diagnosis of CLL and 28 patients with other MBCN. The CLL patients at presentation usually showed typical clinical and hematological characteristics, including the presence of anemia and/or thrombocytopenia in low frequency (16% vs. 6%, respectively). It was also found that patients with CLL had significantly higher mean values of hemoglobin and platelets in comparison with other MBCN patients, which was in correlation with literature data [3].

Detection and counting of peripheral blood B-cell population in patients with MBCN, based on CD19 antigen expression, as well as exploring of clonality status of B-cell populations, based on sIg light chain restriction, represent very important information provided by IFC [2]. All patients with MBCN had significantly increased circulating B-cells at presentation whereas monoclonal status was detected in high frequency (96%). Moreover, it was found that the great majority of CLL patients (95%) had absolute count of monoclonal peripheral blood B cells above

5000 cells/μl. Detection of the increased absolute count of monoclonal peripheral blood B-cells in patients with normal WBC count could be important for early differential diagnosis of MBCN [2].

To estimate the value of each membrane leukocyte antigen in differential diagnosis of CLL, the expression of three B-cell lineage antigens (CD19, CD20, CD22), and five antigens from the CLL scoring system (CD5, CD23, FMC7, CD79b, sIg), including prognostic antigen CD38 was analyzed. Among lineage B-cell antigens, CD19 was expressed in all our patients with MBCN, indicating that it is the only marker consistently expressed on leukemic B-cells. Our results are in the line with literature data which select CD19 as one of the best gating antigen for immunophenotypic analysis of B cell neoplasms, including CLL [12]. However, other two lineage B-cell antigens, CD20 and CD22, were less consistently expressed on CLL cells. The probable reason for that is their low expression levels on CLL cells. In contrast, in other MBCN, all patients expressed CD20 and CD22 at higher expression levels. According to our results, CD20 and CD22 were not important for differential diagnosis of CLL, because these antigens were expressed in virtually all our MBCN cases. Our results are in the line with results obtained by Delgado et al. [13]. On the other hand, the assessment of the CD20 expression in CLL patients by IFC would be of the utmost clinical importance if the anti-CD20 immunotherapy was considered [14].

One of antigens from CLL scoring system is CD5, which is generally considered as pan T-cell antigen, although some restricted B cells, named B1 cells, also express CD5 [15]. In pathological conditions, CD5 is usually expressed in CLL and MCL, in some cases of PLL, diffuse large B-cell lymphoma and HCL [1]. Our results showed that frequency of expression of CD5 antigen as well as $CD5^{+high}$ expression pattern was significantly higher in CLL compared to non-CLL group. Considering these data, CD5 antigen could have important role in differential diagnosis of CLL. Similar results were found in studies by Pangalis et al. and Deneys et al., where the frequency of CD5 expression in CLL was very high (90% vs. 98%) [16, 17].

Considering the CD23 antigen, it was shown that it was expressed in the majority of CLL compared to non-CLL patients, which made it relevant for differential diagnosis of CLL. Our results are in the line with literature data suggesting that CD23 antigen is one of the most important markers for differential diagnosis between CLL and MCL [18]. Addressing this issue, DiRaimondo et al. demonstrated that CLL/$CD23^-$ variant was rare (6%) and that the majority of MBCL patients with $CD23^-CD5^+$ cells had MCL [18]. In these cases, the diagnosis of MCL has to be confirmed by cyclin D1 immunostaining on biopsy and/or by detection of chromosomal translocation t(11;14).

FMC7 antigen is also considered to be reliable marker for differential diagnosis of CLL, distinguishing CLL from other MBCN [5]. Our results support this finding showing that only 8% of CLL patients expressed FMC7, whereas it was expressed in all patients from non-CLL group. Furthermore, some studies have shown wide range of frequency of FMC7 positive CLL cases (12-30%) [13, 17].

