

# The analysis of L-selectin expression intensity (CD62L) on B-lymphocytes in B-cell chronic lymphocytic leukemia cells (B-CLL)

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## Abstract

Decreased percentage of B cells expressing L-selectin in B-cell chronic lymphocytic leukemia (B-CLL) is known from literature. The purpose of our studies was to compare the intensity of L-selectin expression on subpopulation of L-selectin positive B-lymphocytes in untreated and treated patients with B-CLL.

The lymphocytes were obtained from peripheral blood of 70 patients suffering from B-CLL (40 untreated and 30 after the treatment) and 20 healthy blood donors. The percentage of positive cells and intensity of CD62 L-selectin expression (RFI) were measured on CD19+ B lymphocytes using tri-color fluorochrom labeled monoclonal antibodies and flow cytometric analysis.

The lower percentage of L-selectin positive B-cells paralleled with considerable decrease of this selectin intensity was observed in patients with B-CLL in comparison to healthy subjects. Both these changes may contribute to the impaired adhesion and migration of B-cells in B-CLL resulting in the accumulation of these cells in the peripheral blood. In a group of patients entirely responding to the treatment the tendency toward normalization of both measured parameters has been observed.

**Key words:** L-selectin, B-cells, B-CLL.

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## Introduction

The migration of lymphocytes from blood, through peripheral vessel endothelium, to lymph node is regulated by the expression of adhesion molecules. The expression of L-selectin on lymphocyte surface plays a key role during the first phase of adhesion to the endothelium [1]. Published data document decreased percentage of B cells expressing CD62L (L-Selectin) in B-CLL in comparison to healthy donors [2-5]. A loss of L-selectin expression has also been observed on B-cells isolated from patients with lymphoma (e.g. MALT lymphoma [6, 7]).

Using microarray technique, the decreased activity of genes responsible for a synthesis of surface adhesion molecules, including L-selectin, in B-CLL has been reported [8]. Therefore, it is possible that B-cells from B-CLL patients exert impaired expression of L-selectin. However, literature search revealed no data on the intensity of L-selectin

expression (a direct indicator of density of surface expressed L-selectin) on the subpopulation of B-cells that have not lost CD62L molecules.

The purpose of our studies was to compare the intensity of L-selectin expression on subpopulation of L-selectin positive B-lymphocytes in untreated and treated patients with B-CLL.

## Method

### The object of research

We have investigated the lymphocytes obtained from peripheral blood of 70 patients suffering from B-cell chronic lymphocytic leukemia:

- ▶ 40 patients before treatment (BT), without validated contact with cytostatics; Rai 0 – IV; age 65.7±9.8; 22 men, 18 women,

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- ▶ 30 patients after treatment with cytostatics (AT):
  - a) 20 patients with a partial response (AT-PR) after treatment with Chlorambucil or Chlorambucil and Cladribine (2-chlorodeoxyadenosine, 2CdA); Rai 0 – IV; age 60.1±13.1; 14 men, 6 women,
  - b) 10 patients with a complete response to curing (AT-CR) after a Cladribine therapy – as a first – line therapy; age 58.9±5.3; 4 men, 6 women.

The diagnosis of B-CLL based on clinical morphological and immunological criteria (reactivity with monoclonal antibodies: CD5, CD19, CD20, CD23, κ or λ light chain). The clinical stage of disease was evaluated according to the Rai system [9]. The partial response (PR) and the complete response (CR) were defined in accordance with determined by an NCI-Sponsored Working Group criteria [10]. The control group comprised 20 healthy donors.

**Procedure**

Leukocytes were marked with fluorochroms labeled monoclonal antibodies directed against antigens CD62L, CD5, CD19 in tricolor fluorescence scheme. The adequate isotype controls were included in all series. After incubation with antibodies, erythrocytes were lysed using the reagent from Ortho. Cytometric analyses were performed directly after labeling (Cytoron Absolute Ortho flow cytometer). 10,000 cells were counted. The analysis of the material was carried out using the Immunocount II software (Ortho).

The expression of antigen was estimated as a percentage of positive cells (i.e. the percentage of CD62L-positive cells among the population of CD19+ cells). The intensity of

CD62L-antigen expression was found by relative fluorescence intensity (RFI) using mean channels of fluorescence for the antigen and adequate value of isotype control according to Miwa equation [11]:

$$RFI=10 \times [(3.5 \times \text{mean channel of the sample}) \div 255] - 10 \times [(3.5 \times \text{mean channel of the control}) \div 255]$$

(Mean channel of CD62L fluorescence intensity on B-cells in the analyzed sample and the control isotype sample, 255 – number of fluorescence channels in the Cytoron Absolute Ortho cytometer).

