Leukemia Research Reports 20 (2023) 100373

Contents lists available at ScienceDirect

Leukemia Research Reports

journal homepage: www.elsevier.com/locate/lrr

Chronic-phase chronic myeloid leukemia: Incidence of BCR/ABL transcript and its correlation with presenting features, response to treatment, and survival

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ARTICLE INFO

Keywords:
Myeloproliferative disorder
Chronic phase
Philadelphia chromosome
BCR-ABL transcript
Imatinib mesylate

ABSTRACT

Introduction: Chronic myeloid leukemia (CML) is characterized by Philadelphia chromosome resulting in the fusion between the BCR gene, located on chromosome 22, and the ABL gene on chromosome 9. The prognostic significance of BCR-ABL transcript variants in CML is controversial. The aim of the current study was to evaluate the clinico-hematological presentation and evolution of the disease, response to treatment and survival according to transcript type in chronic phase CML patients.

Results: The median age of our population was 50 years with a slight female predominance (sex-ratio 0.78). Sixty percent had the b3a2 transcript and 34% had the b2a2 type. Patients with the co-expression of these two transcripts (4.5%) and those with e19a2 were excluded from the analysis. Patients with b3a2 subtype were associated significantly with thrombocytosis ($p = 0.006$) and higher Sokal score ($p = 0.038$) compared to those with b2a2 transcript. The two isolated transcripts were not significantly associated with gender, age group, blast cell percentage or the identified ranges of spleen size. Complete cytogenetic response at 12 months for b3a2 patients and b2a2 patients was 78.6% and 21.4% respectively. This difference was statistically significant ($p = 0.001$, HR = 9.5, 95% CI 6.5–13.7). Patients with b3a2 transcript had a higher rate of optimal molecular response at 3 months ($p = 0.04$, HR = 4.2, 95% CI 1–17.3) and major molecular response at 12 months ($p = 0.004$, HR = 4.9, 95%CI 1.5–15.1). At the date of last follow-up, most patients achieving deep molecular response (MR4 or deeper) belonged to b3a2 group (79%) ($p = 0.003$, HR = 5.2, 95% CI 1.6–16.4). We did not find a significant difference in OS and EFS between the two groups.

Conclusion: Our study concluded that b2a2 transcript is a prognostic factor in cytogenetic and molecular response but further studies are needed to complete this aspect.

1. Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by clonal damage of the hematopoietic stem cell with the presence of a genetic anomaly: the reciprocal translocation between chromosomes 9 and 22 t(9;22)(q34;q11.2) giving rise to the Philadelphia chromosome. This translocation results in the fusion of two genes Breakpoint Cluster Region (BCR) and Abelson (ABL) encoding a chimeric protein with constitutive tyrosine kinase activity responsible for granular lineage excess proliferation [1]. The breakpoint in the ABL gene on chromosome 9 is usually located in exon a2. In 95% of cases the breakpoints in the BCR gene on chromosome 22 occur in the major BCR (M-BCR), either in exon e13 (b2) or exon e14 (b3), generating two slightly different chimeric transcripts. These breakpoints give rise to various BCR-ABL rearrangements, most commonly the e13a2 (b2a2) and e14a2 (b3a2), which code for a p210 protein [2]. In some cases, there is a co-expression of these transcripts due to alternative splicing (b2a2+b3a2). Less frequently, the BCR breakpoint occur in exon 19 resulting in e19a2 transcript, which codes for p230 protein, and more rarely in exon 1 generating e1a2 transcript coding for p190 protein [3].

The prognostic value of transcript discrimination is controversial. Some studies were performed to determine the influence of BCR-ABL transcript type on the clinical outcome and correlation with treatment response and survival in CML patients. We conducted a retrospective

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https://doi.org/10.1016/j.lrr.2023.100373
Received 8 April 2023; Accepted 25 May 2023
Available online 26 May 2023

study that included patients treated for chronic phase CML. The aim of this study was to evaluate the prognostic impact of the different subtypes of BCR-ABL transcript on the outcome of CML patients treated with the front-line treatment (Imatinib mesylate).