CD79b is one of the B-cell receptor complex components [15] and represents the latest antigen that was incorporated into CLL scoring system [7]. The largest series of CLL so far published has provided the evidence that the membrane expression of CD79b is greatly diminished or even absent in the majority of the typical CLL [19, 20]. In our CLL group, CD79b was expressed with lower frequency compared to non-CLL group (57% *vs.* 96%), whereas $CD79b^{+low}$ expression pattern was detected in the majority of CLL patients, similar to the study by Garcia Vela et al. [20]. These characteristics make this antigen a good marker for differential diagnosis of CLL.

Another component of the B-cell receptor complex is sIg, which is used to determine monoclonality of B-cells by flow cytometry, defined according to the presence of sIg light chain restriction. Although the monoclonality was detected in 95% of our CLL patients, Ig light chains were undetectable at presentation in 5% of patients, representing an aberrant feature of CLL cells [2]. In our CLL group, the frequency of $sIg\kappa^+$ was higher than $sIg\lambda^+$ cases. Likewise, in the study by Matutes et al. [6], it was shown that sIg was expressed in 92% of CLL cases with similar distribution of $sIg\kappa^+$ and $sIg\lambda^+$ positive cases. Our results showed that the majority of CLL patients had significantly higher frequency of sIg^{+low} expression pattern compared to the non-CLL patients, what makes this antigen important for differential diagnosis of CLL.

Concerning CD38 antigen that regulates apoptosis and proliferation of B-cells [15], the frequency of expression was found to be low (32%) in our CLL patients. Similarly, the other studies reported lower frequency of CD38 antigen expression in CLL patients (20%-27%), where this antigen was presented exclusively as prognostic marker [21, 22, 23].

Based on our results, which determined the value of each explored antigen for differential diagnosis of CLL, we could define the specific immunophenotypic profile of CLL cells as follows: $CD19^+$ $CD20^{+low}$ $CD22^{+low}$ $CD5^{+high}$ $CD23^+$ $FMC7^-$ $CD79b^{+low}$ sIg^{+low} (Figure 2). Moreover, it is of note that only the combination of the aforementioned

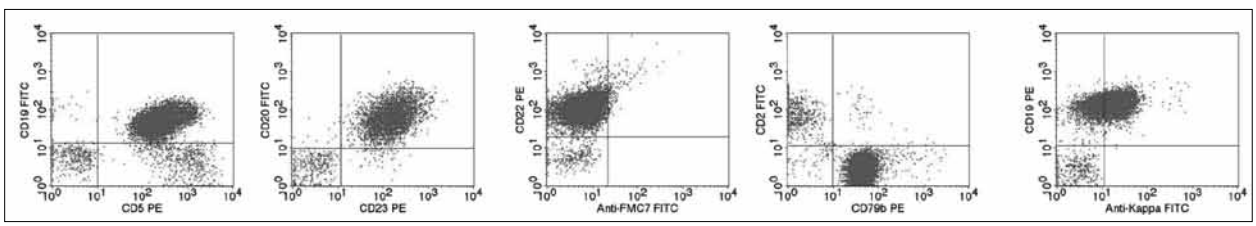


Figure 2. Flow cytometry biparametric dot plots that illustrate specific immunophenotypic profile of the chronic lymphocytic leukemia (CLL) cells

FITC – fluorescein isothiocyanate; PE – phycoerythrin

antigens can be used for reliable differential diagnosis of CLL, distinguishing it from other MBCN.

By applying the CLL scoring system to all our patients with MBCN, it has been shown that the majority of patients with the final diagnosis of CLL (97%) had high score values (5 or 4). In contrast, a small group of our CLL patients had score value of 3 or 2 points (3%), indicating that CLL diagnosis would have to be reconsidered in these cases. Addressing this issue, the rate of CLL with score 3 and 2 points was little higher (13%) in the study of Matutes et al. [6]. The majority of our patients with final diagnosis of non-CLL MBCN (96%) had lower score values (0 - 3), whereas only one patient had score of 4 points (4%). Similarly in the study of Matutes et al. [6], the incidence of non-CLL MBCN with score 4 points was 0.5%. Our results confirmed that CLL score was very practical and useful in differential diagnosis of CLL.

