

Original article



## STUDY OF THE IMMUNOLOGICAL MARKERS CD49d AND CD38 IN EARLY-STAGE B-CLL PATIENTS

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### SUMMARY:

Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of B- lymphocytes into the blood stream, primary and secondary lymphoid organs as a consequence of defect in the apoptosis. Most frequently it affects people aged 67- 72. The greater part of CLL patients are in the early stage of the disease at the time of diagnosis, which gives a chance to delay the therapy. In 2008 the diagnostic criteria for CLL were revised. New prognostic and predictive factors were added in order to make the indications for starting the treatment more precise

The aims of our study were: to determine the frequency of monoclonal B-cell lymphocytosis (MBL) in the subjects with absolute lymphocytosis, to establish a relationship between the flow cytometric markers CD49d and CD38 in patients with early stages Rai O-II / Binet A. To distinguish the patients with B- CLL from MBL as well as to define the expression of immunological markers we used flow cytometric analysis of peripheral blood.

The results of our study have shown that a small number of patients met the criteria for MBL. Flow cytometric markers CD49d and CD38 associated with unfavorable prognosis were negative in most of the early stage patients.

**Keywords:** leukemia, integrin, stage,

### INTRODUCTION:

Chronic lymphocytic leukemia is a clonal disorder. It is characterized by accumulation of small B-lymphocytes ( 90% of cases) [1] The disease has a variable clinical course, and it is an ideal model for personalized medicine [2]

Since 1970 up to the present day reliable prognostic factors have been looked for in order an adequate assessment of the risk categories of patients to be made and consequently the right therapeutic regime to be chosen.

According to literature, the two Rai / Binet clinical staging systems alone are not able to predict the outcome of the disease as well as to discriminate patients with

the stable course of the disease.

In recent years, in the quest for a better understanding of the disease biology, new laboratory prognostic factors have been added, divided by most authors into four major groups: clinical, cytogenetic, serum and flow cytometric

The presented different results from the clinical studies on the prognostic value of the flow cytometric markers CD38 and CD49d led us to look for a correlation between the two markers and the early stage of the disease. According to some authors the different results related to the prognostic value of CD38 could be explained with the lack of standardized laboratory methods for the evaluation of the marker and its change during the course of the disease [3, 4]. Based on the study reports unlike CD38, the level of CD49 remains unchanged in the course of the disease which makes it reliable prognostic factor. [5]

### PATIENTS AND METHODS:

The cohort included 28 patients who were not treated and were followed-up for 24 months. All patients signed an informed consent to participate in the trial, which was approved by the local ethics committee.

To make B- CLL diagnosis, as well as to distinguish CD38 positive patients flow cytometric analysis of peripheral blood was carried out, using FAC Sort/ Becton-Dickinson/ and software product Cell Quest CD 45/ CD 14/ CD 19CD 20/ CD 5 / CD 22/CD 23/ CD 10/HLA-DR/ CD 25/CD 11c/ CD 11a/ CD38/CD 3 / CD 4/ CD 8 / CD 2 /s Ig D / s Ig G / s Ig M/kappa/ lambda. Fresh venous blood was drawn into sodium-heparin tubes, and the results were obtained within 2 hours. Leukocytes were analysed by using a dual-laser FACS Calibur cytometer (Becton Dickinson, Heidelberg, Germany) and Cell Quest Pro software (Becton Dickinson). Briefly, blood cells were stained with fluorescence-conjugated antibodies (FITC and PE). After lysis of erythrocytes (Lysis buffer; Becton Dickinson) and two washes, stained PBMC were re-suspended and fixed with CellFIX (BD Biosciences). Ten thousands of lymphocytes were selected in a forward scat-