2. Patients and methods

2.1. Patients

The current study included newly diagnosed chronic phase CML patients. It was conducted in the Hematology Department of Fattouma Bourguiba University Hospital of Monastir, Tunisia, over the period between February 2003 and April 2022. Patients' data was collected from the medical files by the corresponding author. Peripheral blood smear and bone marrow aspiration were systematically performed at the first presentation of the patient. Cytogenetic and molecular studies confirmed the diagnosis. All patients were treated with 400 mg / day Imatinib. Patients expressing both transcripts (b2a2+b3a2) and e19a2 type were excluded from the analysis.

2.2. Cytogenetic study

The conventional bone marrow karyotype was performed to highlight the presence of the Philadelphia chromosome and additional cytogenetic abnormalities (ACA). It was performed in R-bands. The technique mainly includes culture, blocking of mitoses in metaphase, hypotonic shock, fixation, spreading and finally marking and staining followed by microscopic reading. Fluorescent in situ hybridization (FISH) is not mandatory for the diagnosis, but it was performed to detect chromosomes not detected by conventional karyotype methods. This technique uses genomic probes that targets specific known chromosomal regions and can be performed on both interphase and metaphase nuclei.

2.3. Reverse transcriptase polymerase chain reaction

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) is the technique recommended by the European Against Cancer for the diagnostic confirmation of CML by the detection of the BCR-ABL transcript. It includes the following technical steps:

- Isolation of nucleated cells: Molecular analysis is performed on ribonucleic acid (RNA). The nucleated cells are first isolated from bone marrow or peripheral blood sample after lysis of red blood cells.
- RNA extraction: it is performed on nucleated cells preserved in Trizol according to Chomczynski and Sacchi method [4]. It includes an extraction step with chloroform, then precipitation steps with isopropanol, washing with ethanol, drying and finally a dilution step of the RNA pellet in diethylpyrocarbonate water.
- Retro-transcription: The ribonucleic acids are retro-transcribed into single-stranded complementary Deoxyribonucleic Acid (DNA) using the reverse transcriptase enzyme.
- Multiplex RT-PCR: The transcripts searched are generally the M-BCR transcripts (b3a2, b2a2, b2a3 and b3a3) for "major" breakpoint and m-BCR (e1a2 and e1a3) for "minor" breakpoint. It consists in carrying out 2 PCR in parallel with 2 different pairs of primers framing the fusion point.
- Agarose gel electrophoresis: Once the PCR program is completed, the amplification product is migrated in agarose gel. Electrophoresis will allow the amplicons to migrate and to be identified. It aims to separate molecules according to their size through a gel with an electric field. The revelation of the DNA bands is performed through ethidium bromide which is an intercalating agent of the double-stranded DNA. These bands are visualized with an ultraviolet imager.

2.4. Quantitative reverse transcriptase polymerase chain reaction

The Quantitative Reverse Transcriptase Polymerase Chain Reaction (q RT-PCR) is performed to monitor residual disease after treatment. It is based on a real-time PCR technique developed in the late 1990s and continuously improved to be a largely standardized method [5]. We used the GeneXpert system (Cepheid) which is an automated method for the quantification of BCR-ABL transcript.

