

The Pattern of Chromosome Aberrations and Molecular Markers in the Population of Hematological Patients Diagnosed at the University Clinical Center Tuzla

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ABSTRACT

Introduction: Besides cardiovascular, malignant diseases are one of the leading causes of death in Bosnia and Herzegovina. At the top of this list are hematological diseases. This research aimed to identify cytogenetic and molecular biomarkers in patients treated for different types of hematological neoplasms.

Methods: The retrospective study included 1600 samples of patients with different hematological diseases in the period from January 2006 to May 2022. The Polymerase Chain Reaction (RT-PCR) method was used to determine the presence of genetic rearrangements and to confirm the findings of conventional cytogenetic analysis.

Results: Chromosomal aberrations were found in 739 (46,18%) patients. Using the RT-PCR technique, positive cases were increased by 1,5%. The *BCR-ABL* fusion gene was present in e14-a2 transcript form in 73% of samples, e13-a2 isoform in 21%, e1-a2 in 2%, while e14-a2/e1-a2 transcript coexpression was present in a percentage of 4% of the samples. The *PML-RARA* fusion gene was found in the form of bcr 1 transcripts in 21%, bcr2 32% and bcr3 59% of the samples. In twelve cases A type of the *CBFB-MYH11* fusion transcript was detected. The *MLL-AF4* fusion was found in only one case.

Conclusion: The obtained percentages of frequency of individual molecular gene isoforms are in accordance with the results of most other researchers. This refers to the Balkan population and the Caucasian ethnic group.

Keywords: Chromosomes, genetic rearrangements, hematological diseases.

Submitted : November 18, 2022

Published : December 24, 2022

ISSN: 2593-8339

DOI: 10.24018/ejmed.2022.4.6.1599

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I. INTRODUCTION

In today's modern world, hematological malignancies are the leading cause of mortality. Data updated in September 2022 indicate that approximately every three minutes, in the United States, one person becomes ill with leukemia, lymphoma, or myeloma. It is estimated that a total of 186,400 people in the US will be diagnosed with leukemia, lymphoma, or myeloma in 2022 [1]. Cancer is one of the leading public health problems of modern society. Although considerable efforts are being made in prevention, cancer as the second most important cause of death is a significant health problem for the population of Bosnia and Herzegovina. According to data from the Institute for Public Health of the Federation of Bosnia and Herzegovina (FB&H) from 2013, the proportion of morphologically verified tumors in the FB&H is highest in Tuzla (83.2%) and Central Bosnia Cantons (92.5%). The smallest share was recorded in Herzegovina-Neretva Canton (17.6%) [2].

According to the data from the Croatian Institute of Public Health, the incidence of cancer per 100,000 inhabitants in 2017 was 591.2 [3]. Currently, we do not have data related specifically to hematological malignancies and their frequency in Bosnia and Herzegovina as well as neighboring countries.

From what we know so far, acute myeloid leukemia (AML) is the most common leukemia in adults. This type of leukemia accounts for about half of all leukemias in humans. It shows the least geographical variation in incidence and is 1.5 patients per 100,000 inhabitants per year in the age group of 40 to 44 years. AML is characterized by a large number of gene rearrangements resulting from structural and numerical chromosomal aberrations, among which the following stand out: translocation t(8;21), inversion inv(16), translocations t(16;16) and t(15;17) [4]. Their incidence recorded in studies is 0 to 5% [5].

Chromosome 11 (11q23) aberrations occur in 4% to 10% of AML patients [6]. As far as numerical aberrations are concerned, trisomy 8 is the most common [4].

Acute lymphoblastic leukemia (ALL) is the most common hematological disorder in children, with translocation t(12;21) being the most common abnormality [7]. Another very common and diagnostically important chromosomal aberration is the translocation t(9;22), otherwise known as the Philadelphia translocation (Ph), which is found in about 5% of children and 20-25% of adults with ALL [8].

