Correlation between the type of bcr-abl transcripts and blood cell counts in chronic myeloid leukemia – a possible influence of mdr1 gene expression

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Abstract

The impact of BCR-ABL mRNA type (b3a2 vs. b2a2) on chronic myeloid leukemia (CML) phenotype is still a subject of controversies. We searched for a correlation between the BCR-ABL transcripts type and CML patients’ characteristics, including MDR1 gene expression. Ninety-eight untreated chronic phase CML patients were studied. The type of BCR-ABL fusion transcripts and MDR1 gene expression were determined by reverse transcriptase polymerase chain reaction. B3a2 and b2a2 transcripts were found in 53 [54%] and 44 [45%] patients, respectively. One patient co-expressed b3a2/b2a2 and was excluded from analysis. The only difference in the clinical characteristics between the two groups was the platelets count, that was higher in b3a2(+) patients [791.3±441.3×109/L vs. 440.4±283.4×109/L in b2a2(+)]; P=0.007]. MDR1 over-expression [MDR1(+) ] was observed in 48 patients (49.5%), more frequently in older patients >60 years [71% (24/34) vs. 38% (24/63) in younger; P=0.008], and was associated with a lower white blood cells (WBC) count [105.5±79.8×109/L vs. 143.6±96.5×109/L in MDR1(-) cases; P=0.047]. On performing the analysis only within the MDR1(+) group, the b2a2(+) cases were characterized with a significantly higher platelets count [908.7±470.1×109/L vs. 472.9±356.1×109/L; P=0.006] and a lower WBC count [85.4±61.2×109/L vs. 130±93.9×109/L; P=0.004] compared to b2a2(-) patients. No similar differences were found between b3a2(+) and b2a2(+) groups with normal MDR1 levels. These results indicate that the type of BCR-ABL transcripts correlates with the hematological parameters of CML, however only in the subgroup of patients characterized by MDR1 over-expression.

Introduction

Chronic myeloid leukemia (CML) is characterized by the consistent involvement of the Philadelphia chromosome (Ph) and the BCR-ABL fusion gene, which derives from the reciprocal translocation t(9;22)(q34;q11) between chromosome 9 and 22. In almost all CML patients, the breakpoint in the BCR gene involves the Major breakpoint cluster region (M-bcr). The position of the breakpoint within the M-bcr, after exon b2 (e13) or exon b3 (e14) determines two main types of the fused BCR-ABL mRNA defined as b2a2 and b3a2 transcripts differing by 75 nucleotides. These transcripts encode two 210-kDa tyrosine kinase proteins (p210BCRABL), which differ by 25 amino acids respectively.1

The impact of M-bcr breakpoint position on disease phenotype and its prognosis has been a subject of controversies for a long time. Several reports have suggested that the type of the chimeric mRNA (b2a2 or b3a2) is associated with differences in the clinical and hematological characteristics of CML patients and prognosis, despite that others failed to confirm any significant correlation.2-6 One of the most interesting finding is the association of b3a2 type of fusion transcript with a higher platelet counts with some evidence in favor7,8 and some against.10-12 A great deal of the discrepancy or discordance between the various studies could be due to qualitative and/or quantitative differences in patient or sample selection.12 However, the contradictory results might also be attributed to the presence of unknown biological factors, which can affect the obtained results. This hypothesis is supported by the observation of Shepherd et al.,1995 who failed to detect any significant difference in the presented features at diagnosis for patients with either b2a2 or b3a2 transcripts. However, in a subgroup of patients whose presenting white blood cell (WBC) count was <100×109/L, those with b3a2 transcript did have a significantly higher platelet count.13 On the other hand, it has been reported that early chronic phase CML patients with very similar characteristics (i.e. thrombocytosis, WBC count <50×109/L, and age >50 years) were characterized with elevated levels of Multidrug Resistance 1 (MDR1) gene expression.14 Therefore, it might be possible, that the level of MDR1 gene expression in the individual CML patients could influence the association between the different BCR-ABL transcripts and disease features.