2.5. Operational definitions

International criteria have been used to stratify responses to tyrosine kinase inhibitor (TKI) therapy based on hematological, cytogenetic, and molecular data. The European Leukemia Net (ELN) guidelines define optimal, alert and failure responses to TKI therapy. According to ELN2020 recommendations [6] Complete Hematological Response (CHR) is defined as Leukocyte count $<10 \times 10^9$/L, platelet count$<450 \times 10^9$/L, no immature cells (blasts, promyelocytes, myelocytes) in the peripheral blood and non-palpable spleen. Complete cytogenetic response (CCyR) is defined as the absence of Philadelphia chromosome metaphases. When monitoring residual disease, the expression of the BCR-ABL1/ABL1 ratio according to the international scale (IS) has made it possible to define several thresholds (molecular responses or MRs) to verify therapeutic efficacy based on the IRIS (International Randomized study of Interferon versus STI571) study. These points are defined as a decrease of 3, 4, 4½, or 5 log decimal points from a theoretical baseline at diagnosis (defined as BCR-ABL1/ABL1 = 100%). Thus a value of 0.1% corresponds to major molecular response (MMR or MR3), values of 0.01%, 0.0032%, 0.001% define MR4, MR4.5 and MR5 as deep molecular responses (DMR), respectively. Optimal molecular response at 3 months is defined as BCR/ABL $\leq$ 10%. Overall survival (OS) is defined as the time of Imatinib onset to the date of death from any cause or date of the last follow-up. Event-free survival (EFS) is defined as the time of Imatinib onset to the date of the occurrence of any event. Event is defined as: hematological, cytogenetic or molecular relapse, progression to accelerated or blast phase or death from any cause.

2.6. Statistical analysis

All statistical analyses were performed using the SPSS 21 software. The type of BCR-ABL transcripts were analyzed in correlation to clinical and hematological characteristics of the disease, response of treatment and survival. Comparisons of categorical variables between groups were evaluated by Fisher's exact test. P values less than 0.05 were considered as statistically significant. Time was calculated as months. Survival probabilities were estimated by the Kaplan-Meier method and compared by the log-rank test.

3. Results

3.1. Patients

We included 73 patients with chronic phase CML diagnosed and followed for 19 years. The median age of the study population was 50 years with extremes ranging from 12 to 88 years. A maximum of frequency was observed in the 41–60 years age group (42%). The sex ratio was 0.78. The disease was discovered fortuitously in 40% of cases. At diagnosis, splenomegaly was found in 43 patients (59%). White blood cell (WBC) count ranged from 28×10^9/L to 515×10^9/L with a median of 120×10^9/L. Leukocytosis more than 100×10^9/L was noted in 59% of cases. Anemia was found in 35% of our patients. The median hemoglobin (Hb) level was 10.8 g/dl. It varied between 5.4 and 14.1 g/dl. The platelet count ranged from 91×10^9/L to 2600×10^9/L with a median of 509×10^9/L. Thrombocytosis was found in 33 cases (45.2%). The percentage of basophils varied from zero to 25% with a median of 3%.

Blood blasts ranged from zero to 15% with a median of 1%. The cytogenetic study revealed that Philadelphia chromosome was found in 98%. ACA was found only in one case and that was a structural abnormality of chromosomes 9 and 12. Sixty percent of patients expressed b3a2 transcript and 34% expressed b2a2.

When analyzing the presenting features of the disease according to transcript type, we found that a higher rate of thrombocytosis was significantly noted in patients with b3a2 transcript ($p = 0.006$). These patients presented with higher risk Sokal [7] Hasford [8] and ELTS [9] scores than patients with b2a2. This association was significant only with Sokal score ($p = 0.038$) (Table 1).

3.2. Treatment response according to transcript type

According to the ELN 2020 recommendations, at three months of the Imatinib onset, 97% of our patients obtained a CHR, 60% achieved a CCyR. Optimal molecular response at three months was noted in 80% of our study population. The incidence of cumulative CCyR and MMR at 12 months was obtained in 88% and 82% respectively

When analyzing the therapeutic response according to transcript type, the proportion of patients with b3a2 achieving CCyR at 12 months was higher compared with b2a2 patients (78.6%% vs 21.4%). This difference was statistically significant ($p = 0.001$, hazard risk [HR] 9.5, 95% confidence interval [CI] 6.5–13.7). Additionally, we observed that patients with b3a2 transcript had a higher rate of optimal molecular response at three months ($p = 0.04$, HR = 4.2, CI 1–17.3) and MMR at 12 months ($p = 0.004$, HR = 4.9, CI 1.5–15.1). Similarly, for DMR at the date of the last follow-up, patients with b2a2 had a significantly lower rate of response compared with those with b3a2 ($p = 0.003$, HR = 5.2, CI 1.6–16.4) (Table 2).

