INVESTIGATION OF ZAP-70 EXPRESSION IN THE PERIPHERAL BLOOD OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA USING FLOW CYTOMETRY

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Chronic lymphocytic leukemia (CLL) is the most common form of adult leukemia in the Western world. Two main subtypes of the disease had been previously defined, an indolent form and a more progressive variant.

ZAP-70 is a tyrosine kinase that plays a major role during the early phases of TCR signaling. Interestingly, ZAP-70 is expressed by the leukemic B-cells in a subgroup of CLL patients and has been shown to correlate with a worse clinical prognosis. Although the flow cytometric analysis of ZAP-70 expression in CLL was described more than a decade ago, it is still not included in the diagnostic panel of CLL patients in Pécs due to technical difficulty.

Our main goal was to establish a reliable and reproducible flow cytometric method that could later be introduced into diagnostics. We also wanted to examine the correlation between ZAP-70 expression and the clinical parameters to see if the analysis has any prognostic value.

We performed our experiments on the peripheral blood of CLL patients and used the samples of healthy donors as controls. First we used fluorescently labeled anti-CD5 and anti-CD19 antibodies to distinguish the different lymphocyte subgroups, then performed hemolysis and fixation. We permeabilized the cell membranes with saponin and used ALEXA 647 conjugated anti-ZAP-70 antibodies for the intracellular labeling of ZAP-70 kinase. The samples were then analyzed using a FACS CANTO II flow cytometer. Cells which had higher fluorescence intensities than the autofluorescence of normal B-cells were considered ZAP-70 positive. The cut-off point of ZAP-70 positivity in the patients was defined as 20 percent of leukemic cells based on the literature.

Our experiments took place between Dec. 2013 and Sept. 2014. During this period 25 patients have been tested; five among them on more than one occasion with reproducible results. 40% (n=10) of the patients were found ZAP-70 positive which is similar to the results of previous studies. The median elapsed time between the diagnosis and the experiments was 7 years in the ZAP-70 positive and 8.3 years in the negative group. There was no significant difference regarding the ratio of treated patients (46% in the negative vs. 40% in the positive group, respectively), however, the median time to treatment in the negative group was longer (7.16 years) than in the positive (4.25 years). Positive patients close to the threshold seemed to belong to the indolent clinical subtype. Increasing the threshold of positivity to 30% of cells reflected better the progressivity, as in this case treatment was necessary in 57% of the positive and 39% of the negative patients. Yet, the final cut-off point of positivity still remains to be defined after further clinical evaluation.

Besides flow cytometric analysis, the confirmation of our results on ZAP-70 expression with Western-blot and PCR is in progress, together with the examination of the correlation with the clinical parameters in more detail.

Poszterként szeretnénk bemutatni, klinikai jellegű.