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Original article

CLINICAL STUDY OF GENE EXPRESSION PROFILE AND SOME OF THE MOST COMMON CHROMOSOMAL ABERRATIONS IN UNTREATED B-CHRONIC LYMPHOCYTIC LEUKAEMIA PATIENTS

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ABSTRACT;

Aim: Chronic lymphocytic leukemia (CLL) is one of the most common leukemias in adults. Unlike myeloid and acute lymphoblastic leukemia, treatment can be delayed after diagnosis. Exploration of the unique biology of the disease has led to the identification of more than 35 prognostic factors reported in the literature. In practice, however, the number of validated clinical and biological prognostic factors is significantly smaller. The purposes of our study are: to investigate the distribution of untreated CLL- patients according to Binet staging as well as to analyse the frequency of one of the most common chromosomal aberrations in the group. To study the correlation between mutational status (determined by evaluation of lipoprotein lipase/ ADAM29 ratio), and the identified chromosomal aberrations in the group of 48 untreated patients.

Materials and methods: We studied 48 patients with a confirmed flowcytometric diagnosis B-CLL, using the 2008 IWCLL-NCI criteria. The interphase fluorescence in situ hybridization (FISH) was applied to determine the chromosomal abnormalities. Analysis of mutational status was performed as described by Pablo Oppezzo with slight modification. Documentary and statistical methods were used for data processing by Fisher's Exact test.

Conclusion: We found that a large proportion of patients were diagnosed with early stage A of the disease. In most untreated CLL- patients isolated del13q was detected. Statistically significant association was found between del13q, del11q, del17p / p53 and mutational status.

Keywords: lymphocytic leukemia, lipoprotein lipase, ADAM29, del13q14, prognosis,

INTRODUCTION:

CLL affects persons over 65 years of age, predominantly male. The disease is more common in Western countries [1].This difference in geographical distribution has served as a basis for studying the genetic predisposition to the disease and excluding the effects of external factors. Between 9 and 17% of CLL patients have relatives with the lymphoproliferative disease [2].

The clinical course of CLL is exclusively variable. In some patients, no treatment is needed for the rest of their lives, while in others, the disease takes an aggressive clinical course [3]. This diversity has led to the extensive study of molecular and cellular markers with prognostic and predictive value [4]. Differences in tumor proliferation and apoptosis levels explain the unpredictable clinical manifestation of the disease, reflecting both genetic differences and the activity of external signaling mainly through the B-cell receptor pathway(BCR) [4].

The two staging systems of Rai and Binet are currently an essential part of most prognostic models. When analyzed together with the lymphocyte count, they reflect indirectly the tumor burden, but are not sufficient to predict which patients in the early and intermediate stages will progress and need treatment [5]. In the late 1990s, the first reports about correlation between the mutational status of IgV (H) genes and the clinical outcome of the disease were published [6].In the early 2000s Dohner and colleagues, based on regression analysis, created a hierarchical model of 5 subgroups of cytogenetic disorders, graded by the predictive value as follows: del17p13> del11q22-23> t12> no aberrations> del13q14 [7,8,9]. Recently, extensive genome analysis has led to the identification of gene mutations [NOTCH1, SF3B1, BIRC3] which correlates with the clonal evolution and clinical

outcome. However, these new-generation sequencing techniques are not currently available in all countries, that is why in the clinical practice some unverified surrogate markers such as ZAP70, CD38 and others are used. Studying the prognostic factors, the so called 'traditional' (stage of disease, lymphocyte count) and tumor-specific genetic and molecular markers, provides a better opportunity to determine the time to treatment, the risk of developing a relapse as well as choice of therapeutic regimen. Expanding the knowledge in this area and applying it in clinical practice would lead to an increase overall survival of CLL patients.