3.3. Survival outcomes according to transcript type

Five-year probabilities of OS and EFS for our entire population were 96% and 63% respectively. We compared the survival outcomes in different transcripts groups. Patients with b3a2 had a slightly higher survival rates than b2a2 group but without a significant difference. The five-year OS for patients expressing b3a2 and b2a2 was estimated at 97% and 95% respectively ($p = 0.57$). Five-year probabilities of EFS were 65.8% and 59% respectively ($p = 0.43$).

Table 1
Distribution of the clinical and hematological characteristics according to transcript type.

		Transcript b2a2 ($n = 25$)	b3a2 ($n = 44$)	*P* value
Sex	Male	44.4%	55.6%	0.25
	Female	30%	70%	
Age	<40	45.5%	54.5%	0.28
	≥40	31.5%	68.5%	
SMG (below costal margin)	<10cm	44%	56%	0.3
	≥10cm	28.5%	71.5%	
WBC	$<100 \times 10^9$/L	33.3%	66.7%	0.4
	$\geq 100 \times 10^9$/L	38.5%	61.5%	
Platelets	$\leq 450 \times 10^9$/L	55.5%	44.5%	0.006
	$>450 \times 10^9$/L	21%	79%	
Blasts	<5%	37.7%	62.3%	0.6
	≥5%	28.5%	71.5%	
Basophils	<5%	37%	63%	0,89
	≥5%	35%	65%	
Sokal score	Low	58%	42%	0.038
	Intermediate	35%	65%	
	High	21%	79%	
Hasford score	Low	50%	50%	0.4
	Intermediate	27.5%	72.5%	
	High	38.5%	61.5%	
ELTS score	Low	33.5%	66.5%	0.9
	Intermediate	47%	53%	
	High	37%	63%	

SMG: Splenomegaly; WBC: White blood cells.

Table 2
Distribution of therapeutic responses according to transcript type.

	Transcript type b2a2 ($n = 25$)	b3a2 ($n = 44$)	*P* value
CHR at 3 months	38%	62%	0.27
CCyR at 3 months	33.5%	66.5%	0.88
CCyR at 12 months	21.4%	78.6%	0.001
Optimal MR at 3 months	32.3%	67.7%	0.04
MMR at 12 months	22%	78%	0.004
DMR	21%	79%	0.003

CHR: complete hematological response; CCyR: complete cytogenetic response; MR: molecular response; MMR: major molecular response; DMR: deep molecular response.

4. Discussion

CML is a rare hematological malignancy that belongs to the myeloproliferative neoplasms. It represents 15% of all leukemia cases and affects one to two individuals per 100,000 inhabitants [10]. Blood count and smear are the key test for the diagnosis of CML. The most common blood abnormalities are hyperleukocytosis with a predominance of neutrophils, normocytic normochromic anemia and thrombocytosis [11]. A conventional cytogenetic karyotype is essential for the diagnosis of CML. It is performed on bone marrow or a blood sample. It reveals the Philadelphia chromosome resulting from the translocation (9,22) in 95% of cases or any ACA. The conventional cytogenetic karyotype may be normal in 5% of cases if there is a cryptic insertion of chromosomal material undetectable by conventional cytogenetic techniques, FISH is therefore necessary [10]. Despite the major contribution of molecular biology in CML, cytogenetic testing remains essential for diagnosis and follow-up of the disease until the CCyR is achieved. It is also essential in the event of TKI resistance or cytogenetic relapse [12].

Molecular biology is an essential step in the diagnosis and the management of CML. At diagnosis, it allows the detection of BCR-ABL transcript by the qualitative RT-PCR method and its type to be specified [13]. The b2a2 or b3a2 transcripts are present in more than 95% of cases in the literature [10]. The higher frequency of the b3a2 transcript compared to b2a2 has been described in different published series from North Africa [2] Eastern countries [14] and Europe [15]. This was noted in our study (60% of cases for b3a2 versus 34% for b2a2). On the other hand, the predominance of the b2a2 transcript has been described in different studies from Western countries [16], as well as in some African series [17].

