Journal of BUON **16:** 104-107, 2011

ORIGINAL ARTICLE

Frequency of *BCR-ABL* fusion transcripts in Serbian patients with chronic myeloid leukemia

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Summary

Purpose: *The aim of this study was to analyze the occurrence of the most frequent BCR-ABL transcript variants (b3a2, b2a2 and e1a2) in Serbian patients with chronic myeloid leukemia (CML) and compare it with the occurrence reported in other populations.*

Methods: *We analyzed peripheral blood and bone marrow samples of 136 Serbian patients with CML by RT-PCR and cytogenetic methods.*

Results: *In 100 patients (73.5%) the b3a2 and in 34 (25%) the b2a2 forms of BCR-ABL were detected. One (0.75%) patient was BCR-ABL negative, but in lymphoblastic transformation he expressed the e1a1 transcript of BCR-ABL. One (0.75%) patient displayed both b2a2 and b3a2 forms of BCR-ABL.*

Analysis of this group according to karyotype showed b3a2 predominance (79%) in patients with classic t(9;22); b2a2 was found in 20% and both b2a2 and b3a2 forms in 1%. In variant translocations b3a2 in 65% and b2a2 in 35% of the patients were detected. In contrast, the subgroup with normal karyotype expressed slight predominance of the b2a2 form (50%); b3a2 was found in 43% of the patients and one patient (7%) displayed e1a2.

Conclusion: *Predominance of the b3a2 form in Serbian patients with CML is in concordance with other relevant investigations, conducted mostly on Caucasian ethnic groups, but in contrast to the study performed on the Mestizo ethnic group in Ecuador. Slight predominance of the b2a2 form was also noticed among the patients with normal karyotype.*

Key words: *BCR-ABL*, chronic myeloid leukemia, Serbia, transcript variants

Introduction

CML is a clonal malignant disorder of a pluripotent hematopoietic stem cell characterized by the presence of the Philadelphia (Ph) chromosome in more than 90% of the patients [1]. Ph chromosome was the first consistent, neoplasia-associated chromosomal abnormality in humans described by Nowell and Hungerford in 1960 [2]. Ph chromosome is a product of t(9;22)(q34;q11) translocation which transposes the 3' portion of *ABL* oncogene from 9q34 to 5' portion of the *BCR* gene on 22q11.2. The crucial pathogenetic consequence of this translocation is the creation of a chimeric *BCR-ABL* gene in the breakpoint region of the derivative chromosome 22 which encodes 210kD protein (p210 $^{BCR-ABL}$) with elevated tyrosine kinase activity. In 5-10% of the cases the Ph chromosome may originate through other rearrangements that involve additional chromosomes. Those rearrangements are named variant translocations. In 5-10% of the cases, CML patients have a normal karyotype [1].

The breakpoint in the *ABL* gene can occur anywhere within a >300 kb segment at the 5' end of the gene, either upstream of the first alternative exon Ib, between exons Ib and Ia, or downstream of exon Ia. In the vast majority of CML patients the breakpoint in the *BCR* gene is found within a 5.8 kb region known as the major breakpoint cluster region (M-bcr). This region spanning 5 exons, historically is named b1 to b5, now known to be exons 12 to 16 forming e13a2 (b2a2) or e14a2 (b3a2)

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Received 13-06-2010; Accepted 03-07-2010

junction, differing in 75 nucleotides [3]. In sporadic CML cases, breakpoint can occur between exons e19 and e20 (μ-bcr) displaying e19a2 junction or between alternative exons e2 and e2' (m-bcr) forming e1a2 junction. Rarely *BCR/ABL* junctions may derive from different breakpoints, giving rise to atypical fusions [4-6].

The frequencies of *BCR-ABL* mRNA transcripts in CML have been reported in several studies, but no study exists carried on Serbian population. Owing to this, the aim of this study was to analyze the occurrence of the most frequent *BCR-ABL* transcript variants (b3a2,b2a2 and e1a2) in Serbian patients diagnosed with CML for additional comparison with the occurrences reported in other populations.

Methods

Patients and samples

We analyzed peripheral blood and bone marrow samples of 136 Serbian patients with CML, diagnosed in the Military Medical Academy and the Institute for Hematology, Clinical Center of Serbia. The diagnosis of CML was established according to clinical presentation, morphologic criteria of bone marrow aspirates and cytogenetic analysis.

