Genetic markers useful in the diagnosis, prognosis, and personalized therapy of patients with chronic lymphocytic leukemia.

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Background: It is more and more evident that intratumoral genetic variability not only facilitates clonal evolution and leukemic progression but also provides the basis for the development of therapeutic resistance. For this reason, the evaluation of these biomarkers is recommended before treatment initiation by most current guidelines for the management of chronic lymphocytic leukemia (CLL). The identification of new therapeutic targets and the development of targeted therapies can contribute to improving the quality of life and survival of patients with CLL.

Objectives of the study were the extensive genetic investigation of patients with CLL, risk stratification, and assessment of the prognosis of each patient based on the identified genetic anomaly.

General methodology: The study was conducted in several stages. In the first stage, CNV (copy number variations) analysis was performed using the MLPA technique with a kit containing specific probes for chromosomal regions frequently involved in the pathogenesis of CLL, as well as three probes for the *NOTCH1* c.7541-7542delCT, *SF3B1* c.2098A>G, and *MYD88* c.794T>C mutations. The genetic investigation continued by testing the mutational status of the *IGHV* gene and analyzing the *IL-10* (rs1800896 and rs1800872) and *TNF-α* (rs361525 and rs1800750) polymorphisms. The study included 239 healthy individuals with the same age and sex distribution (representing the control group).

Study 1: In this study, we investigated possible associations between CNVs and/or gene mutations and the biological and clinical characteristics, as well as the survival of CLL patients. Another aim of the study was to evaluate the effectiveness of the MLPA technique in the genetic investigation of CLL patients. The analysis revealed genetic alterations in 65% of the patients. The most frequent CNVs identified, in a decreasing order, were: del(13q), del(11q), trisomy 12, del(7p), del(14q), and dup(10q). Additionally, a patient with concomitant trisomy of chromosomes 12, 13, and 19 was identified. Somatic mutations (*NOTCH1* and *SF3B1*) were frequently observed in patients over 60 years old. The presence of the *SF3B1* mutation or multiple CNVs was associated with an increased white blood cell count. The *NOTCH1* mutation was associated with lymphadenopathy in over 78% of patients ($p<0.001$). The longest survival was observed in patients with isolated del(13q), followed by del(13q) associated with other CNVs, del(11q), *SF3B1* mutation, those without genetic alterations, trisomy 12, del(14q), *NOTCH1* mutation, and del(17p). Survival was also reduced in patients with multiple CNVs or CNVs associated with somatic mutations (especially *NOTCH1*).

Conclusions: *Elderly patients are more prone to developing somatic mutations, which may be attributed to age-related clonal hematopoiesis. Patients with CNVs and/or somatic mutations have an increased white blood cell count, which is considered an unfavorable prognostic factor. Somatic mutations (especially NOTCH1) and CNVs influence the early onset of clinical manifestations and could therefore trigger the clinical progression of the disease.*

Study 2: Somatic hypermutation (SHM) of the *IGHV* gene (variable region of the immunoglobulin heavy chain) is a natural biological process by which *IGH* gene rearrangements in B cells undergo genetic changes when exposed to antigens. Thus, the absence of hypermutation is associated with an unfavorable prognosis. Through this study, we aimed to classify patients into a low-risk or high-risk prognostic group based on the mutational status of *IGHV*. The absence of hypermutation was observed in 67.2% of patients, being more frequent in women and associated with reduced survival. The absence of hypermutation was associated with the presence of CNVs (p=0.009), and this combination of genetic alterations was associated with anemia, thrombocytopenia, and leukocytosis, but also had an impact on survival compared to the isolated absence of the mutation.

Conclusions: *The absence of hypermutation in the IGHV gene has prognostic value and is associated with reduced survival. Survival in patients without IGHV hypermutation was reduced in the presence of CNVs and/or associated somatic mutations.*

Study 3: The objective of the study was to investigate the effects of cytokine polymorphisms (SNPs) in the *IL-10* (rs1800896 and rs1800872) and *TNF-α* (rs361525 and rs1800750) genes. There were no significant differences in the genotype distribution between the analyzed groups, namely patients with CLL and the control group. Variant genotypes (including those with combined two, three, or four variant genotypes) did not impact the clinical characteristics or survival of the patients. None of the haplotypes were associated with the risk of developing CLL. Finally, we analyzed the concomitant presence of all genetic risk factors and observed that none of the patients showed a negative result for all the investigated risk factors; the vast majority of them (95.2%) associating multiple factors. The most frequent association was between unmutated *IGHV*, the variant allele of *IL-10* rs1800896, and CNVs, a combination identified as a combined risk factor for CLL.

Conclusions: *The presence of somatic mutations and the variant allele of IL-10 rs1800872, as well as the association of CNVs with the variant allele of IL-10 rs1800896, were identified as risk factors associated with reduced overall survival. IL-10 rs1800896 modulated survival in patients without IGHV hypermutation and with associated CNVs. Based on existing data, our study is the first to investigate the role of TNF-α rs1800750 and rs361525 polymorphisms in CLL.*

Study 4: The study describes data from the literature regarding genome editing techniques in leukemias, starting with meganucleases and leading up to the discovery of the CRISPR/Cas9 technique. The CRISPR/Cas9 system has been used more frequently for acute myeloid leukemia compared to other types of leukemia. Mutations in genes encoding cytokine signaling mediators, epigenetic modifiers, and transcription factors were edited, thereby reproducing the possible combinations of mutations observed in patients. Ongoing clinical studies are investigating the safety and efficacy of CRISPR-edited cells in the treatment of relapsed or refractory hematological malignancies.

General Conclusions: *Genetic testing holds a central position in the multidisciplinary management of CLL, providing valuable information for disease prognosis and useful guidance for therapeutic decisions. Techniques such as MLPA, IGHV gene testing, and the investigation of SNPs in cytokine genes have provided a deeper understanding of the genetic complexity of the disease and its implications for prognosis and treatment. For these reasons, extensive genetic testing of CLL patients is essential, especially considering that in most cases, multiple genetic risk factors are present, and the combination of several such alterations could have a significant impact on prognosis.*