MATERIALS AND METHODS:

Forty-eight patients with confirmed diagnosis of B-CLL, according to the 2008 IWCLL-NCI revised criteria were included in the study. All patients participating in the study signed an informed consent, approved by the Local Ethics Committee of the Medical University-Pleven.The Binet staging system was used to stage the patients. X-ray of the chest, abdominal ultrasound and CT scan before starting treatment were performed. From each patient, 3ml of venous blood was collected in a EDTA-containing vacuum tube. All samples were processed within 6-24 hours of collection. Leukocytes were analyzed using a two-laser FACS Calibur cytometer [Becton Dickinson, Heidelberg, Germany] and Cell Quest Pro Software (Becton Dickinson). Lymphocytes were separated by CD45 / SSC gating. Thereafter, all subsequent steps of the assay were performed on CD19 + B cells.

Fluorescence in situ hybridization (I- FISH) was performed according to standard Vysis protocol (Vysis®; Abbot Molecular Inc., Abbott Park, Illinois, USA) on interphase nuclei from peripheral blood isolated nuclear cells. Locus-specific deletion DNA probes were used for the study of the p53, ATM and DLEU1 genes: Vysis TP53 / CEP17 FISH Probe Kit, Vysis ATM / CEP11 FISH Probe Kit, Vysis D13S319 / 13q34 FISH Probe Kit.

A modification of the method of Pablo Oppezzo [10] was used to determine the expression of LPL and ADAM29 (disintegrin and metalloproteinase domain 29-containing protein) genes. Multiplex polymerase chain reaction after reverse transcription [RT-PCR] on RNA in complementary DNA (cDNA) was carried out. The availability of RNA for analysis and the efficiency of reverse transcription was evaluated by amplification of the b-Actin gene. Mutated IGVH CLL- status was determined in case of presence of distinct amplification products including 1 band corresponding to ADAM29 expression or 2 products corresponding to ADAM29 and LPL. Unmutated IGVH CLL- status: presence of distinct amplification product including 1 band corresponding to the LPL.

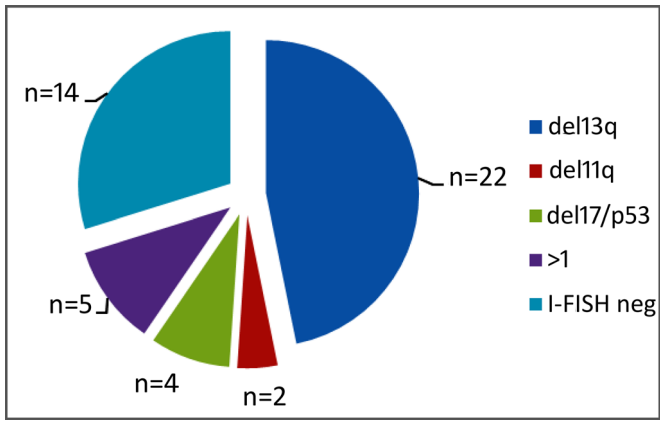
In the case of ADAM29 and LPL amplification products, regardless of the presence of the b-Actin amplification product, the corresponding sample was classified as undetermined IGVH CLL- status.

RESULTS:

The distribution of patients by sex was as follows: 65% (31/ 48) of patients were male and 35% (16/ 45) female, respectively. Patients' age ranged from 43 to 84 (median age of 68). At the diagnosis, 32 (66,66%) patients were in stage A, 9 (18,75%) in B and 7 (14, 58%) in stage C. Similar distribution of CLL patients , based on the staging system is presented in most scientific reports.