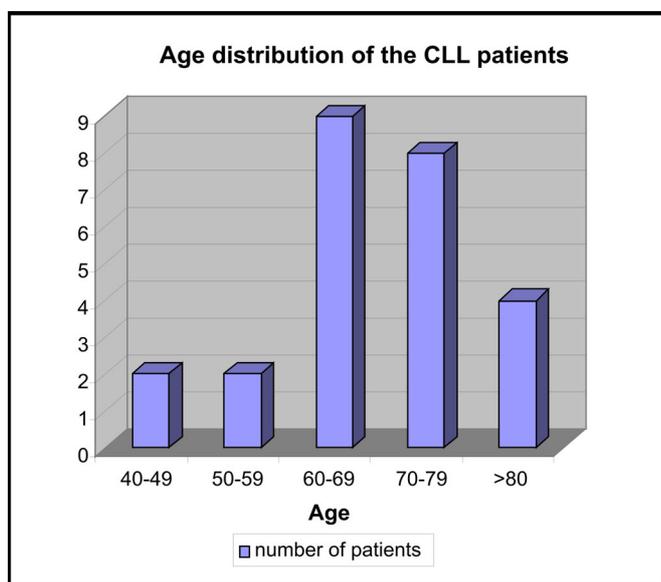
ter/side scatter (FSC/SSC) lymphocyte gate and saved together with the other leukocyte populations. Data are presented as a percentage of the lymphocyte gate. The cytometer was calibrated daily with appropriate single-stained samples for setting compensation and acquired data were analysed by FACSComp software©2007 Becton Dickinson. Fluorescence conjugated antibodies by Becton Dickinson Pharmingen™ were used to identify cell population CD19B-lymphocytes, CD49dPE, CD29 PerCP.

To stage the patients Rai staging system was used.

**RESULTS:**

28 patients aged 42 to 84 (mean 67) were included in the study. Of these, 64% (16/ 25) are men and 36% (9/ 25) are women. The distribution of patients by age is shown in figure 1.

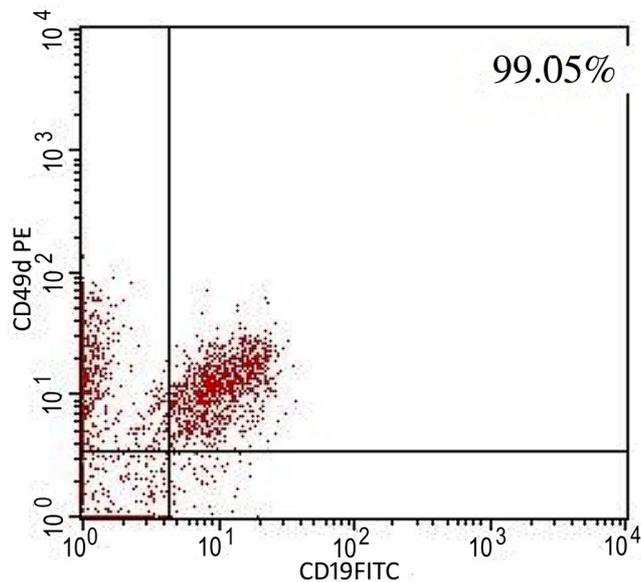
**Fig. 1.** Age characteristic at the diagnosis



Based on the revised IWCLL criteria, three of the Rai O-stage patients were reclassified as monoclonal B-cell lymphocytosis. Neither of the above mentioned patient hasn't report for family history for CLL. The other 25 responded to the diagnostic criteria for B-CLL. The malignant B cell population has showed immunophenotype CD5+,CD19+, CD20+ and CD 23+, specific for B- CLL. 14 (25) of the patients were asymptomatic without enlarged lymph nodes, spleen, liver and without constitutional symptoms. The only pathological expression of the disease was presenting absolute lymphocytosis more than 5G/l, evaluated by differential counting and flow cytometric analysis of peripheral blood. These patient were assessed as low risk patients, using Rai staging system at the time of the diagnosis. The rest of them 11(25) were in I or II clinical stage respectively [intermediate risk] due to the presence of absolute lymphocytosis, lymphadenomegaly, splenomegaly and missing constitutional symptoms. To determine the expression of CD49d it was evaluated in combination with CD 5 and CD 19 positive B- lymphocytes. The result was interpreted

as positive in cases when the expression of the immunological marker was higher than 30% of B- lymphocytes. In three of 25 patients CD49d was evaluated as positive. (figure 2)

**Fig. 2.** CD49d expression in positive B- CLL patient



As far as CD38 is concerned we accepted as a positive result, level of expression more than 30% in CD19 + cells. The levels of expression were more than 30% in two of our patients and only in one of them the two markers were positive. (The results are shown in table 1)

