Original article

Does *BCR-ABL* transcript type influence the prognosis of patients in chronic myelogenous leukemia chronic phase?

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ABSTRACT

Introduction and objective: In this study, we evaluated the influence of the transcript type on hematological and clinical parameters, as well as the event-free survival of 50 patients in the Chronic myeloid leukemia chronic phase.

Methods: We reviewed the medical records of 55 patients with Chronic myeloid leukemia. The eligibility criteria were based on the availability of hematological and clinical baseline data in the medical records. Data on *BCR-ABL* transcripts were obtained from medical records.

Results: Eighteen patients (36%) had the *b2a2* transcript, 24 (48%) had *b3a2* and 8 (16%) had *b2a2/b3a2*. The median platelet count for transcripts *b2a2*, *b3a2* and *b2a2/b3a2* was $320.65 \times 10^3/L$, $396 \times 10^3/L$, and $327.05 \times 10^3/L$, respectively ($p = 0.896$). We could not find any differences in relation to the other hematological parameters, when compared to the transcript type. Comparison between spleen and liver size and type of transcript did not differ inside the groups ($p = 0.395$ and $p = 0.647$, respectively) and the association between risk scores and transcript type did not show statistical significance ($p > 0.05$). The 21-month probability for event-free survival was 21%, 48% and 66% for the transcripts *b2a2*, *b3a2* and *b2a2/b3a2* respectively ($p = 0.226$)

Conclusion: We conclude that the expression *BCR-ABL* transcripts have no influence on hematological, clinical and event-free survival parameters of patients in the Chronic myeloid leukemia chronic phase.

Introduction

Chronic myelogenous leukemia (CML) is characterized by the presence of a reciprocal and balanced translocation, the t(9;22) (q34;q11), which originates the Ph chromosome. The *BCR-ABL* chimeric gene formation, that encodes a BCR-ABL protein with constitutive tyrosine kinase activity, is the main result of this translocation.[1] This hybrid gene commonly results from the *BCR* gene cleavage in the major breakpoint cluster region (M-BCR) with the fusion of the *ABL* gene at the exon 2 (a2) level. The *BCR* gene breakdown in the M-BCR may occur after exon 13 (e13 or b2) or after exon 14 (e14 or b3), thereby generating the *BCR-ABL* gene, which can form *b2a2* or *b3a2* mRNA molecules after transcription. Around 5–10% of the patients may also present alternative splicing events that allow simultaneous expression of the transcripts *b2a2* and *b3a2*.[2–5]

The prognostic significance of these transcripts has been investigated over time; however, their real contribution has not been established. Many studies suggest that hematological and clinical parameters can be influenced by the transcriptional variant type that is expressed.[6–8] Studies in this field have shown controversial results in terms of platelet counts: although most studies have failed to demonstrate any significant correlation, others have demonstrated higher platelet counts in patients with the *b3a2* transcript.[6,9–11]

A considerably smaller number of studies attempted to demonstrate transcript influence on the event-free survival outcome of patients expressing different transcripts. Although no significant results were found, a previous study found that patients with the *b2a2* transcript showed a lower event-free survival (EFS) trend.[10,12]

In this study, we evaluated the relevance of hematological and clinical parameters, as well as possible survival outcomes in CML-chronic phase patients expressing different types of *BCR-ABL* transcripts.

Materials and methods

Patients

The medical records of 55 patients with CML treated at Walter Cantídeo University Hospital (HUWC) between November 2000 and November 2015 were included in this study, performed under approval by the Federal University of Ceará Research Ethics Committee. The eligibility criteria were based on the availability of hematological and clinical baseline data in the medical records. Patients expressing the transcripts *e13a2* (*b2a2*), *e14a2* (*b3a2*) or co-expressing both *e13a2* (*b2a2*) and *e14a2* (*b3a2*) transcripts were included in the analysis. Patients younger than 18 years, pregnant women, and those in the accelerated and blast phases at diagnosis were excluded. Overall, the patients were submitted to an initial cytoreductive therapy with oral hydroxyurea (HU). After therapy with HU, patients were treated with first-generation tyrosine kinase inhibitors (ITKs) and when necessary, second-generation ITKs were used. Data on *BCR-ABL* transcripts were obtained from medical records, not necessarily at the diagnosis.