An example of B-lymphocytes stained with anti L-selectin antibody from patients and healthy donors is presented in Fig. 1. Obtained values are presented as means ±SD and ±SE. The statistical analysis was made by Mann Whitney U Test (p<0.05 were considered as statistically significant).

**Results**

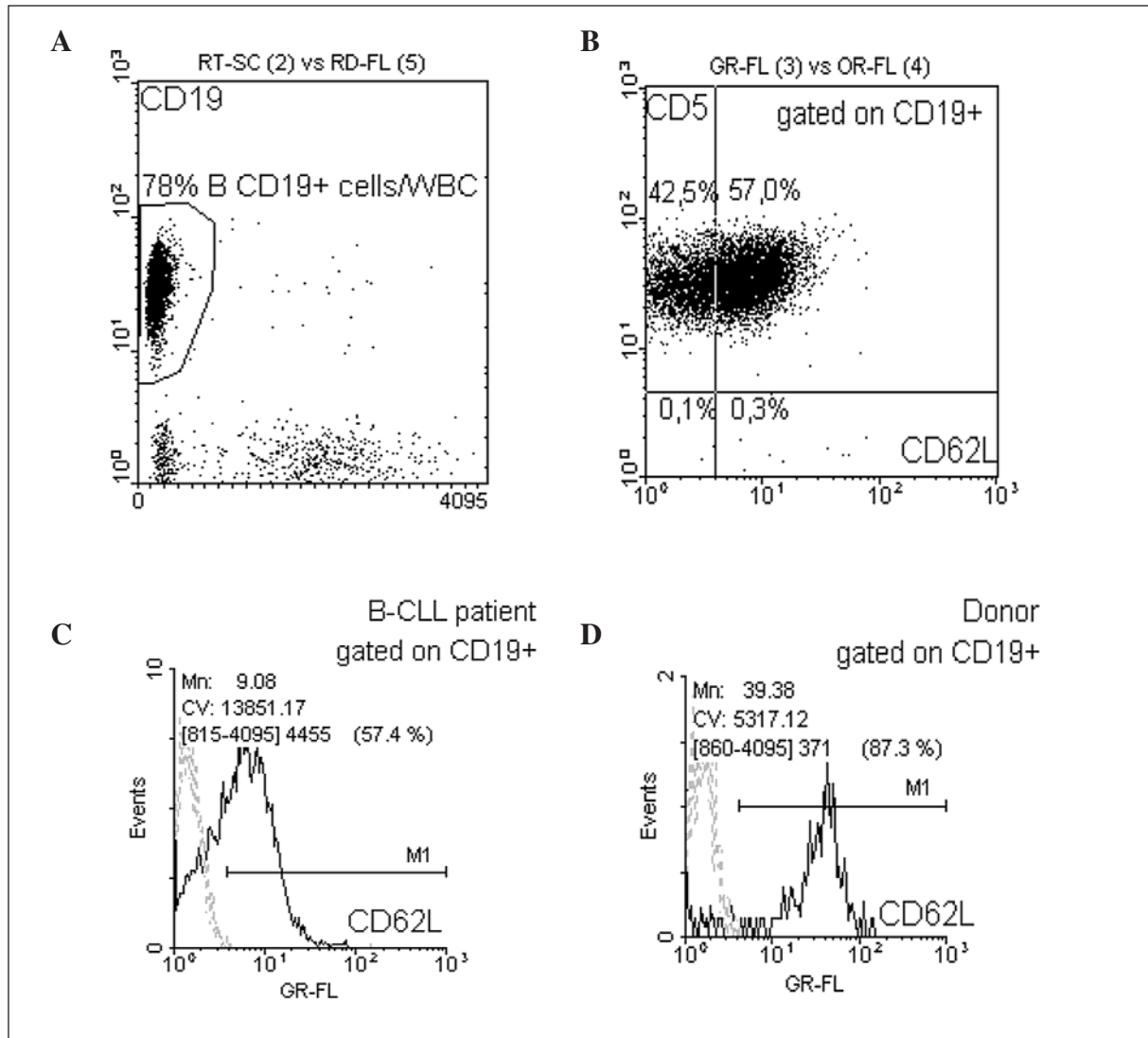
**▶ Changes of percentage of B-cells with CD62L-expression**

In peripheral blood the percentage of B CD19+ lymphocytes with CD62L expression below the lower limit of control varied from 10% to 48% of tested patients respectively in CR and PR groups (Fig. 2).