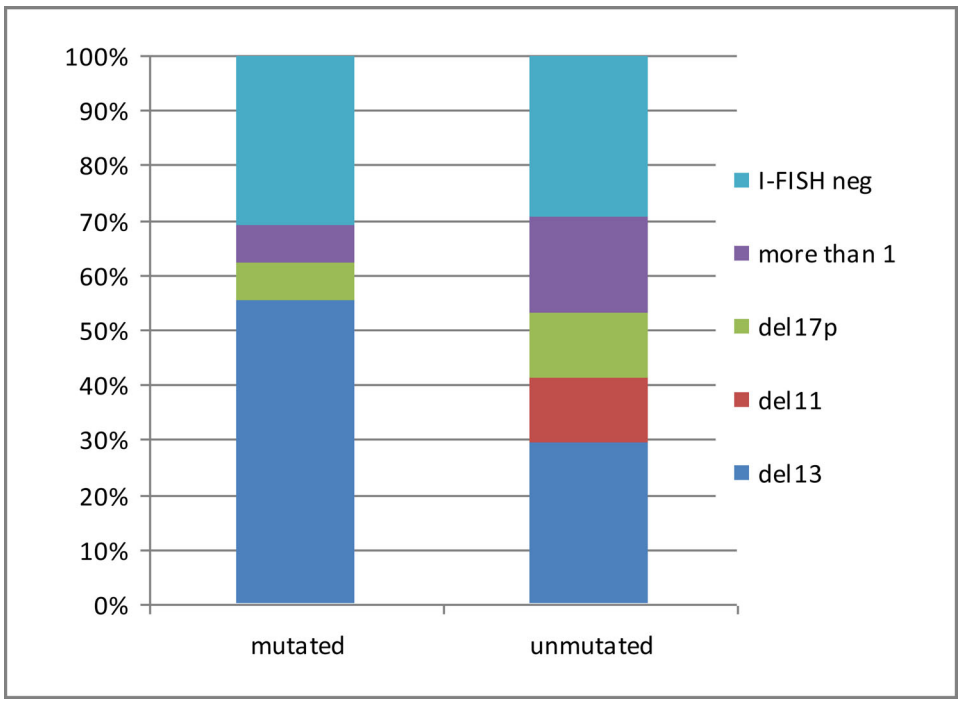
Using the I- FISH method, to identify one of the most common structural chromosomal abnormalities, del13q was detected in 45,83% (22/48) of the patients. In 29.16% (14/48) del13q, del11q, del17p / p53 were not detected. The group of patients with two chromosomal lesions and del17p / p53 was significantly smaller 10.41% (5/48). One result of the FISH analysis was unrepresentative. The distribution of the studied chromosomal abnormalities is presented in Fig. 1.

Fig. 1. Distribution of genetic disorders by FISH analysis in the group of 48 CLL patients: del 13q; del11q; del17p/p53; > 1-more than one aberration; I-FISH neg- none of the above mentioned chromosome aberration was identified.



The patients with del13q (16/ 48) and those who do not have all the above-mentioned structural damage (10/48) in stage A were predominant in number. We applied a modified Pablo Oppezzo method, as an alternative method for determination of the mutational status of CLL-patients. Based on the results, the patients were divided into two groups as follows: mutated CLL- patients (MT) 32 and unmutated CLL- patients (UM) 16 (fig 2). By using Fisher's exact test, statistically significant relationship was found between the chromosomal abnormalities and mutational status (p = 0,_035)

Fig. 2. Distribution of the chromosomal aberrations according to the mutationl status.



Deletion 13q was the most common chromosomal abnormality (50%) in the group of patients, where the mutational status was determined as mutated, by using alternative method based on PCR analysis, while 18.75% of patients with UM - CLL had more than one chromosomal aberration. A deletion of 17p / p53 was found in 6.25% (2/32) of MT-CLL patients and in 12.5% (2/16) of UM-CLL patients.