Chronic myeloid leukemia (CML) is a white blood lineage disease. It is diagnosed by the presence of the *BCR-ABL1* fusion gene, which is associated with the translocation t(9;22)(q34;q11). Thanks to Nowel and Hungerford, who discovered a small marker Ph chromosome in the sixties of the last century, and later Janet Rowley, who gave the exact translocation formula, today we have a diagnostic and prognostic marker for CML [9], [10]. Since it doesn't exist a register of cytogenetic and molecular changes was established in Bosnia and Herzegovina, this research provides the first information on the genetic basis of hematological disorders of part of the population of Bosnia and Herzegovina. At the same time, the goal of the research is to compare our results with studies from other countries. The combination of the obtained data will help in achieving better diagnostics, which will improve the overall treatment and monitoring of hematological diseases.

II. PATIENTS AND METHODS

Retrospective research was carried out in the laboratory for cytogenetic and molecular medicine, Polyclinic for laboratory diagnostics, University Clinical Center Tuzla (UCC Tuzla). The results of cytogenetic and molecular gene tests were analyzed on 1,600 bone marrow and peripheral blood samples from patients with a suspected hematological disorder, in the period from January 2006 to May 2022. Routine cytogenetic analysis was performed on bone marrow aspirate samples immediately after collection. During the cultivation and chromosome harvesting, the standard protocol of the cytogenetic laboratory was used, as well as the GTG chromosome banding technique (Giemsa banding).

The results of the analysis were interpreted in accordance with An International Cytogenetic Nomenclature [11].

Total RNA was obtained from a sample of 10^7 peripheral blood leukocytes using a commercially available RNA extraction kit, the QIAamp RNA Blood Mini Kit (Qiagen, Hilden, Germany). Complementary DNA (cDNA) was synthesized using the High-Capacity cDNA reverse transcription kit (Thermo Fisher Scientific, USA), according to the manufacturers manual.

Reverse transcriptase polymerase chain reaction (RT-PCR) was applied to prove the presence and splice site of chimeric genes *AML1-ETO*, *PML-RARA*, *CBFB-MYH11*, *BCR-ABL* and *MLL-AF4* using primers and protocol according to [12]. The products of the gene amplification were analyzed by horizontal electrophoresis on a 2% agarose gel and visualized under UV light.

III. RESULTS

Out of 1,600 analyzed samples, 891 (55.6%) male patients and 709 (44.3%) female patients were recorded. The age of the patients ranged from 15 to 87 years, with a median value of 51 years. The diagnoses dominated in this research group are as following: ALL (4.5%), myelodysplastic syndrome-MDS (10.5%), myeloproliferative neoplasms-MPN (32%), CML (4%), AML (22.5%), and a group of lymphoproliferative diseases (Non-Hodgkin's lymphoma, Hodgkin's lymphoma, Chronic lymphatic leukemia, Small lymphocyte lymphoma, Burkitt's lymphoma, etc. in the percentage of 26.5% (Fig. 1; Table I).

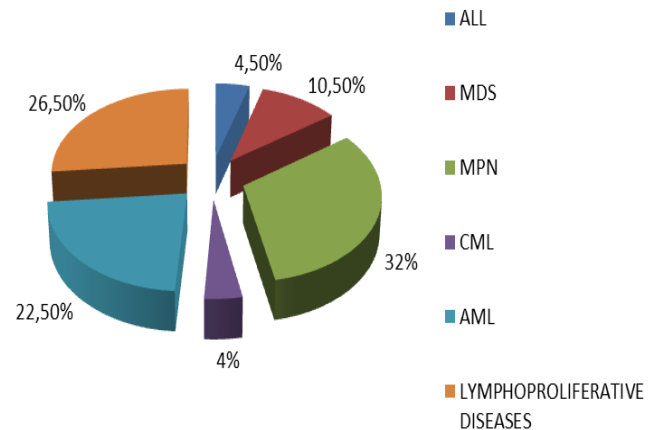


Fig. 1. Distribution of hematological diseases in the patients' study group.

