Frequency of BCR-ABL1 splice variants in Bosnian patients with chronic myeloid leukemia

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ABSTRACT

Objectives: To calculate the frequency of BCR-ABL1 splice variants (e14a2, e13a2 and e1a2) in a group of Bosnian patients with chronic myeloid leukemia (CML) and compare it with the data reported in other populations. Comparisons between cytogenetic and therapy outcomes in patients with BCR-ABL1 e13a2 and e14a2 transcripts was also aim of this study.

Methods: Forty six (46) CML patients, hospitalized at the University Clinical Center Tuzla, were subjected to cytogenetic and RT-PCR analysis.

Results: Out of 46 patients, 33 (72%) patients expressed e14a2, followed by e13a2 seen in 10 (22%) patients. Two patients (4%) displayed both e14a2 and e1a2 forms of BCR-ABL1. One patient (2%) showed e1a2 transcript of BCR-ABL1. In a subgroup of 19 CML patients, treated with Imatinib, patients with e13a2 transcript had higher complete cytogenetic response (CCgR) compared to those with e14a2 transcript.

Conclusion: The frequency of BCR-ABL1 e13a2 and e14a2 molecular isoform in patients with CML included in our research is in concordance with other researches. Continuation of the study with a larger number of patients is required to confirm these preliminary observations.

Key words: BCR-ABL1 transcripts, CML, cytogenetic response, Philadelphia chromosome.

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INTRODUCTION

Chronic myeloid leukemia (CML) belongs to a group of myeloproliferative diseases and it is characterized by clonal proliferation of mature hematopoietic blood cells and bone marrow. The molecular genetic basis of the disease makes translocation between the BCR gene located on chromosome 22 and the ABL1 proto-oncogene located on chromosome 9 [1]. BCR-ABL1 fusion transcripts, formed as a result of balanced reciprocal translocation t(9;22)(q34;q11), can vary depending on the site of the breakpoint in the BCR gene. The breakpoints in the BCR gene are located on the intron between exons e13 and e14 or on the intron between exons e14 and e15. These breakpoints are a part of major breakpoint cluster region (M-BCR), located between BCR exon 12 and 16. In most cases, breakpoints on the ABL1 gene are located 5' of exon a2. Thus, in many patients with CML specific transcripts are e13a2 or e14a2. These splice variants of the BCR-ABL1 rearrangements encoding abnormal 210 kDa protein with oncogenic activity. The BCR-ABL1 e1a2 fusion transcript can be detected in a rare CML cases. In this rearrangement sequence of the first BCR exon, located in the minor breakpoint cluster region (m-BCR), is connected with the second exon of the ABL1 gene (e1a2). Sequence of the BCR-ABL1 e1a2 junction is encoding abnormal 190 kDa protein, related to an acute lymphoblastic leukemia (ALL) and also found in some patients with CML. BCR-ABL1 e19a2 junction can be detected in several CML patients. This is a longer version of the BCR-ABL1 fusion transcript connecting BCR exon 19, lying in the micro breakpoint cluster region (μ-BCR), and ABL1 exon 2. This BCR-ABL1 sequence is encoding a 230-kDa BCR-ABL1 protein [2]. Genetic abnormalities can cause the formation of fusion oncogenes. This phenomenon is usually controlled by environmental and lifestyle factors that vary in different geographical areas [3]. Frequency of fusion...
oncogenes associated with leukemia can vary in different ethnic groups, which may have a significant influence on the management and prognosis of this type of malignant blood diseases [4][5].

Although CML is one of the most common diseases of adult hematopoietic tissues in Bosnia and Herzegovina, there are no published data on the prevalence of certain BCR-ABL1 isoforms. Our study aimed to calculate the frequency of BCR-ABL1 splice variants (e13a2, e14a2 and e1a2) in patients with CML and compare it with the data reported in other populations. Comparisons between cytogenetic and therapy outcomes in patients with BCR-ABL1 e13a2 and e14a2 transcripts was also the aim of this study.

**MATERIAL AND METHODS**

We performed a retrospective research study to determine types and incidence of BCR-ABL1 fusion gene transcripts using medical records data of 46 patients with CML, diagnosed in the Department of Hematology, Oncology and Radiotherapy, University Clinical Center Tuzla in the period from January 2005 to August 2013. Ethics Committee of the University Clinical Centre Tuzla has approved use of patients' medical records data due to perform this study. Available data included results of biochemical, cytogenetic and molecular tests. Pretreatment patients' characteristics are given in Table 1.

**Table 1. Pretreatment patients' characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age mean (range)</td>
<td>58 (21-79)</td>
</tr>
<tr>
<td>Male</td>
<td>16</td>
</tr>
<tr>
<td>Female</td>
<td>30</td>
</tr>
<tr>
<td>Platelet count x10^5/L (mean)</td>
<td>106</td>
</tr>
<tr>
<td>Hemoglobin g/L (mean)</td>
<td>97</td>
</tr>
</tbody>
</table>

After making definite diagnosis of CML, patients were therapy treated. In Bosnia and Herzegovina and other developing countries, the therapy management of CML is financially limited and dependent of the national health insurance. The Health insurance in the Federation of Bosnia and Herzegovina has funded imatinib mesylate insurance. The Health insurance in the Federation of Bosnia and Herzegovina is financially limited and dependent of the national health insurance. The Health insurance in the Federation of Bosnia and Herzegovina has funded imatinib mesylate insurance. The Health insurance in the Federation of Bosnia and Herzegovina has funded imatinib mesylate insurance.