**Tabl 1.** Distribution of the patients according to the immunological markers CD38 and CD49d

| Marker/status             | CD38 | CD49d |
|---------------------------|------|-------|
| <b>positive</b>           | 1    | 2     |
| <b>negative</b>           | 23   | 22    |
| <b>number of patients</b> | n=24 | n=24  |

**DISCUSSION:**

In 2008, the B-CLL diagnostic criteria were corrected, it was assumed that cases of > 5 × 10<sup>9</sup> / L peripheral B-lymphocytes coexpressing CD5, CD19, and CD23 for more than three months correspond to diagnosis B – CLL [6].That led to the reclassification of part of the patients from O-stage into a separate group of so-called monoclonal B cell lymphocytosis. The definition includes asymptomatic patients with less than 5 x 10<sup>9</sup>/ L clonal B-cells in the peripheral blood [7]. According to the literature, the incidence of monoclonal B- lymphocytosis increases with age, and it is about 0.12 to 18% in the overall population [7]. In the our study 10% of the patients with leuko- and lymphocytosis met the MBL criteria, which is in accordance with results in the reports. Neither of them have evolved to be chronic lymphocytic

leukemia in the next 24 months.

CD49d is a surface marker expressed by B- lymphocytes, and it is alpha 4- integrin. It mediates cell-cell interactions, lymphocyte migration, invasion in the tissues by binding to fibronectin and VCAM-1 [8, 9, 10]. CD49d in combination with lipoprotein lipase, metalloproteases and chemokines take part in activation of cell proliferation in patients with B- CLL. The high level in patients with B-CLL is associated with an aggressive course of the disease and is considered to be one of the negative prognostic markers [11, 12]. In the three patients, two of which were in the second clinical stage and one in the first, the marker was positive. We can conclude by these results that a small number of B- CLL early stage patients have high level of CD 49 expression. Due to the absence of symptoms and progress of the disease treatment was not started.

CD38 is a transmembrane glycoprotein and it could be present in 1/4 of the B- CLL patients [13]. It is expressed by B- lymphocytes during the different stages of the cell maturation and differentiation [14]. In most of the scientific sources the levels of immunological marker more than 30% are considered positive and are related to a poor prognosis [15]. But in 2004 Tait D and co-authors quote data showing that the flow cytometric marker undergoes changes in the course of the disease in 10-25% of the cases. These facts as well as the absence of the standardized methods of evaluation of the immunological marker make it difficult to interpreted as a prognostic one [16]. For this reason, we chose a group that met the criteria - untreated patients, at an early stage of the dis-

ease (Rai 0, I, II). Out of a total of 25 patients, only two were CD38.

Unfortunately, the small number of cases did not allow a final conclusion on the existence of a link between the two flow cytometric markers in early-stage patients to be drawn. Two patients who were negative for CD38 and CD49d but with high levels of  $\beta$ 2-microglobulin after a period of 12, 15 months were in need of treatment due to a significant increase in leukocyte count, the emergence of new groups of lymph nodes and the symptoms of the disease. Pre-treatment FISH analysis showed del (13) (q14) in 18% of the interphase nuclei in the first one and 85% in the other patient.

#### CONCLUSIONS:

We support the fact that the two staging systems Rai and Binet are an important part of the risk- adapted therapy [17]. The interpretation of the immunological markers should be in combination with the rest of the markers connected to the biology of the disease such as mutations in IgVH genes and cytogenetic abnormalities. Due to small number of the patients in our study, we didn't find correlation between the flow cytometric markers CD38, CD49d and the time to first therapy. Further, long-term clinical studies comprising a larger number of untreated patients with different stages, would make it possible to assess the prognostic value of CD38 and CD49d and their influence on the optimal time of treatment of the patients.

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*Please cite this article as:* Popova V, Blazheva S, Lukanov T, Naneva S, Tzvetkov N. Study of the immunological markers CD49d and CD38 in early-stage B-CLL patients. *J of IMAB*. 2018 Jan-Mar;24(1):1883-1886.

DOI: <https://doi.org/10.5272/jimab.2018241.1883>

Received: 20/06/2017; Published online: 24/01/2018



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