TABLE I: CYTOGENETICS FINDINGS IN DIFFERENT HEMATOLOGICAL DISEASES

Disease	Cases with suspected disease n / %	Cases with chromosome abnormalities n / %	Cases with normal karyotype n / %	NO metaphases n / %
ALL	72 / 4,5	47 / 66,6	25 / 33,3	-
MDS	168 / 10,5	128 / 76,2	16 / 9,5	24 / 14,3
MPN	512 / 15,5	115 / 22,6	330 / 64	67 / 12,9
CML	65 / 4	50 / 77	15 / 23	-
AML	360 / 22,5	240 / 66,7	40 / 11,1	80 / 22,2
Lymphopr. diseases (BL, CLL, NHL, TCL, FL, SLL)	423 / 26,5	159 / 37,5	223 / 53	41 / 9,8

Chromosome abnormalities in the karyotype were found in the group of 739 (46.18%) patients, while a normal karyotype was observed in 649 (40.56%) samples. Due to the absence of metaphases, 212 (13.25%) samples were not analyzed. If we look at the types of chromosomal aberrations, the most numerous are structural (412/739; 55.8%) followed by numerical (194/739; 26.2%). Complex karyotypes were found in a percentage of 18% (133/739).

In the group of structural changes, translocations are found in 213 (51.7%) karyotypes, deletions in 88 (21.3%), inversions in 68 (16.5%) and additions in 43 (10.6%) pathological karyotypes.

As far as numerical aberrations are concerned, all types of this type of chromosomal changes were found.

The most frequent are trisomies of chromosomes 8, 20 and 22. In addition to individual numerical aberrations, tetraploidy was found in 1.6% (12/739) of karyotypes, as well as 0.67% of cases (5/739) with octaploid karyotype.

Table II shows the most common chromosomal aberrations in individual hemoblastoses.

TABLE II: THE MOST COMMON CHROMOSOMAL ABERRATIONS IN INDIVIDUAL HEMOBLASTOSIS

Hemoblastosis	Chromosome aberration
AML	t(8;21)
	t(15;17)
	inv(16)
	del(16q)
	t(9;11)
	+8 -7
ALL	t(9;22)
	t(1;19)
	t(4;11)
	11q23 rearrangements
CML	t(8;14)
	t(9;22)(q34;q11)
	Variant Philadelphia translocations
Lymphoproliferative diseases	14q rearrangements
	t(8;14)
	t(14;18)
MDS	del(5q)
	-5
MPN	+8, +20, +22

To prove the possible presence of chimeric genes, in samples where it was not possible to obtain metaphases (12.9% MPN, 22.2% AML), as well as in cases with a normal karyotype (11.1% AML; 64.5% MPN; 23 % CML, 25% ALL) the RT PCR method was applied.

On this occasion, in 8 AML patients (6.66%; 8/120), *AML-ETO* and *PML-RARa* amplification products were proven, while 5 MPN patients (1.25%; 5/397) carrying the *BCR-ABL1* fusion gene. In one patient, the presence of the *MLL-AF4* fusion gene was determined. The obtained results increased the percentage of newly diagnosed patients by 2.22% AML, 1% MPN and 1.4% ALL compared to the results obtained by conventional cytogenetic analysis.

In all previously proven cases of AML with translocation t(8;21), the presence of the *AML-ETO* fusion transcript was determined. We identified bcr1 (21%), bcr2 (32%) and bcr3 (59%) fusion transcripts in AML M3 patients carrying the *PML-RARa* chimeric gene. The *CBFB-MYH11* E form fusion gene was present in 12 subjects (3.33%).

In the population of patients with chronic myeloid leukemia (CML), i.e. translocation t(9;22), the fusion gene *BCR-ABL1* is present in the form of e14a2 transcript in 73% of samples, e13a2 in 21%, e1a2 in 2%, while co-expression of e14/e1a2 transcript was found in 4% of samples.

IV. DISCUSSION

In recent years, hematological malignancies in Bosnia and Herzegovina represent one of the main causes of mortality. To date, we have limited knowledge about their genetic characteristics. There are only test results on the type of *BCR-ABL1* gene and its frequency in a small population of

subjects [13]. This research presents the genetic characterization of newly diagnosed patients with different hematological diseases. These genetic tests and their results provide a better understanding of the evolution and prognosis of the disease. The presented data were obtained through routine diagnostics of hematological diseases at the Polyclinic for Laboratory Diagnostics of UCC Tuzla since 2006 when cytogenetic and molecular diagnostics has been established.