The prognostic impact of BCR-ABL transcript variants has always been a controversial issue. In our study, we evaluated the impact of BCR-ABL transcripts on several epidemiological, clinical, and biological characteristics including gender, age, spleen size, hyperleukocytosis as well as platelet count, percentage of blast cells and basophils at diagnosis. Our study did not reveal a significant association between BCR-ABL transcripts and a particular profile or clinico-biological presentation, which is in accordance with several studies in the literature [18]. Ghalesardi et al. showed that the ratio of male to female was higher in the b2a2 group compared to b3a2 [19]. In a Mexican study, a higher leukocyte count in patients with the b2a2 transcript was reported [20]. However, in our patients, hyperleukocytosis $\geq 100 \times 10^9$/L was found to be more associated with the b3a2 transcript type without statistically significant results, which is consistent with other studies [21]. Some authors showed no correlation between platelet count and BCR-ABL transcript variants [22]. Others found a higher platelet count in patients carrying b3a2, which is in accordance with our study results. They also suggested an influence of this transcript on thrombopoiesis [19]. Our results showed that the b3a2 transcript was frequently correlated with a high Sokal score ($p = 0.038$). In contrast to some studies, where

patients with b2a2 variant had higher Sokal, Hasford, and EUTOS scores [23]. Other authors revealed no difference between transcript variants and their impact in the different prognostic scores [18]. As for the impact of M-BCR transcript types on the response to Imatinib therapy, we found a better cytogenetic and molecular response in patients with the b3a2 transcript. For the studies conducted before the Imatinib era, there was no incrimination of the transcript type on the clinical outcome [22]. However, since the introduction of the Imatinib as a front-line treatment, the results have been controversial. Studies including a small number of patients, at different stages of the disease, suggested that patients with the b2a2 transcript responded better to Imatinib in terms of hematological and molecular responses [23] while other studies which included a higher number of patients showed a better molecular response to Imatinib for patients with the b3a2 transcript [24]. Pagnano et al. found that b3a2 patients had higher rates of CCyR at six months and higher rates of optimal molecular response at three months compared with b2a2 patients [25]. In a recent Spanish study, Marcé et al. showed no differences in the cumulative incidence of cytogenetic responses or in the DMR, but they observed that b3a2 transcript had a positive impact on MMR at six months [18]. Some other studies demonstrated that patients with b3a2 type had prolonged DMR [26].

In our study, the analysis of survival according to transcript type did not find a significant difference between the two groups. This was in agreement with some studies, such as the German group study [27] and the Brazilian study [25], but in disagreement with the findings of some authors. Indeed, Jain et al. found that b2a2 was associated with lower rate of EFS, but this result was not statistically significant [24]. Analyzing 559 CML patients, Castagnetti et al. showed that b3a2 patients responded faster and more deeply to Imatinib, and had a significantly higher seven-year OS and EFS [28]. Pagnano et al. showed no difference at five-year OS but a better 10-year OS was observed in patients with b2a2 transcript. Furthermore, Marcé et al. found that b2a2 was significantly associated with a better OS [18]. A larger study on 1494 CML patients treated with Imatinib found no significant difference in OS and EFS rates in the two groups [29]. On the other hand, some authors suggested that b2a2 patients had a higher risk of progression to acute leukemia [24].

5. Conclusion

In conclusion, b3a2 transcript type was found to be associated with higher Sokal score for chronic phase CML patients treated with Imatinib. On the other hand, b3a2 patients seem to respond well to the Imatinib therapy. Given the small sample size of our population, more studies are needed to complete this aspect and to validate the association between the transcript variants and cytogenetic and molecular responses.

Informed consent

Authors declare that consent was obtained by all participants in this study and that this article has not been published elsewhere.

Authors' contributions

BL and NS wrote the manuscript. WB helped in collecting data. IO and MAL revised the manuscript. All authors approved the final manuscript.

Ethic approval

Authors declare that consent was obtained by all participants in this study and that this article has not been published elsewhere.

Funding

The authors declared that they did not receive any financial support.

Declaration of Competing Interest

The authors declare that no competing interests exist.

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