CONCLUSION

Our results have shown that specific immunophenotype which differentiates CLL from other MBCN is defined with the following marker combination - $CD19^{+}$ $CD20^{+low}$ $CD22^{+low}$ $CD5^{+high}$ $CD23^{+}$ $FMC7^{-}$ $CD79b^{+low}$ sIg^{+low}. This study has also shown that none of the explored antigens can separately discriminate CLL from other MBCN. It is only the combination of these antigens that makes possible to distinguish CLL from non-CLL MBCN. Furthermore, the CLL score value is of the utmost importance in the differential diagnosis of CLL based on the fact that the majority of CLL patients at presentation have score values of 5 or 4 points. Besides, all CLL patients at presentation have a significant increase of the circulating monoclonal B-cells, estimated by flow cytometry, which could be important for early diagnosis of disease.

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doi: 10.2298/SARH1404197D

Улога имунофенотипизације у диференцијалној дијагнози хроничне лимфоцитне леукемије

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КРАТАК САДРЖАЈ

Увод За постављање прецизне дијагнозе хроничне лимфоцитне леукемије (ХЛЛ) неопходна је примена имунофенотипизације проточном цитометријом ради поуздане диференцијације од других зрелих Б-ћелијских неоплазми (ЗБЋН).

Циљ рада Главни циљ овог рада је дефинисање имунофенотипског профила ХЛЛ ћелија.

Методе рада Узорци периферне крви 211 болесника са ЗБЋН су анализирани при дијагностиковању болести применом имунофенотипизације и проточне цитометрије.

Резултати Просечна вредност апсолутног броја Б-лимфоцита је била статистички значајно повећана у групи болесника са ЗБЋН у поређењу с вредношћу у контролној групи здравих испитаника (p<0,05). Моноклоналност Б-лимфоцита је утврђена код 96% болесника са ЗБЋН на основу експресије лаких ланаца мембранских имуноглобулина (*mIg*). Б-ћелијски антигени *CD19*, *CD20* и *CD22* експримирани су с веома високом учесталошћу код болесника са ХЛЛ, као и код болесника с осталим ЗБЋН. Код болесника са ХЛЛ учесталост експресије *CD5* и *CD23* антигена била је статистички значајно виша (p<0,0001), док је учесталост експресије *FMC7* антигена била статистички значајно нижа (p<0,0001), у односу на болеснике с осталим ЗБЋН. Болеснике са ХЛЛ су одликовали ниски обрасци експресије *CD20*, *CD22*, *CD79b* и *mIg* (p<0,0001), те висок ниво експресије *CD5* антигена (p<0,05). Корелација између коначне дијагнозе ЗБЋН и вредности ХЛЛ скора показала је да 97% болесника са ХЛЛ има високе вредности скора (5 или 4), док 96% болесника са осталим ЗБЋН има ниже вредности скора (0-3).

Закључак Резултати испитивања су показали да типичан имунофенотипски профил ХЛЛ ћелија, који може диференцирати ХЛЛ од других ЗБЋН, представља комбинацију следећих маркера: $CD19^{+}$ $CD20^{+nizak}$ $CD22^{+nizak}$ $CD5^{+visok}$ $CD23^{+}$ $FMC7^{-}$ $CD79b^{+nizak}$ mIg^{+nizak}. Вредности ХЛЛ скора од 5 или 4 поена указују на дијагнозу ХЛЛ с високом поузданошћу.

Кључне речи: хронична лимфоцитна леукемија; имунофенотипизација; проточна цитометрија

Примљен • Received: 09/05/2013 **Прихваћен • Accepted:** 18/06/2013