RT-PCR analysis

Total RNA was extracted from 10^7 peripheral blood leukocytes with TRIzol® Reagent (Invitrogen, Carlsbad, USA). Reverse transcription was performed on 1 μg of total RNA after heating at 65° C for 15 min, with the 1st Strand cDNA Synthesis Kit for RT-PCR (AMV, Roche Diagnostics Corporation, Indianapolis, USA) according to the manufacturer's manual. A volume of 5 μl cDNA was diluted with 45 μl of PCR mixture (PCR Core Kit, Roche Diagnostics Corporation, USA). *BCR-ABL* was amplified with slight modification as described by Moravcova et al. [7] and *ABL* as described by Gabert et al. [8]. PCR products were separated and visualized on a 2% ethidium bromide stained agarose gel.

Cytogenetic analysis

Twenty metaphases from bone marrow samples were analyzed after direct and/or 24h culture preparations according to standard method. Chromosomes were G-banded with trypsin-Giemsa stain (GTG banded). The karyotypes were described according to the International System for Human Cytogenetic Nomenclature [9].

Results

The fragment size of the PCR products were 327 bp for the b3a2, 252bp for the b2a2 and 307bp for the e1a2 form. The quality of RNA and efficiency of cDNA synthesis were analyzed by amplification of *ABL* gene as internal control. The amplified *ABL* product was 123bp.

Among 136 CML patients, 100 (73.5%) expressed the b3a2 form of *BCR-ABL* rearrangement, and 34 (25%) the b2a2 form of *BCR-ABL* fusion. One patient (0.75%) was *BCR-ABL* negative, but in lymphoblast transformation we detected e1a1 transcript of *BCR-ABL*. One patient (0.75%) displayed both b2a2 and b3a2 forms of *BCR-ABL* (Figure 1).

We additionally analyzed this group according to karyotype. In the subgroup of 105 patients with classical t(9;22), the b3a2 form predominated (83 patients, 79%); the b2a2 form was detected in 21 patients (20%), while one patient (1%) displayed both b3a2 and b2a2 forms. In the subgroup of 17 patients with variant Ph translocations, b3a2 was expressed in 11 (65%), and b2a2 in 6 (35%) patients. There were also 14 patients with normal karyotype. Contrary to all our results, in this group we found slight predominance of the b2a2 transcript in 7 patients (50%) in comparison to 6 patients (43%) with the b3a2 and one (7%) patient with the e1a2 form in blastic transformation (Figure 2).

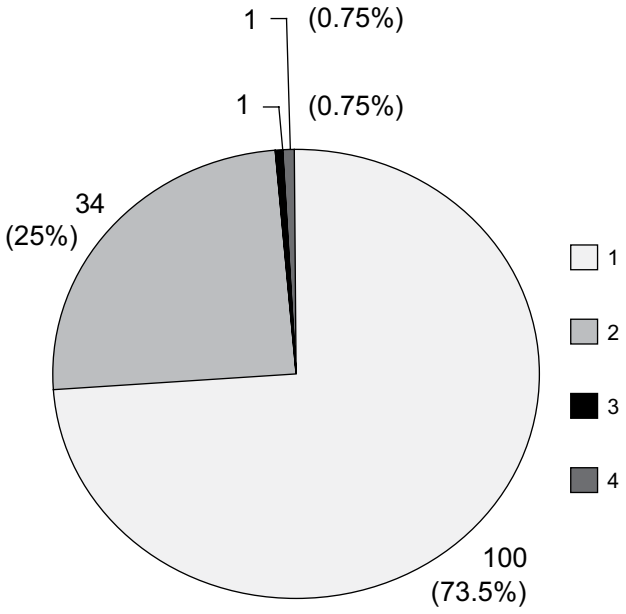

Figure 1. Frequencies of *BCR-ABL* fusion transcripts in Serbian patients with CML: **1.** b3a2 form detected in 100 patients (73.5%). **2.** b2a2 in 34 patients (25%). **3.** both b3a2 and b2a2 in one patient (0.75%). **4.** e1a2 in one patient (0.75%).

