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# *Expression of tumor necrosis factor (TNF) receptors on T cells from patients with B-cell chronic lymphocytic leukaemia**

Ekspresja receptorów czynnika martwicy nowotworu (TNF) na limfocytach T pacjentów z przewlekłą białaczką limfatyczną B-komórkową

## INTRODUCTION

Tumor necrosis factor (TNF) is a pleiotropic cytokine that is a major mediator of immunological and pathophysiological reactions. This cytokine is important for the growth and survival of the leukemic cells in B-cell chronic lymphocytic leukemia (B-CLL) [7].

The wide range of TNF activities is mediated by the presence of two distinct types of TNF receptors, termed TNF-R1 (p55, CD120a) and TNF-R2 (p75, CD120b) with a molecular mass of 55 kDa and 75 kDa, respectively [4,12,14]. TNF-R1 is the receptor responsible for mediating most of the effects exerted by TNF such as cytotoxicity, prostaglandin synthesis and cellular adhesion [14]. One of the most widely studied aspects of TNF-R1 function is as a primary mediator of TNF induced cell-death, only TNF-R1 possesses the death homology domain [4]. TNF-R2 transduces proliferation signals to malignant B cells in B-CLL following binding to TNF [12]. All biological activities attributed to TNF-R2 can also be exerted by TNF-R1 [13]. Unlike TNF-R1 it has no cytoplasmic death domain, but it can directly bind TRAF1 and TRAF2 (TNF-R2 associated factors 1 and 2), and in this way give activation signals and modulator activities of TNF-R1 [6].

B-CLL is a clonal lymphoproliferative disorder accompanied by a variety of immunoregulatory abnormalities [7]. These included impaired humoral and cellular im-

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mune response in B-CLL patients, which appears to involve defects in the function of T cells or an imbalance of T-cell subpopulations [2]. In the past, various abnormalities of the nonmalignant T-cell population in B-CLL have been reported, such as impaired proliferative response to mitogens, abnormal cytokine responsiveness, defects in helper and supressor activities, an abnormal composition of T-cell subsets in B-CLL patients [2, 5, 9].

In this study, we examined the expression of TNF receptors on the surface T cells from patients with B-CLL. The aim of our study was to assess the expression of TNF receptors is associate with stage of disease.

## MATERIALS AND METHODS

***Patients***

Peripheral blood (PB) and bone marrow (BM) were obtained from thirty four newly diagnosed, untreated patients with B-CLL (23 men and 11 women, aged from 49 to 79 years). Diagnosis of B-CLL was made on the basis of clinical, morphological and immunological criteria. Patients were graded according to Rai et al. staging system [10] as follows: stage 0 (13 cases), stage I (8 cases), stage II (6 cases), stage III (1 case), stage IV (6 cases). The control group consisted of peripheral blood from seventeen healthy donors and bone marrow from seven healthy donors.

***Reagents***

Fluorescein (FITC) or phycoerythrin (PE) conjugated monoclonal antibodies (MoAbs) were used in this study. TNF-R1 FITC (Clone 16803.161, IgG1) and TNF-R2 FITC (Clone 22235.311, IgG2a) were obtained from R&D Systems (Germany); CD3 PE (Clone UCHT1, IgG1), from Dako (Denmark). Isotype-specific anti-mouse IgG1 or IgG2a was purchased from Becton Dickinson (USA). FcR-Blocking Reagent (human IgG) was obtained from Miltenyi Biotec (Germany).

***Cell preparation***

Mononuclear cells were separated by density gradient centrifugation on Lymphoprep (Nycomed, Norway) for 25 minutes at 400 g at room temperature. Interphase cells were removed and washed twice with phosphate-buffered saline (PBS).

***Analysis of TNF receptors***

To minimize FcR-mediated MoAb binding, cells were stained in the presence of FcR-blocking reagent containg human IgG. After that cells ($1x10^5$) were incubated for 30 minutes at 4°C with 10$\mu$l of specified MoAb against surface antigen (CD3) and 10 $\mu$l of anti-TNF-R1 or anti-TNF-R2. Following this incubation, unreacted reagents were removed by washing the cells in PBS supplemented with 0.5% bovine serum albumin (BSA) and analysed flow cytometry. The data were shown as the percentages among the CD3+ cells.