MDR1 gene, located on chromosome 7q21 encodes a 170-kDa membrane transporter P-glycoprotein [P-gp] that acts as an energy-dependent, efflux pump. Over-expression of MDR1/P-gp confers resistance to the cytotoxic effects of a broad range of structurally unrelated compounds (i.e. anthracyclines, epipodophyllotoxins and vinca alkaloids) and is one of the most common mechanisms of so-called multidrug resistance (MDR) in various malignancies. Over-expression of the MDR1 gene has been found in a significant proportion of patients with different hematological malignancies and several observations suggest its poor prognostic value.15

The data concerning the incidence of MDR1 over-expression in chronic phase CML patients are controversial ranging from total absence to presence in 20-65% and up to in 100% of patients.14,16,20 Even less is known about the relationship of the MDR1 levels to clinical data at presentation in CML.14 The association between the MDR1 levels and type of BCR-ABL transcripts to our knowledge has not been studied yet.

In this study we analyzed the correlations between the BCR-ABL mRNA types and CML patients’ characteristics at presentation, including the possible impact of MDR1 mRNA levels.
Materials and Methods

Patients

Ninety-eight untreated consecutive chronic phase CML patients (59 males and 39 females) with a mean age 50.5±14.1 years were included in this study. All patients signed informed consent form before entry on study. The diagnosis of CML was established on the basis of the peripheral blood parameters and morphological analysis of peripheral blood and bone marrow aspirates and confirmed by the presence of Philadelphia chromosome and/or fusion BCR-ABL gene by conventional cytogenetics and reverse transcription polymerase chain reaction (RT-PCR) respectively.

Methods

RNA extraction and complementary DNA (cDNA) synthesis

Bone marrow and/or peripheral blood samples were collected after obtaining informed consent at the time of CML diagnosis. Mononuclear cells were separated after lysis of red blood cells with a lysis buffer (155 mM NH4Cl, 10 mM KHCO3, 0.1 mM EDTA). Total cellular RNA was isolated using Trizol Reagent (Invitrogen) according to the manufacturer’s protocol. cDNA was synthesized by reverse transcription of 1 μg of RNA in a reaction medium with final volume of 20 μL containing: 1x first-strand buffer, 200 U MMLV reverse transcriptase (Thermo Scientific, ABGene, UK), 1 mM of each dNTPs, 20 U RNAsin (Promega) and 5 μM random hexamers (Roche Diagnostics), by consecutive incubation of the samples at 37°C for 1 hour and at 99°C for 3 minutes. Random hexamers instead of specific primers were used so that different PCR analyses could be performed on the same cDNA sample.

PCR assay for BCR-ABL

The presence of P210BCR-ABL rearrangement and the type of the respective fusion transcripts were determined by a single step RT-PCR using primers (ABL-a3B: gtttgggcttcacactccatctggcagtaa (for MDR1); together with B2-M1: acctcactgaaagtgtca/b2-M2: acctcactcatgatg (Genset).25 The reaction started with denaturation at 94°C for 5 min; proceeded with 25 cycles of amplification at 94°C for 30 sec; at 57°C for 30 sec; at 72°C for 30 sec; and terminated at 72°C for 10 min. Amplification products were run in a 3% agarose gel, stained with ethidium bromide, visualized after UV illumination and photographed. For all RT-PCR reactions, K562 (b3a2) and/or BV173 (b2a2) cell lines were used as a positive control and RNA from healthy donors as a negative control.

PCR assay for MDR1 gene expression

P CR was carried out by simultaneous amplification of the MDR1 and β2-microglobulin (β2-M) RNA, as an internal control. Briefly, 5 μL of cDNA were amplified in 50 μL medium, containing 1x PCR buffer, 1.5 mM MgCl2; 200 μM each of dNTPs; 1 U Taq polymerase (Promega) and 0.4 μM each of primers MDR1-TL9: tcaacagttcagcaagcagag / MDR1-TL10: gtttcagccatccattataagcaca (for MDR1); together with B2-M1:acctcactgaaagtgtca/b2-M2:acctcactcatgatg primers (for β2-M) (Genset).25 The reaction started with denaturation at 94°C for 5 min; proceeded with 25 cycles of amplification at 94°C for 30 sec; at 57°C for 30 sec; at 72°C for 30 sec; and terminated at 72°C for 10 min. Amplification products were run in a 3% agarose gel, stained with ethidium bromide, visualized after UV illumination, and photographed. For all RT-PCR reactions, K562 (b3a2) and/or BV173 (b2a2) cell lines were used as a positive control and RNA from healthy donors as a negative control.

