

3 SUMMARY

Introduction: Chronic lymphocytic leukaemia displays variable clinical course in an individual patient. For this reason, it is essential to estimate prognostic factors in any patient.

Aims: To evaluate the prognostic value of the expression of ZAP-70 in malignant cells of chronic lymphocytic leukaemia patients by flow cytometry.

Patients and Methods: This study was approved by Ethics Committee of Medical School and University Hospital in Hradec Králové. This study was supported by Ministry of Health, Czech Republic, research projects IGA NR/8373-3/05, MZO 00179906. Total of 138 patients, 94 males, 44 females, median of age 59 years, were investigated. The expression of ZAP-70 molecule was determined by flow cytometry using antiZAP-70 monoclonal antibody (clone 1E7.2). Two different approaches were utilized, either based on isotypic control or mean fluorescence intensity (MFI) method. The stability of the expression of ZAP-70 molecule in the course of disease was followed in 44 patients. ZAP-70 expression was correlated with selected clinical parameters, expression of CD38 molecule, and mutational status of IgVH, respectively.

Results: Compared to isotypic control approach, MFI method seems to be superior to identify patient's prognosis in terms of "time to treat" period and survival of patients. Based on these findings, MFI method was only used in combined and multi parameter analyses. Both, the period without treatment ($p=0.0003$), and overall survival ($p=0.001$) were lower in ZAP-70 positive patients compared to ZAP-70 negative patients. Combined ZAP-70 expression and mutational status of IgVH, we found, that the longest time to treat ($p=0.0001$) and overall survival ($p=0.008$) revealed patients with ZAP-70 negativity and mutated IgVH genes. Combined ZAP-70 expression and CD38 expression, we found, that the longest time to treat ($p=0.0001$) and overall survival ($p=0.047$) revealed patients with both ZAP-70 and CD38 negativity.

If the only expression of ZAP-70 was included in multivariate analysis the threshold significance was reached ($p=0.053$). The only other followed parameter with significant prognostic value was the age of patient ($p=0.004$).

The significant changes in the expression of ZAP-70 molecule in the natural course of disease were found in 15 patients using isotypic control method and in 9 patients using MFI method. Majority of ZAP-70 expression changes into positivity was associated with unmutated IgVH status and disease progression or relapse.

Conclusions: The determination of ZAP-70 expression in CLL B cells was found by us as a clinically relevant parameter with adverse predictive value to precise individual risk in CLL patients, especially in those patients with the most favourable prognosis. We found that the determination of ZAP-70 molecule is of prognostic value in patients with no access to modern molecular genetic prognostic parameters. Our findings, that the changes in the expression of ZAP-70 into the positivity in the course of disease, are associated with either disease progression or relaps of disease seems to be original. It could be concluded from this study that serial ZAP-70 determination could identify CLL patients with progression of the disease.

Keywords: chronic lymphatic leukaemia, ZAP-70, CD38, prognosis