***Flow cytometric analysis***

Samples were analysed by two-color flow cytometry technique using Becton Dickinson FACSCalibur instrument. A minimum of 10,000 events were acquired and analysed using CellQuest software. In these experiments, the mean percentage of cells

expressing TNF receptors was analysed. Furthermore, the level of expression (the number of receptors expressed on cell), indicated by the mean fluorescence intensity (MFI) was analysed.

***Statistical analysis***

The Wilcoxon paired test was used to compare results in peripheral blood and bone marrow. Mann–Whitney U test was applied for the statistical comparison results between patients with B-CLL and healthy donors. Spearman's rank test was used to assess the relationship between expression of TNF receptors and the stage of disease. Differences were considered as statistically significant when the p-value was $<0.05$. Summary statistics are given as the mean ± SD.

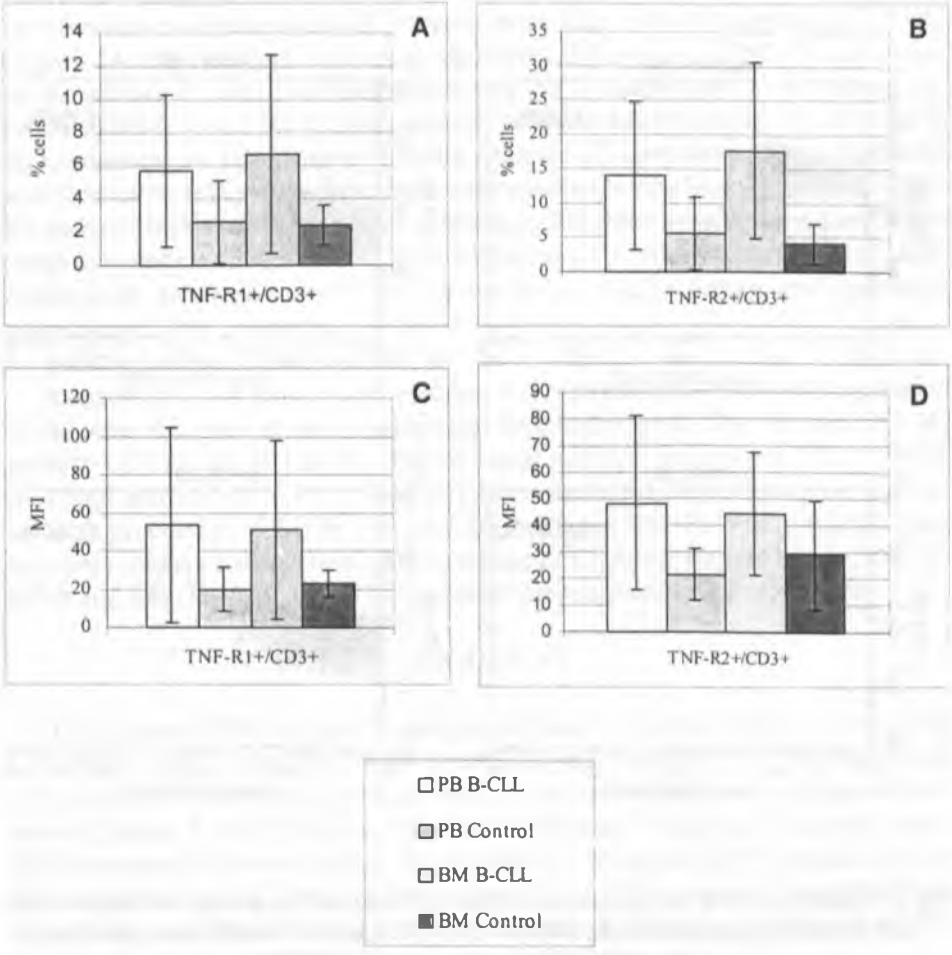


Fig. 1. TNF-R1 and TNF-R2 expression on T lymphocytes in patients with B-CLL and healthy controls. (A,B) percentage of T lymphocytes with TNF-R1 or TNF-R2, (C,D) mean fluorescence intensity (MFI) of TNF receptors