Results

In this study we analyzed the type of BCR-ABL transcripts and MDR1 gene expression in 98 untreated adult patients with chronic phase CML and correlated the findings with clinical, hematological and molecular data (Table 1).

Types of BCR-ABL transcripts in correlation to basic patients’ characteristics at presentation

RT-PCR was used to confirm the diagnosis of CML by detection of fusion BCR-ABL gene, and to reveal the type of transcripts depending on the M-bcr region breakpoint location. All studied patients were found to be BCR-ABL positive. Fifty-three of them expressed b3a2 transcripts [54%], whereas forty-four of the remaining cases were positive for b2a2 transcripts [45%]. Co-expression of both types of transcripts (b2a2 (+= b3a2) was observed in one patient [1%], who was excluded from further analysis. All patients were analyzed to determine the relationship between the type of transcripts and the presenting features at diagnosis including age, sex, white blood cells (WBC) count, platelets and hemoglobin (Hb) concentration. The platelets count was statistically higher in the subgroup of patients expressing b3a2, than those with b2a2 transcript (791.3±441.3×109/L vs. 440.4±283.4×109/L; P=0.007). The remaining variables were not significantly different in both groups (b2a2 and b3a2) (P>0.05). (Table 2).

MDR1 expression in correlation to basic patients’ characteristics at presentation

The pattern of MDR1 amplification in CML

Table 1. Basic demographic and hematological features of patients included in the study.

| Total number of patients [n=] | 98 |
|---|---|
| Gender | | |
| Males [n=] | 59 [60%] |
| Females [n=] | 39 [40%] |
| Age | | |
| Mean ± SD [years] | 50.5±14.12 |
| WBC | | |
| Mean ± SD [×109/L] | 125.2±90.4 |
| Platelets | | |
| Mean ± SD [×109/L] | 615.8±406.3 |
| Hemoglobin | | |
| Mean ± SD [g/L] | 111.7±27.0 |
patients varied considerably from case to case with a different intensity of the reaction ranging from negative to strongly positive. RT-PCR revealed moderate or strong positive reaction, corresponding to over-expression of MDR1 gene [MDR1(+)], in 48 out of 97 patients (49.5%). No product of MDR1 amplification, corresponding to a normal level of MDR1 expression [MDR1(-)], was seen in the remaining 49 patients (50.5%). The mean WBC count was significantly lower in MDR1(+) patients [105.5±79.8±10⁹/L vs. 143.6±96.5±10⁹/L (P =0.047) in MDR1(-)]. In addition, MDR1 gene over-expression was significantly more frequent in elderly patients with age >60 years - 71% (24/34), compared to that in younger patients - 38% (24/63) (P =0.008), although, the mean age did not differ between the MDR1(+) and MDR1(-) patients (P =0.05). No significant differences in regard to sex, type of BCR-ABL transcripts, platelet count and hemoglobin concentration between the two groups of patients expressing either normal or elevated MDR1 levels were found (P > 0.05) (Table 3).

The impact of MDR1 status of patients on the association between the types of BCR-ABL transcripts with basic characteristics at the presentation

In order to determine the impact of MDR1 status of patients on the association of the type of transcripts with clinical and biological characteristics, both groups were additionally divided into subgroups with normal and elevated MDR1 levels. The analysis of the b3a2 patients revealed a lower mean WBC count in MDR1(+) cases compared to the respective value in MDR1(-) patients [85.4±61.2±10⁹/L vs. 150.8±93.9±10⁹/L (P=0.004)]. However, no differences in the patients’ characteristics were found in the b2a2 group according to the MDR1 status. Applying the same approach by stratifying the patients according to the MDR1 status, no significant differences in patients’ characteristics were observed between b3a2 and b2a2 cases with normal MDR1 levels (Table 4). In contrast, within the group with MDR1 over-expression, the b3a2-positive cases were characterized with a significantly higher platelets count (P =0.006) and lower WBC count (P =0.004) compared to b2a2-positive patients (Table 4).