The considerable differences in the number of B-cells with CD62L expression were observed among patients from BT and AT-PR groups. Therefore, wide range of values and consequently high values of standard deviations are reported (Tab. 1).

**Table 1.** The characteristic of BCD19+ cells from peripheral blood of patients suffering from B-CLL before treatment (BT), after treatment with a partial response (AT-PR) and a complete response (AT-CR) to the treatment. p – a p value (Mann Whitney U Test), a statistically significant difference compared to the control group

		Donors n=20	BT n=40	AT-PR n=20	AT-CR n=10
CD19 + cells as a % of lymphocytes	mean (±SD)	11.4 (±2.6)	87.8 (±8.6)	77.4 (±19.9)	13.8 (±13.1)
	min-max	7.5-16.5	56.0-98.7	19.9-32.9	2.2-46.0
	p		<0.0001	<0.0001	0.52
% CD5 cells in B CD19 + cells	mean (±SD)	4.9 (±2.5)	83.3 (±16.4)	88.8 (±15.3)	33.0 (±31.8)
	min-max	2.2-10.5	38.8-99.9	57.6-100	3.0-94.8
	p		<0.0001	<0.0001	0.0001
% CD5 + CD62L + cells in B CD19 + cells	mean (±SD)	4.7 (±2.5)	77.5 (±19.7)	71.5 (±18.7)	25.0 (±25.9)
	min-max	1.8-10.0	29.7-99.1	32.1-98.0	3.0-92.8
	p		<0.0001	<0.0001	0.0014
% CD62L + cells in B CD19 + cells	mean (±SD)	93.8 (±5.0)	87.4 (±17.7)	80.7 (±20.9)	86.2 (±15.6)
	min-max	85.0-98.6	29.7-99.9	31.1-99.7	50.8-97.0
	p		0.80	0.16	0.059



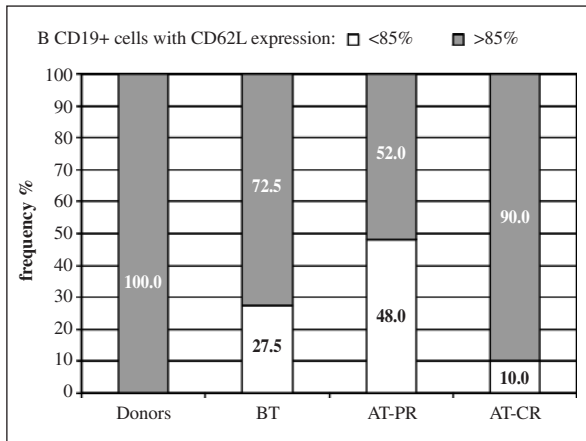
**Fig. 1.** An example of tricolor cytometric analysis of B CD19+ cells in peripheral blood in patient suffering from B-CLL (cytograms A and B). Cells were labeled with antibody anti-CD19 RPE-Cy5 in a combination with FITC-anti-CD62L and with RPE-anti-CD5. CD19+ cells were gated in side scatter vs fluorescence RPE-Cy5 (A). Among CD19+ four populations of cells are visible (B). The histograms show examples of CD62L expression on B CD19+ cells in a patient suffering from B-CLL (C) and healthy donor's blood (D). The gray line – the negative control. The black line – cells after staining with anti-CD62L antibody

#### ► The analysis of CD62L expression intensity on B CD19+ cells

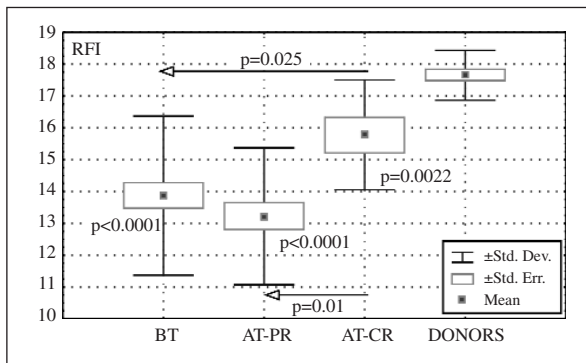
The preliminary comparison of CD62 L-selectin expression intensity in BT and AT-PR groups of patients (n=10 in each group) revealed no significant differences between B CD19+CD5-positive (leukemic) cell and B CD19+CD5-negative cell subpopulations ( $p>0.05$ ). In the BT group the value of CD62L RFI was  $13.7\pm 2.3$  vs  $15.3\pm 2.1$ ; in the AT-PR group –  $15.3\pm 1.9$  vs  $16.2\pm 2.5$ . Thus, in the next analysis of CD62L expression intensity in these groups was done using entire B CD19+ cell population.