Statistical analysis

The Kruskal–Wallis test or one-way ANOVA was used for the analysis of quantitative variables of the three groups, while the χ^2 test was performed for categorical variables. The probability of EFS was estimated by the Kaplan–Maier method and compared by the log-rank test. The EFS was measured from the diagnosis date until the date when the event was observed. Events considered for EFS analysis were loss of hematological, cytogenetic, and molecular response, presence of neutropenia or thrombocytopenia, clonal evolution, disease progression to accelerated or blast phases and treatment failure. Values of $p < 0.05$ were considered significant.

Results

Patient distribution for the demographic, hematological, clinical, and survival analysis was performed according to data availability and eligibility criteria (Figure 1). Fifty-five records of CML patients with clinical, hematological, and molecular diagnosis were reviewed. Of the 55 patients, 50 (90.9%) were diagnosed in the chronic phase of the disease and included in the analysis. Four patients (7.2%) were diagnosed in the accelerated phase and 1 (1.9%) in the blast phase and thus were excluded from the analysis. Of the 50 patients, 22 (43%) were females and 28 (56%) were males. The mean age at diagnosis was 42.8 ± 14.2 years (ranging from 18 to 71). Eighteen patients (36%) had the *b2a2* transcript, 24 (48%) had *b3a2* and 8 (16%) had *b2a2/b3a2*.

Demographic, hematological and clinical data analysis

The comparative analysis between the demographic and hematological data with the different BCR-ABL transcripts is shown in Table 1. Patients with the *b2a2/b3a2* transcript showed the lowest mean age; however, there was no statistical difference when they were compared with the patients who were carriers of transcripts *b2a2* and *b3a2* ($p = 0.167$). Statistical analysis showed that platelet counts did not change with the type of transcript, and the median of 320.65×10^3/L, 396×10^3/L, 327.05×10^3/L corresponded respectively to transcripts *b2a2*, *b3a2* and *b2a2/b3a2* ($p = 0.896$). As with platelets, the other hematological data did not show any statistical difference ($p > 0.05$), when compared with the type of transcript (Table 1).

Comparison of transcript types with clinical characteristics (hepatosplenomegaly and risk scores) are summarized in Table 2. The Kruskal–Wallis test revealed that spleen and liver size did not differ between the different types of transcripts ($p = 0.395$ and $p = = 0.647$, respectively). The association between risk scores and type of transcript did not show any statistical significance ($p > 0.05$).

Analysis of event-free survival

The EFS analysis for the transcriptional variants of *BCR-ABL* is shown in Figure 2. The 21-month probability for EFS was 21%, 48% and 66% for the transcripts *b2a2*, *b3a2* and *b2a2/b3a2*, respectively ($p = 0.226$).
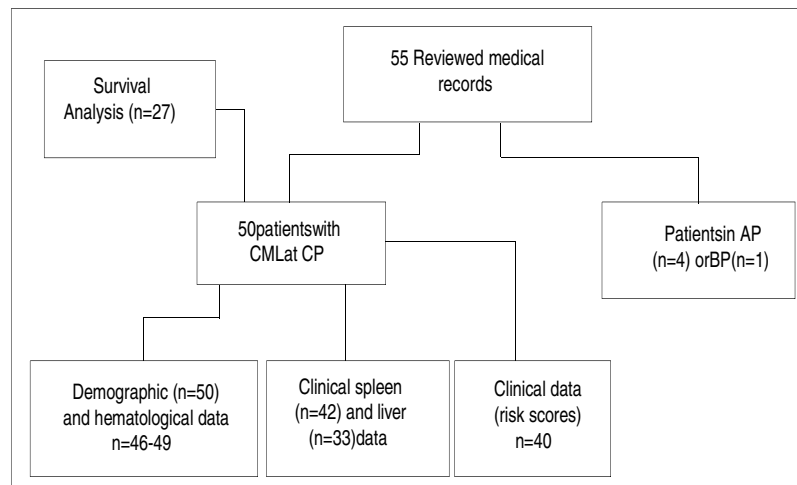
Figure 1 – Study flowchart. Chronic myeloid leukemia (CML); chronic phase (CP); accelerated phase (AP); blast phase (BP).