Structural and numerical chromosomal abnormalities were detected in 46.18% of cases. These data are important for a high-quality and timely diagnosis, but also for the prognosis of each patient's disease, and their specific therapeutic response. In AML, MPN and ALL cases when metaphase could not be obtained, or the karyotype was normal, the RT-PCR method was used. This made it possible to detect an average of 1.5% more newly diagnosed cases within the specified group of patients. All newly diagnosed cases with AML, ALL, MPN and CML are characterized by the following chromosomal aberrations: translocations: t(8;21), t(15;17), inv(16), t(9;22) and 11q rearrangements. These translocations are characterized by the following gene fusions: *AML1-ETO*, *PML-RARa*, *CBFB-MYH11*, *BCR-ABL1* and *MLL-AF4*. Previous studies have identified at least 20 translocation partners of chromosome 11, including the *MLL* gene (11q23) [14]. However, this study found only one case with *MLL-AF4* gene rearrangement.

The Philadelphia chromosome, using a conventional cytogenetic test, was detected in 77% of CML samples. Analyzing MPN samples without metaphases, but also those with a normal karyotype, using the RT-PCR method, 5 new cases with the presence of the chimeric *BCR-ABL1* gene were identified, which places them in the group of patients with CML. Compared with cytogenetic results, PCR analysis increased the sensitivity of the detection of the Ph chromosome and *BCR-ABL1* gene in CML by 1.5%.

Although this research focused on the molecular and cytogenetic characteristics of hematological neoplasms in the population of patients treated at the UCC Tuzla. The focus of the study was also on the frequency of different types of fusion genes. Variations in breakpoints were correlated with leukemia phenotype [15]. RT-PCR method proved the presence of *AML1-ETO* fusion in subjects with t(8;21) translocation.

The frequency of *BCR-ABL1* isoforms is as follows: e14a2 (73%), e13a2 (21%), e1a2 (2%), e14a2/e1a2 (4%). The dominance of the e14a2 transcript in our population is consistent with the results of most other studies [16], [17].

As far as the Balkan countries are concerned, the results correlate with the above. Thus, in the Serbian population, the percentage of *BCR-ABL* e14a2 (73.5%) is almost three times higher compared to the e13a2 *BCR-ABL* isoform (25%) [18]. A group of authors from Croatia reached the same results. In their research on a sample of 26 patients, they published results in which the *BCR-ABL1* e14a2 fusion transcript was present in 73% of patients, while the *BCR-ABL* e13a2 transcript was present in 27% of them [19].

This research established that the isoforms of the *PML-RAR* gene are represented differently. Thus, the frequency of *PML-RARa* bcr 1 is 21%, bcr 2 32% and bcr 3 69%.

Comparing the results obtained with the results of other similar studies, the frequency of individual *PML-RAR* isoforms does not differ significantly [20]. Only in the population of Ecuadorian subjects the absence of the bcr 1 variant of the *PML-RAR* gene fusion was recorded, while the prevalence of bcr 2 is somewhat lower, at 5% [21]. The presence of a certain variant in the patient population can be extremely important when it comes to therapeutic selection and the final outcome of treatment [22].

The presence of the *CBFB-MYH11* gene fusion in bone marrow or blood samples is associated with a good prognosis [23]. In this study, in all patients with a proven fusion gene, type A transcript was determined, which is the most common transcript present in more than 85% of positive cases. Transcripts D and E represent about 5%, while the others are unique cases [24]. Differences in the frequencies of individual gene fusion types largely depend on the studied population.

The dominance of some types of fusion genes in our population is consistent with the results of most other researchers. This particularly applies to the Balkan population and the Caucasian ethnic group. Significant differences in the frequency of individual genes are particularly pronounced in populations composed of different ethnic groups, such as the South American population composed most of the native Spanish populations [21].

V. CONCLUSION

This research aimed to identify cytogenetic and molecular biomarkers in patients treated for different types of hematological neoplasms. The detection of chromosomal aberrations and the expression of pathological genes in patients suffering from hematological diseases is significant because of their predictive value. This means that based on these analyses, it is possible to determine which hematological neoplasms will most likely give the maximum effect on the applied therapy. In the era of personalized medicine, when we try to use applied therapy to act on the target type of tumor, according to its characteristics, both morphological and molecular, this is of extreme importance. It is very important to emphasize that these diagnostic methods and the biological markers found allow us to apply the drug in a targeted manner for those patients in whom we expect the maximum effect.

CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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