**Discussion**

Data concerning the association between the type of BCR-ABL transcripts and CML patient characteristics remain contradictory. Therefore, we compared the main clinical fea-

### Table 2. Association between clinical and laboratory data and the type of fusion BCR-ABL transcripts for the 97 chronic myeloid leukemia patients.

| Parameter | b3a2 | b2a2 | P |
|-----------|------|------|---|
| Patients  | 53 [55%] | 44 [45%] | NS |
| Age       | 51.0±13.7 | 49.9±4.8 | NS |
| Gender    | Males [n=] | 28 [53%] | 25 [47%] | NS |
|           | Females [n=] | 31 [70%] | 13 [30%] | NS |
| WBC       | 119.5±81.7 | 132.4±100.8 | NS |
| Platelets | Mean ± SD [×10⁹/L] | 791.3±441.3 | 440.4±283.4 | 0.007 |
| Hemoglobin| Mean ± SD [g/L] | 116.0±19.1 | 107.9±32.5 | NS |

### Table 3. Clinical parameters at diagnosis of CML patients in chronic phase expressing normal and elevated levels of MDR1 gene.

| Parameter | MDR1(+) patients | MDR1(-) patients | P |
|-----------|------------------|------------------|---|
| Patients  | 48 [49.5%] | 49 [50.5%] | NS |
| Age       | 52.4±15.4 | 48.7±12.6 | NS |
| Patients  | >60 years [n=] | 24/34 [71%] | 10/34 [29%] | 0.008 |
|           | <60 years [n=] | 24/63 [38%] | 38/63 [62%] | NS |
| Gender    | Males [n=] | 27 [56%] | 32 [65%] | NS |
|           | Females [n=] | 21 [44%] | 17 [35%] | NS |
| BCR-ABL transcripts | b3a2 [n=] | 25 [52%] | 28 [57%] | NS |
|           | b2a2 [n=] | 23 [48%] | 21 [43%] | NS |
| WBC       | Mean ± SD [×10⁹/L] | 105.5±79.8 | 143.6±96.5 | 0.047 |
| Platelets | Mean ± SD [×10⁹/L] | 647.2±447.8 | 591.0±381.4 | NS |
| Hemoglobin| Mean ± SD [g/L] | 110.6±38.6 | 112.4±17.2 | NS |

### Table 4. Clinical and laboratory data of patients with different types of fusion BCR-ABL transcripts and MDR1 expression.

| Parameter | MDR1(+) patients | MDR1(-) patients | P |
|-----------|------------------|------------------|---|
| Age: mean±SD [years] | 53.0±15.2 | 51.6±16.0 | 49.3±12.1 | 47.9±13.8 | NS |
| Patients  | >55 years [n=] | 12 [48%] | 13 [52%] | 7 [35%] | 5 [24%] | NS |
|           | <55 years [n=] | 11 [44%] | 14 [56%] | 16 [70%] | 21 [75%] | 16 [76%] | NS |
| Gender    | Males [n=] | 11 [44%] | 16 [70%] | 17 [61%] | 15 [71%] | 13 [61%] | NS |
|           | Females [n=] | 14 [56%] | 7 [30%] | 11 [39%] | 6 [29%] | 16 [58%] | NS |
| WBC       | Mean ± SD [×10⁹/L] | 85.4±61.2 | 130±93.9 | 150.8±86.6 | 134.6±109.5 | NS |
| Platelets | Mean ± SD [×10⁹/L] | 908.7±470.1 | 472.9±356.1 | 727.3±434.0 | 403.8±189.1 | NS |
| Hemoglobin| Mean ± SD [g/L] | 123.5±18.6 | 103.1±46.2 | 112.7±19.4 | 112.1±15.6 | NS |
tures of BCR-ABL-positive CML patients to the type of transcripts. The only variable, that showed significant difference between the two groups, was the platelet count, which was significantly higher in patients who expressed b3a2, providing additional evidence that b3a2 transcripts seem to be associated with a higher thrombopoietic activity in CML.17,18

In addition, the most interesting finding in our study was that the significant correlation between the elevated platelet count and b3a2 transcripts compared to b2a2 was restricted only to the subgroup of patients with MDR1 over-expression. Moreover, the MDR1 over-expression status outlined a significant association between the WBC count and the type of transcripts as we observed lower counts in the b3a2-positive cases compared to b2a2. No similar differences were found between b3a2- and b2a2-positive patients within the subgroup with normal MDR1 levels.