DISCUSSION:

According to the literature, there is a significantly higher number of patients in the early and intermediate stage, at the diagnosis of the disease and the our results confirm these facts. Of the 48 patients, only 7 were in advanced stage C at diagnosis

Some of the most commonly discussed chromosomal disorders associated with loss of regions in chronic lymphocytic leukemia are: del17p13 and del11q22-23. The two chromosomal abnormalities in most reports have been cited as poor prognostic factors associated with rapid disease progression, treatment resistance, and advanced stages [11, 12]. This is not true about del13q14. It is one of the most common chromosomal aberrations (50-60% of cases) and is considered to be an early event in the pathogenesis of the disease [13]. According to most scientific reports, the isolated presence in CLL patients is associated with a favorable prognosis [13, 14]. In 2009 and 2010, Hernandez et al., Van Dyke et al. described a correlation between, the number of CLL cells carrying 13q deletion, expressed as a percentage of deleted nuclei, the disease outcome and time to first treatment (TTFT) [15]. Esther M. Orlandi et al reported a surprisingly short treatment free survival (TFS - 22 months) in patients with †e" 70% deleted cells [16]. Nedeva et al. did not find significant differences in TTFT, using cut- off value of 80% of the cells with del13q nuclei, but reported differences in PFS in patients with e" 80% 13q deleted nuclei [17]. What's more, some authors have analyzed prognostic value of del13q in combination with mutational status and have reported shorter time to treatment for those with del13q and UM status unlike those with del13q and MT [18].

For 20 years, IGVH mutational status has been undoubtedly considered to be one of the most reliable prognostic factors that does not change during the course of the disease [19]. This dividing of the CLL patients into two categories (mutated - good prognosis, unmutated - short survival) based on the gene expression profile has been widely accepted due to different clinical course and probably different cellular origin. A recent study of Gina Eagle et al. on the expression of proteins by B-CLL cells, found correlation between mutation status and altered transcription and translation processes, leading to greater adhesion and decreased lymphocyte migration in patients with unmutated status [20]. Unfortunately, sequencing methods as well as iTRAQ methods are not widely applicable for routine clinical practice. In 2005 Pablo Oppezzo et al., suggested using the ratio of the genes of lipoprotein lipase and ADAM29 as a surrogate marker of IGVH mutation status [10]. For this purpose they used multiplex polymerase chain reaction after reverse transcription polymerase chain reaction on cDNA. At present, the effect of lipids on CLL biology is not clear enough. Their participation in the structure of the cell membrane is indisputable. The so-called "membrane rafts" are rich of cholesterol and sphingolipids. Lipid rafts are heterogeneous and highly variable and can facilitate amplification of BCR signaling upon binding to the corresponding ligand. In contrast to the normal B-lymphocytes, increased expression of the LPL gene has

been reported in unmutated B-CLL lymphocytes [21]. The other gene, whose product is expressed in CLL cells with respect to IGVH status, but is not expressed in normal B-lymphocytes is ADAM29 [22]. Over the years, several scientific reports have confirmed the use of the LPL/ ADAM29 as a surrogate marker of IGVH mutational status.

Cytogenetic disorders can occur at different stages of the course of CLL and have different predictive and prognostic value, which implies the necessity of being analyzed in combination with others prognostic factors. According to most authors, the determination of cytogenetic disorders and mutational status should be performed before initiation of therapy, in order to select correct treatment. But the aforementioned prognostic factors are an integral part of current systems for risk stratification of the patients who are in the early and intermediate stages and should be monitored till progression. The question remains when they should be investigated at the diagnosis and/ or during the course of the disease and if there is a correlation between cytogenetic and molecular disorders. Moreover, unlike cytogenetic disorders, the mutational status remains stable throughout the disease (23). Some clinical researches have reported a correlation between mutational status and cytogenetic disorders, so detection del 11q and 17p-, whose presence is associated with poor prognosis , are more likely to occur in patients with unmutated status (24).

CONCLUSION:

Based on the results of our study, we found that the majority of CLL patients were diagnosed with early-stage of the disease. The isolated del13q was one of the most common chromosomal aberrations proved by FISH method in our patient cohort. Ànalyzing the assotiation between mutational status (determined by the modified Pablo Oppezzo method) and cytogenetics aberrations, our results confirm the presence of correlation between the two prognostic factors, but having in mind the small group of patients and the variety of chromosomal aberrations, additional verification in a larger group is required.

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