**Bone marrow and blood sampling**

Forty-six bone marrow aspirate (BM) and peripheral blood (PB) samples were obtained with a time of referring diagnosis of Ph positive (Ph+) CML. All samples were done as a part of routine hematological diagnostic procedure which also included histopathological analysis, flow cytometry, molecular and cytogenetic analysis and other relevant laboratory procedures. Bone marrow aspiration and peripheral blood venipuncture were performed at the Department of Hematology, Oncology and Radiotherapy, University Clinical Center Tuzla.

Blood samples for Reverse Transcriptase Polymerase Chain reaction (RT-PCR) were undertaken at the Polyclinic for laboratory diagnostic, University Clinical Center Tuzla.

**Methods**

**RNA extraction and cDNA synthesis**

Total RNA was extracted from 107 peripheral blood leukocytes using a commercially available RNA extraction kit QIAamp® RNK Blood Mini Kit (Qiagen, Hilden, Germany). The quality of RNA was obtained by examining the integrity of the 28S and 18S bands on 2% agarose gel. RNA was reverse transcribed to cDNA for using as template in PCR reaction. Complementary DNA (cDNA) was synthesized using first strand cDNA synthesis kit (Invitrogen Corp., San Diego, USA). The synthesis was carried out with 10 µL of the RNA solution with Super Script III reverse transcriptase (Invitrogen Corp., San Diego, USA) using random primers in a total reaction volume of 20 µL for 50 min at 50°C, according to the manufacturer’s manual.

**PCR amplification**

RT-PCR was performed in two rounds on 5 µL of the cDNA product with BCR-ABL1 t(9;22) translocation assay (In Vivo Scribe Technologies, San Diego, California) according to the manufacturer’s manual. The PCR products of the second PCR step were run on 2% agarose gel, stained with ethidium bromide and visualized under UV light. During PCR reaction the positive controls (BCR-ABL1 e1a2 RNA and e14a2 RNA) contained in the kit were also used. Complementary DNA (cDNA) of these positive RNA controls was synthesized using a method described above, and later used in the PCR amplification. We have used RNase-DNAse free water as a negative control in or-
order to detect contamination of reagents. The quality of RNA and efficiency of cDNA synthesis was analyzed by amplification of the ABL1 gene as internal control.

**Cytogenetic analysis**

Bone marrow aspirate was introduced into culture medium and two different culture methods were used: one culture was harvested on the same day, while second culture was harvested at 24 hours. The harvest and slide preparations were done according to standard procedures. Chromosomes were Giemsa-trypsin banded. Cytogenetic response (CgR) was evaluated by morphologic cytogenetics of at least 20 marrow metaphases. CgR was further categorized as complete CgR (no Ph + cells), partial CgR (1–35% Ph + cells), minor CgR (36–65% Ph + cells), minimal CgR (66–95% Ph + cells), and No response (95% Ph + cells), according to recommendations of the European LeukemiaNet for the management of chronic myeloid leukemia [6,7].

**RESULTS**

Using PCR method, four types of amplification products were detected as: 186bp, 371bp, 126bp, 311bp, 242bp for the BCR-ABL e1a2/e14a2 form (Figure 1A, lines 2,3,4,5,6); 126bp, 311bp and 242bp for e14a2 form (Figure 1B, lines 4,5,6); 186bp and 371bp for e1a2 form (Figure 1C, lines 2,3); 236bp for e13a2 (Figure 1D, line 5). The amplified ABL1 product was 94bp (Figures 1 A, B, C, D, line 1). All examined patients were positive for some type of BCR-ABL1 rearrangement.

Table 2 shows the number of the patients with specific BCR-ABL1 fusion transcript types. During cytogenetic monitoring eighty four percent (16/19) of patients treated with imatinib mesylate achieved a cytogenetic response (CgR) while three patients (3/19;16%) achieved not CgR. Patients expressing the e13a2 isoform have a slight higher rate of complete CgR than patients expressing e14a2 BCR-ABL1 molecular isoform (Table 3).