A possible explanation is that the significant differences in the platelets and WBC count in patients with b3a2 and b2a2 transcripts only in patients with MDR1 over-expression reflect a preferential activation of a common specific signaling cascade in the b3a2- positive cases, which is not active in the remaining patients. This might be related to variations in the level of BCR-ABL transcripts, since a dose-dependent hierarchy of BCR-ABL induced activation of signaling pathways and biological effects exists.25 Moreover, it has been reported that b3a2 transcripts may affect the thrombopoietic activity in CML also in a dose-response manner.26 Alternatively, it might be also possible that MDR1 over-expression due to additional molecular abnormalities might interfere with differences in the structure of two p210BCR-ABL proteins preferentially promoting the interaction of b3a2 with the cytoketosin of megakaryocytes27 resulting in the particular CML phenotype presented by thrombocytosis and relatively lower leukocytosis.

However, this hypothesis is only speculative and needs to be confirmed because the precise molecular mechanisms that underlie an elevated MDR1 expression in untreated CML patients are still unknown. In general, data concerning MDR1 expression in CML are still insufficient and contradictory. Our study provides some interesting information in regard to the incidence of MDR1 over-expression and the related patients’ characteristics. We found elevated levels in approximately 50% of untreated patients. This result was in agreement with the data, reported earlier by Giles et al. (1999) and Weide et al. (1998), who using alternative methods revealed high levels of MDR1/P-gp expression in 57% and 41% of CML cases, respectively.14,26 In our study, there were no differences in the prevalence of the different types of transcripts, as well as in the gender, platelets count and hemoglobin level between patients with normal and elevated levels of MDR1. However, the incidence of MDR1 gene over-expression was significantly more frequent among the older patients. Similar results were observed not only in chronic phase CML patients,14 but also in acute leukemias.27,28 Moreover, an age-related increase of P-gp expression was found on peripheral blood cells of healthy individuals.29 All these data suggest that common biological factors may contribute to the drug resistance in the elderly.

Additionally, we and others14 found that MDR1 over-expression in CML was associated with a lower WBC count compared to the patients with normal MDR1 levels. Interestingly, in acute leukemias, the MDR1 over-expression has been reported to be associated with hyperleukocytosis, both in AML27 and ALL.28 To our knowledge, no explanation for these differences has been reported so far. Some observations suggest that the MDR1 gene over-expression in CML might be one of multiple cellular alterations caused by the p210BCR-ABL. Physiologically, the regulation of MDR1 gene expression involves various signaling pathways (i.e. p53, SP1, NF-Y, AP-1, NF-kb, CEBP and RAS).30 A key role in this process plays Jun NH2-terminal protein kinase (JNK), which in response to stress-signals activates the heterodimeric AP-1 transcription factor, which in turn interacts with the MDR1 gene promoters and enhances transcription.14 Given that it has been shown that p210BCR-ABL may activate most of these pathways,1 including JNK,30 it seems possible that the hybrid oncoproteins may also affect MDR1 expression in this manner.31 This hypothesis is also supported by the observation that transduction of normal cells with b3a2 cDNA leads to upregulation of the expression of P-glycoprotein, the product of the MDR1 gene.31 Alternatively, the differences in the MDR1 expression in CML patients might be related to defects in the methylation machinery as a distinct molecular pathway leading to malignant transformation14 or might be a random event associated with nucleotide and/or haplotype variants of the MDR1 gene, which seems to be important for interindividual differences of its expression.35-38

In conclusion, our preliminary results indicate that the type of BCR-ABL transcripts correlates with the hematological parameters of CML patients, however only in the subgroup of patients characterized by over-expression of MDR1 gene. Further studies need to elucidate the molecular events underlying the relationship between the type of BCR-ABL transcripts and expression of MDR1 gene in CML.

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