L-selectin expression intensity on B CD62L+ cells in B-CLL was considerably lower than L-selectin expression intensity on normal control B-lymphocytes in peripheral blood ( $p<0.0001$ , Fig. 3). It was noticed that in the majority of patients from BT and AT-PR groups (respectively: 65% and 76%) a subpopulation of B-lymphocytes exerted values of RFI CD62L below the minimal value of L-selectin expression intensity on B CD19+CD5+ lymphocytes in healthy donors (RFI=15.4).

In the AT-CR group the mean value of expression intensity of CD62L on B CD19+ lymphocytes (RFI= $15.8\pm 1.7$ ) was closer to control values (RFI= $17.6\pm 0.8$ ) than in untreated



**Fig. 2.** The frequency of cases with a proper (>85%) and a diminished (<85%) percentage of B CD19+ with CD62L+ expression in peripheral blood of patients suffering from B CLL before treatment (BT), with a partial response (AT-PR) and a complete response (AT-CR) to the treatment



**Fig. 3.** RFI of CD62L on B CD19+ cells in peripheral blood of patients suffering from B CLL before treatment (BT), with a partial response (AT-PR) and a complete response (AT-CR) to the treatment.

p – a p value (Mann Whitney U Test), a statistically significant difference compared to the control group and between patients groups (arrows)

patients or those with a partial response to the treatment (Fig. 3). Because in some patients (7/10) from the AT-CR group the percentage of B CD5+ cells was lower than in other groups and became normalized, the analysis of CD62L expression intensity was carried out on separately gated B CD5-positive and B CD5-negative lymphocytes. In the healthy donors' peripheral blood RFI CD62L index was  $16.2 \pm 0.7$  for B CD5+ and  $17.5 \pm 0.6$  for B CD5-, and the difference was statistically significant ( $p=0.0013$ ). In the AT-CR group the mean value of RFI CD62L on B CD5-positive cells was  $14.9 \pm 2.4$ , on B CD5-negative cells ( $\text{RFI}=16.5 \pm 1.4$ , and did not differ considerably from control values (respectively:  $p=0.2$ ,  $p=0.09$ ).

## Discussion

The decrease in the percentage of B-cells with L-selectin expression in B-CLL has been demonstrated in literature [2-4], as well as in our patients. The observations carried out in this study indicate that these changes of L-selectin expression depend on many factors, including response to treatment.

Our key observation is that the decreasing of percentage of B-cells with L-selectin expression is accompanied with the lower CD62L intensity expression on the B-cells, that preserved this expression. Therefore, in patients with B-CLL, B CD62L+ cells possess less L-selectin than the control B-lymphocytes from healthy donors.

The reduction of L-selectin expression intensity in B-cells in CLL may be related to impaired glycosylation of this molecule, as described in literature [12]. However, it is most likely that alterations of expression and expression intensity of L-selectin in B-cells in B-CLL is linked with lower activity of genes responsible for CD62L-selectin expression [8].

Beside a lower susceptibility to apoptosis, the diminished percentage of B CD62L+ cells in B-CLL, may influence the accumulation of cancer B-cells in peripheral blood [13]. This notion is based on the impaired adhesion and migration of these cells through an endothelium related to a lack of L-selectin expression [5, 14].

Described in this study declined L-selectin expression intensity on B CD62L+ cells, may also contribute to the process of accumulation of leukemic B-lymphocytes in peripheral blood of patients suffering from chronic lymphocytic leukemia, untreated or with a partial response to the treatment.

The AT-CR group of patients was treated with 2CdA. The introduction of new purin analogues in the treatment of proliferation of low malignancy B-cells gives wider possibilities to achieve a complete clinical and hematological response, that is noticed in 29%-47% of patients suffering from B-CLL [15, 16].

The analysis of L-selectin expression and L-selectin expression intensity on B-cells in the AT-CR group of patients shows that both: the amount of B-cells with L-selectin expression and the CD62L expression intensity exert tendency to normalize. It is possible that a longer period of time is needed to normalize the selectin expression intensity.

To summarize: the decreased percentage of B-cells with L-selectin expression in B-CLL is associated with a significant decline of the expression intensity in B CD62L+ cells compared to control. Interestingly, the drop in the expression intensity in these cells is mostly pronounced in untreated patients or in patients with a partial response to the treatment. Both these changes could be related with an impaired adhesion and migration of B-cells in CLL, and as a consequence, their accumulation in peripheral blood. In the group of patients with a complete response to the treatment (CR), the percentage of B-cells

with L-selectin expression and CD62L expression intensity exert tendency to normalize.

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