Table 1 – Demographic and hematological characteristics of the patients according to the type of transcript.

Variables	N_T ($n_{b2a2}/n_{b3a2}/n_{b2a2;b3a2}$)	Mean ± standard deviation or median (range)			p
		b2a2	b3a2	b2a2/b3a2	
Age (years)	50 (18/24/8)	46.28 ± 12.86	42.88 ± 15.96	34.75 ± 8.10	0.167
Hemoglobin (g/dL)	48 (17/23/8)	9.94 ± 1.67	10.70 ± 1.35	9.73 ± 1.45	0.167
Leukocytes (10^3/L)	49 (18/23/8)	152.5 (30.54–574.4)	153.7 (43–606)	188.385 (30.7–394)	0.741
Basophiles (%)	47 (17/23/7)	2.0 (0–9)	2.0 (0–14)	3 (0–6)	0.937
Eosinophils (%)	46 (17/23/6)	1.0 (0–6)	2.0 (0–15)	2.0 (0–3)	0.492
Myeloblasts (%)	47 (17/23/7)	1.0 (0–8)	2.0 (0–11)	2 (0–13)	0.298
Platelets (10^3/L)	49 (18/23/8)	320.65 (179–1568)	396 (166–1165)	327.05 (227–703)	0.886

Table 2 – Clinical characteristics of the patients according to the type of transcript. Absolute number (no.).

Variables	Transcripts			p
	b2a2	b3a2	b2a2/b3a2	
Spleen and liver size, median; no. (range)				
Spleen (cm)	9; 15 (0–30)	6; 20 (0–25)	10; 7 (4.5–15)	0.395[a]
Liver (cm)	3; 15 (0–8)	0; 12 (0–10)	1; 6 (0–4.5)	0.647[a]
Sokal score, no. (%)				
Low	4 (28.5)	5 (25)	2 (33)	0.824[b]
Intermediate	4 (28.5)	7 (35)	3 (50)	
High	6 (43)	8 (40)	1 (17)	
Hasford score, no. (%)				
Low	7 (50)	5 (25)	4 (67)	0.104[b]
Intermediate	3 (21.5)	12 (60)	2 (33)	
High	4 (28.5)	3 (15)	0 (0)	
EUTOS score, no. (%)				
Low	11 (79)	16 (80)	6 (100)	0.470[b]
High	3 (21)	4 (20)	0 (0)	
ELTS score, no. (%)				
Low	6 (43)	11 (55)	3 (50)	0.695[b]
Intermediate	2 (14)	3 (15)	2 (33)	
High	6 (43)	6 (30)	1 (17)	

Spleen size: centimeters below left costal margin; liver size: centimeters below the right coast margin.

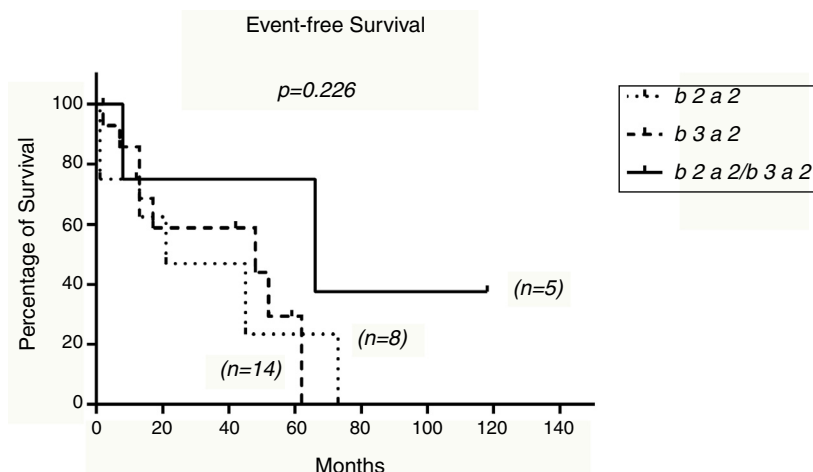[a] Kruskal–Wallis test.
[b] Chi-square test.

Figure 2 – Event-free survival analysis for the different transcripts.