**DISCUSSION**

Results of several studies point to the fact that the knowledge of the BCR-ABL1 fusion transcript types have influence on the clinical outcome. The studies suggest that patients with the presence of one of these variant transcript types have higher survival and short-
er latency to develop leukemia [8]. Likewise the literature results shows that many of the reports proposed that CML patients with e13a2 transcript type do better (longer chronic phase) than those with e14a2 fusion transcript. However, other studies have not been able to confirm any significant correlation between M-bcr isomers and disease outcome. The discrepancy of the results between the various studies could be due to qualitative differences in sample selection. Some of these studies also provided evidence of hematological differences between the M-bcr types of breakpoints [9]. On the clinical and diagnostic ground BCR-ABL1 transcript type significantly correlated with leukemia phenotype. Three main variants of the BCR-ABL1 gene (e13a2, e14a2, e1a2) encode for the p190(BCR-ABL1), P210(BCR-ABL1), and P230(BCR/ABL1) proteins. Thus major BCR breakpoint (e13a2, e14a2) correlates with CML. The BCR-ABL1 e1a2 fusion transcript type in CML is rare (approximately 1%), but may have a poorer prognosis compared to typical CML BCR-ABL1 fusion types [10]. In this study, the frequency of BCR-ABL1 splice variants in patients with CML, hospitalized in our health institution, was determined using RT-PCR method. RT-PCR methodology is one of the more sensitive techniques, which refers to the quality measurement of the quantity of product which does not refer to the amount of input RNA and DNA. Thus, this type of PCR makes quality tools for detecting the presence or absence of specific and required RNA or DNA [11]. In our study we found, the frequency of e14a2 and e13a2 was 72% and 22% respectively. The frequency of e14a2 is more than three times higher than e13a2 (Table 2). A series of studies have assessed the frequency and relationship of major BCR-ABL1 transcripts type (e13a2 and e1a2). In a study of UK patients with CML, the e14a2 form was detected in 55% and e13a2 in 40% of them [12]. In a USA group of 1134 patients, the e14a2 form was detected in 52% and e13a2 in 38% of them [13]. In a study carried out by an Italian cooperative study group the frequencies of e14a2 and e13a2 were 57% and 43% respectively [14]. Polampalli [15] found the incidence of e13a2 and e14a2 transcripts in Central India CML patients to be 32% and 68% respectively. A group of 27 patients in Russia displayed predominance of the e14a2 form (66.6%); e13a2 was detected in 33.3% of the cases [16]. In Serbian CML patients, frequency of e14a2 and e13a2 transcripts were 73.5% and 25% respectively, where the number of patients with e14a2 was almost 3-fold the number of patients with e13a2 [17]. Vranić et al. in the Croatian CML group, found the incidence of e14a2 and e13a2 transcripts in CML patients to be 73% and 27% respectively [18]. Todorić cited Paz-y-Mino and coauthors where added to completely different distribution in the Mestizo ethnic group in Ecuador. Among 40 CML patients studied in Ecuador, 94.6% had e13a2 and only 5.4% e14a2 rearrangement. A possible explanation proposed by the authors was a different genetic component in the Ecuadorian population when compared to Caucasians [17,19]. In the group we studied, two patients (4%) displayed transcripts, e14a2 and e1a2. The favored explanation could be that the appearance of e1a2 (p190) transcripts in p210 expressing CML patients heralded the emergence of acute transformation or blast crisis [20]. Saglio et al. detected appreciable amounts of p190 protein in addition to p210 in cells from two of their patients [21]. In a study carried out by Abdall and co-authors, in Sudanese patients group, the frequencies of M-bcr/m-bcr were 5.4% (8/147) [e14a2/e1a2 3/147 (2%); e14a2/e1a3 2/147 (1.4%); e13a2/e1a2 2/147 (1.4%) and e13a3/e1a2 1/147 (0.6%)] [22]. In terms of the lack of connection with any clinical characteristic of either CML or ALL, detection of low levels of e1a2 transcripts in p210 BCR-ABL positive leukemia is probably of no pathogenetic significance [23]. One patient (2%) in our study group displayed the e1a2 form of a transcript. Although, this type of transcript can be detected in CML Ph+ cells this result should nevertheless be viewed with caution. Some cases of acute leukemia may represent lymphoid blast crisis of CML following a clinically silent chronic phase [21]. So, the formation of the e1a2 transcript we detected probably was a secondary event in the lymphoblastic transformation of CML. Several authors have investigated the response to imatinib mesylate therapy according to the form of BCR-ABL1 transcript. In testing of 26 patients, de Lamos et al. reported reduction in mean BCR-ABL1 expression at 6 months for CML patients with e13a2 compared to those with e14a2 junction (p<0.002) [25]. Lucas et al. in their article published that patients with the e13a2 BCR-ABL1 isoform have inferior responses to imatinib compared to patients with the e14a2 isoform [24]. Possible differences in research methodology and analysis could lead to differences in obtained results [2]. In comparison to these results, among the patients treated with imatinib in our study group, it was shown that after 24 months of therapy the e13a2 form displayed CCGr in 67% of the cases, in comparison to 57% in the e14a2 form (Table 3). These results should be viewed with suspicion because here we have a small sample of patients with BCR-ABL1 e13a2 splice variant.
**Conclusion**

In conclusion, the frequency of BCR-ABL e13a2 and e14a2 molecular isoform in our study group patients with CML is in concordance with most other investigations. The knowledge about the rate of occurrence of these transcripts associated with CML can be of very significance as it can lend a hand to further understanding the pathobiology of Phyladelphia positive leukemic cells. Moreover, it will also assist in prognosis, treatment and management of these CML transcripts types, and can be included in future clinical trials of tyrosine kinase inhibitors. Continuation of the study with a larger number of patients is needed to confirm these preliminary observations.

**Declaration of interest**

The authors declare no conflict of interest for this study.

**References**