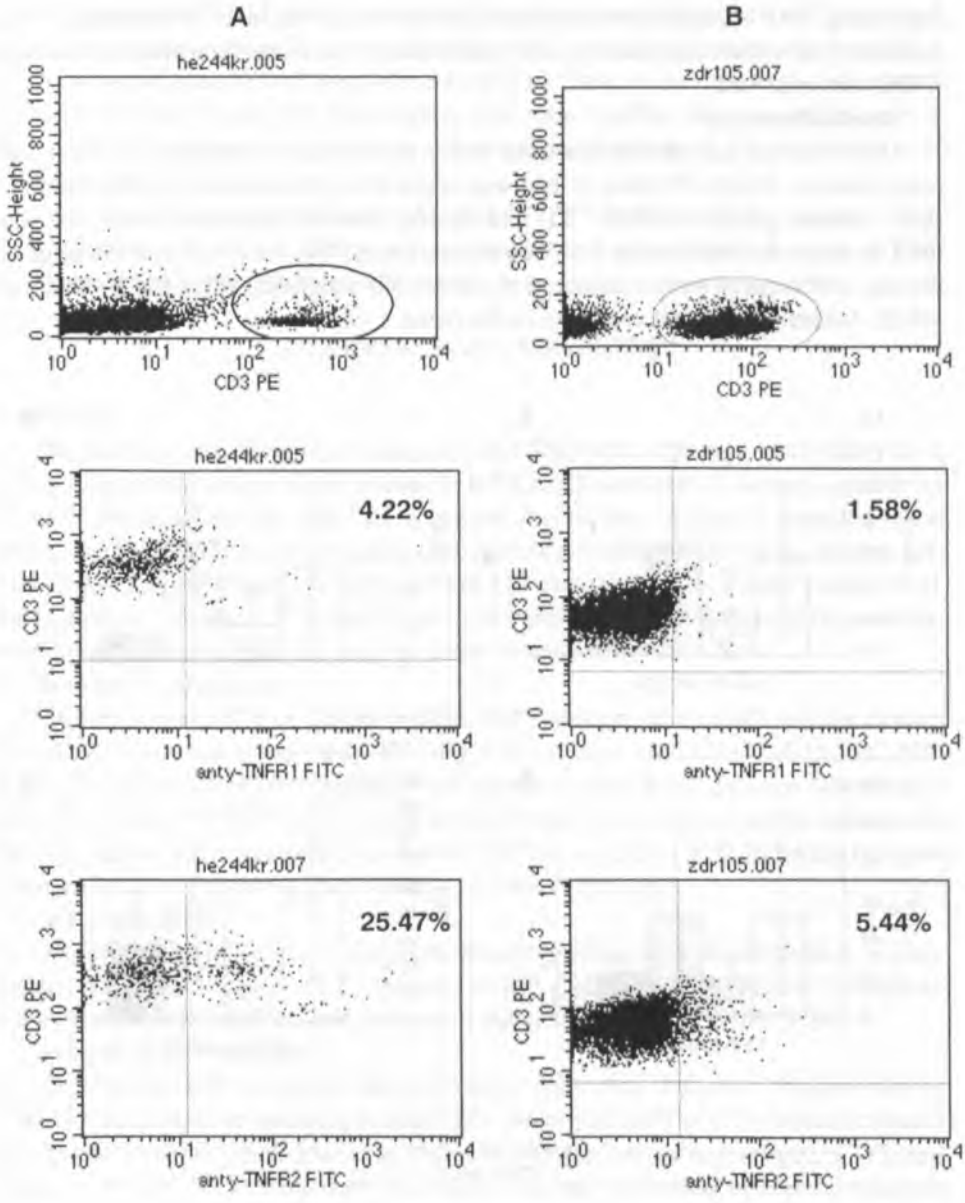


Fig. 2. TNF-R1 and TNF-R2 expression on CD3+ cells in a B-CLL patient (left column – A) and in a healthy individual (right column – B). Percentages in the right upper guadrant indicate the CD3+ cells positive for TNF-Rs.