Discussion

In the present study, the *b3a2* was the most frequently found transcript in patients in the chronic phase of CML. In addition, the type of *BCR-ABL* transcript had no influence on the hematological and clinical parameters, nor on the survival outcome of these patients.

The frequency of *BCR-ABL* gene transcripts in different countries is shown in Table 3. As in most previous studies, our data showed a higher frequency of the *b3a2* transcript, but other studies have also reported, less frequently, a higher incidence of the *b2a2* transcript. The different frequencies found could be explained by an inadequate selection of samples or due to genetic components associated with the ethnicity of the target population.

Many studies have evaluated the levels of hemoglobin, leukocytes, basophils, myeloblasts, platelets, spleen size and Sokal score in patients with different transcripts.[8,9,11,13,14,15] In the present study, eosinophil counts, liver size, Hasford score, EUTOS score, ELTS score and event-free survival were evaluated.

The analysis of our data was confirmed by previous studies, in which there was no difference in the levels of hemoglobin, leukocytes, basophils and myeloblasts, nor in spleen size, and Sokal score among the types of transcripts.[6,8-11,13-15] As for platelet counts, some studies have shown higher values of this parameter in patients expressing the *b3a2* transcript.[7,10,11] However, our data suggest that platelet counts are independent from the type of transcript expressed, with this finding having been confirmed by other studies.[6,8,9,13-14] These discrepancies could be explained by sample selection bias or insufficient sampling.

A study comparing liver size and eosinophil counts with the type of transcript found no difference between the groups, which reinforces our findings that these parameters are not influenced by the transcriptional variants of *BCR-ABL*.[7]

In the literature, there is a relatively small number of studies associating risk scores with the type of transcript. These previous studies did not find an association between

Table 3 – Frequency of transcripts.				
Countries	Source	Type of transcript (%)		
		b2a2	b3a2	b2a2/b3a2
Current study	Brazil	36.0	48.0	16.0
13	USA	38.0	52.0	10.0
14	United Kingdom	40.0	55.0	5.00
15	Italy	43.0	57.0	0.00
16	Poland	29.5	62.3	8.20
17	India	27.0	56.5	5.00
18	Brazil	41.0	59.0	0.00
19	Russia	33.3	66.6	0.00
23	Brazil	33.0	55.0	12.0
6	India	39.0	54.0	7.00
20	United Kingdom	41.0	46.0	0.00
7	India	32.0	68.0	0.00
21	Sudan	53.5	41.9	2.30
22	Ecuador	94.6	5.40	0.00
10	USA	42.0	41.0	18.0

the Sokal risk, Hasford, EUTOS and ELTS scores and the *BCR-ABL* transcript type.[6,8,10,14,16] In the present study, there was no association between these risk scores and the type of transcript; however, further studies are necessary to verify whether there is an association between these parameters and the type of transcript expressed by the patient.

Besides not influencing the hematological and clinical parameters, the type of transcript does not influence the outcome of EFS. Previous studies have shown no difference in EFS according to the type of transcript, reinforcing the results of our analysis.[10,17]

A recent study reported that the presence of 25 extra amino acids encoded by the BCR exon 14, which is absent in the *e13a2* (*b2a2*) transcript, promotes structural changes in the SRC (SH1, SH2 and SH3) homology domains and in the DNA binding domain.[18] It is believed that these structural changes present in the different transcripts may affect protein tyrosine kinase activity, as it has been demonstrated that this activity is greater in patients with the *b2a2* transcript.[12] However, we believe that the type of transcript alone is not sufficient to explain differences between the clinical, hematological and EFS parameters. We hypothesize that other factors might alter these parameters, such as the time variable between disease onset and diagnosis, implying in different evolutions and masking the influence of the type of transcript. Moreover, variations in the immune response inherent to each person could affect the evolution of the disease, distorting the contribution of the different *BCR-ABL* transcripts.

Conclusions

In summary, we showed that the expression of *BCR-ABL* transcripts did not influence the hematological, clinical, and EFS parameters in patients with chronic-phase CML. However, further studies are needed to circumvent possible interfering factors and to determine the actual contribution of *BCR-ABL* transcripts to patient prognosis. If the role of these molecules is confirmed, they can be used to monitor patients with CML in the future.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

r e f e r e n c e s

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