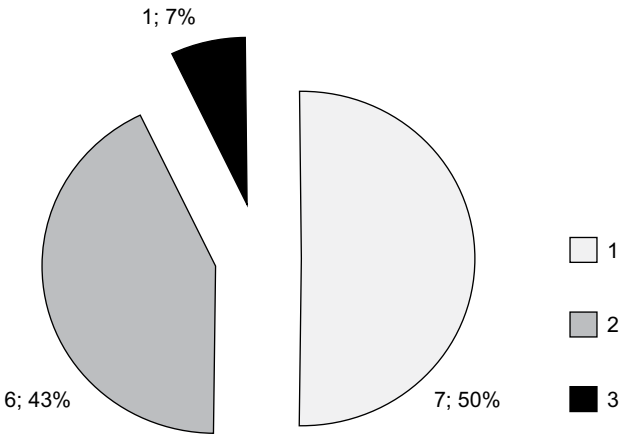

Figure 2. BCR-ABL forms in the group with normal karyotype. Among the patients with normal karyotype (n=14): **1.** Predominance of b2a2 form in 7 patients (50%). **2.** b3a2 form in 6 patients (43%). **3.** e1a2 form in one patient (7%).

Discussion

The b3a2 *BCR-ABL* form was found in 73.5% of the Serbian patients with CML, which is almost 3-fold higher than that of b2a2 (25%). The predominance of the b3a2 transcript is in concordance with the majority of other investigations. In a study of USA patients with CML, the b3a2 form was detected in 67.9% and b2a2 in 30.2% of them [10]. Two-third preponderance of the b3a2 form was found in a group of 119 patients studied in UK [11], while in a group of 37 UK patients from a different study, b3a2 transcript was detected in 62% and b2a2 in 32% of them [12]. Similarly, in a French group of 152 patients, the b3a2 form was detected in 63% and b2a2 in 34% of them [13]. In a study from Thailand 99 patients were studied and b3a2 was expressed in 61% and b2a2 in 31% of them [14]. An Eastern India study with 202 patients showed that 68% of them had the b3a2 and 32% the b2a2 form [15]. A group of 75 patients in Iran displayed predominance of the b3a2 form (62%); b2a2 was detected in 20% of the cases [16].

However, there is a completely different distribution in the Mestizo ethnic group in Ecuador. Among 40 CML patients studied in Ecuador, 94.6% had b2a2 and only 5.4% b3a2 rearrangement. A possible explanation proposed by the authors was a different genetic component in the Ecuadorian population when compared to Caucasians [17].

One patient (0.75%) in the group we studied displayed both transcripts, b2a2 and b3a2. The proposed explanation for the dual expression of b2a2 and b3a2 is the presence of adenine to guanine polymorphism in the putative branch point of *BCR* intron 13. It was concluded that *BCR* intronic polymorphism is associated with activation of a cryptic branch point resulting in reduced efficiency of RNA splicing and exon 14 (b3) skipping in *BCR* and *BCR-ABL* [18]. Another possible explanation for the expression of both transcripts is the coexistence of two cell populations displaying different transcripts.

One patient (0.75%) in our group displayed the e1a2 form of transcript in lymphoblastic transformation of CML. In the chronic phase of the disease he was *BCR-ABL* negative. There is a possibility that in the chronic phase this patient had atypical form of *BCR-ABL* transcript with breakpoints that skipped out of site of the used primers. The formation of the e1a2 transcript probably was a secondary event.

Investigations of the types of *BCR-ABL* transcripts is important for understanding the biology and pathogenesis of CML. Detection of b3a3 and b2a3 fusion transcripts led us to the important conclusion that a2 exon is unnecessary for the pathogenesis of classic CML [12].

There are some studies that investigated the response to imatinib therapy according to the form of *BCR-ABL* transcript, however with non consistent results. In a clinical study of 78 patients, it was shown that after 12 months of therapy the b3a2 form displayed complete cytogenetic response in 54% of the cases, in comparison to 24% in the b2a2 form [19]. In another study 22 patients received a 6-month imatinib treatment. The authors of that study concluded that patients with the b2a2 form had significantly less *BCR-ABL* transcripts compared with those with the b3a2 form [20]. Polampalli and coworkers, in their group of 202 patients, didn't find any difference in response to imatinib therapy between the two forms of transcripts [15].

Contrary to these results, among the patients selected by their karyotype, we detected slight predominance of b2a2 form in the group of patients with normal karyotype. The study of the frequencies of the different *BCR-ABL* transcript variants involved in CML in different ethnic groups may be a useful approach to better understand the reasons that lead to different transcript variants. The predominance of the b3a2 form in Serbian patients with CML is in concordance with other investigations, conducted mostly on Caucasian ethnic groups, but in contrast to the study performed on the Mestizo ethnic group in Ecuador.

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