## RESULTS

Peripheral blood and bone marrow T cells from patients with B-CLL expressed both TNF receptors. Statistical analysis revealed that the mean percentage of T cells positive for TNF-R2 (14.01±10.92%) from PB was significantly higher than that of T cells positive for TNF-R1 (4.59±5.69%) ($p<0.0001$). Likewise in BM the proportion of TNF-R2+/CD3+ cells (17.69±12.96%) was significantly higher than TNF-R1$^+$/CD3$^+$ (6.71±5,97%) ($p<0.0001$) (Figure 1A, 1B).

Figure 2 shown the expression of TNF receptors on CD3+ lymphocytes from individual samples of B-CLL patient and healthy volunteer. In PB and BM we detected statistically significant higher percentage of TNF-R2$^+$/CD3$^+$ cells in patients than in control groups ($p<0.003$). However we did not find a statistically significant difference between patient and control groups in percentage of T cells expressing TNF-R1 (Figure 1A,1B). Although there were significant differences in TNF-Rs expression on the surface T cells. The results show that PB T lymphocytes from patients expressed TNF-R1 and TNF-R2 (respectively: 53.60±45.94 MFI and 48.39±32.84 MFI) more intensely in comparison with healthy volunteers (respectively: 19.86±11.69 MFI and 21.53±9.56 MFI), these differences were statistically significant ($p<0.02$ for TNF-R1 and $p<0.0006$ for TNF-R2). Nevertheless, in BM there were no significant differences in membrane expression of TNF-Rs between the two groups (Figure 1C,1D). Additionally we found no correlation between the stage of disease and membrane expression of TNF receptors.

***Relation between TNF-R1 and TNF-R2***

Expression of one type of TNF receptor was markedly correlated with expression of the other receptor. When we compared the percentage of TNF-R1 and TNF-R2 positive CD3+ cells in PB and BM we found positive correlations (PB: $R=0.73$; $p<0.0001$ and BM: $R=0.81$; $p<0.00001$). Likewise we detected positive correlations between proportion of T cells with TNF-R1 in PB and BM ($R=0.45$; $p<0.02$). Similary correlations we shown between percentage of T lymphocytes presenting TNF-R2 in PB and BM ($R=0.45$; $p<0.02$). Representative data are shown in Figure 3.

## DISCUSSION

The action of TNF requires binding to specific cell surface receptor. Thus, TNF sensitivity could be controlled at the level of receptor expression [3]. Scheurich et al. [11] have shown that TNF$\alpha$ enhances both proliferative and functional capacities of normal human T cells. They also reported that resting T lymphocytes do not express TNF receptors. However, similar to interleukin 2 receptors, TNF receptors are induced upon activation. Similarly, Owen-Schaub et al. [8] have not observed TNF binding to fresh, unstimulated PB T lymphocytes. They reported that expression of TNF receptors on human PB T cells is activation dependent.

In our study the proportion of CD3+ cells expressing TNF-R2 was higher in patients than in normal controls. Likewise, we detected significant differences in mean

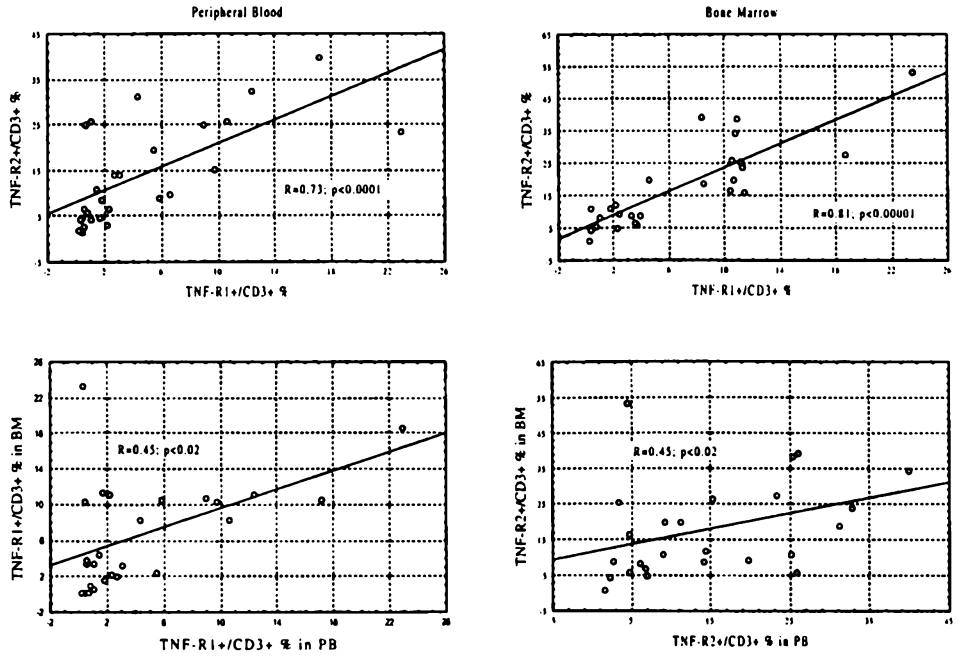


Fig. 3. Correlations between expression of TNF-R1 and TNF-R2 on T cells from PB and BM

fluorescence intensity (MFI) of TNF receptors between both groups. The results show that T lymphocytes from patients with B-CLL expressed TNF-R1 and TNF-R2 more intensely in comparison with healthy donors. The patients included in our study did not have any clinical sign of bacterial or viral infections as a cause of activation of T cells.

Although the neoplastic process in B-CLL involves the B lymphocyte compartment, phenotypic and functional defects in T lymphocyte population implicate their possible role in the pathogenesis of the disease [1]. In B-CLL, an increased absolute number of circulating T cells has been observed [2, 7]. Cooperation of T cells with accesory cells is required for several aspects of immunological activation. As accesory cells in B-CLL patients are predominantly composed of malignant B cells, the activation of T cells might result in a biologic response differing from that observed in normal individuals [2]. Although we did not find correlations between the stage of disease and expression of TNF receptors our results may confirm functional abnormalities of the non-malignant T cells and that the circulating T-cell compartment is dysregulated in patients with B-CLL. On the other hand the higher number of T cells expressing TNF receptors in B-CLL patients may suggest that the activated T lymphocytes try to act against malignant cells.

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## STRESZCZENIE


Celem naszych badań była ocena expresji receptorów czynnika martwicy nowotworu TNF-R1 (p55, CD120a) i TNF-R2 (p75, CD120b) na limfocytach T krwi obwodowej i szpiku. Badaniami objęto 30 nieleczonych chorych z B-PBL i 17 zdrowych dawców. W przeprowadzonych badaniach stwierdziliśmy statystycznie istotnie wyższy odsetek limfocytów CD3+ wykazujących expresję TNF-R2 w grupie pacjentów w porównaniu z grupą zdrową. Nie stwierdziliśmy jednak istotnych różnic w odsetku limfocytów T wykazujących expresję TNF-R1 pomiędzy badanymi grupami. Wykazaliśmy natomiast statystycznie istotne różnice w średniej intensywności fluorescencji (MFI). MFI było istotnie wyższe u pacjentów z B-PBL w porównaniu z grupą kontrolną. Wykazane różnice w expresji TNF-R1 i TNF-R2 pomiędzy grupą pacjentów i grupą kontrolną mogą potwierdzać anomalie limfocytów T u chorych na B-PBL oraz ich udział w patogenezie B-PBL.