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BCR-ABL1 transcript types showed distinct laboratory characteristics in patients with chronic myeloid leukemia

A.P. Vasconcelos[1], I.F. Azevedo[2], F.C.B.C. Melo[3], W.B. Neves[3], A.C.A.C. Azevedo[3] and R.A.M. Melo[2,3]

[1]Universidade Federal de Pernambuco, Recife, PE, Brasil
[2]Universidade de Pernambuco, Recife, PE, Brasil
[3]Fundação de Hematologia e Hemoterapia de Pernambuco, Recife, PE, Brasil

Corresponding author: A.P. Vasconcelos
E-mail: andrezapamelav@gmail.com

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ABSTRACT. In chronic myeloid leukemia (CML) two main types of messenger RNA (e14a2 and e13a2) can be produced by *BCR-ABL1* gene rearrangement. Due to conflicting results, the clinical value of these transcripts remains controversial. The aim of this study was to identify associations of e14a2 and e13a2 transcripts with laboratory variables and also the response to treatment. This study included 203 adult patients with CML treated with Imatinib as first-line drug in a reference hematology center in Northeast Brazil. Clinical and laboratory data were obtained after informed consent. Samples were collected for RNA extraction and analyzed by reverse transcription-polymerase chain reaction (PCR), according to the international protocol BIOMED-1. The LeukemiaNet 2013 criteria were used to establish the molecular response. The frequency distribution of the *BCR-ABL1* transcripts was e14a2 (64%), e13a2 (34%), and double positives (2%). The results showed a statistically significant association of the e14a2 transcript type

with thrombocytosis (P = 0.0005) and the e13a2 with higher leukocyte count (P = 0.0491). In a subgroup of 44 patients, the molecular response to treatment with Imatinib was assessed by quantitative PCR at 3 months (*BCR-ABL1* ≤ 10%), 6 months (*BCR-ABL1* ≤ 1%), or 12 months (*BCR-ABL1* ≤ 0.1%). Although patients with the transcript e14a2 showed higher frequency of good responses than patients with the transcript e13a2, this difference was not statistically significant. In agreement with published data, our results showed association of the *BCR-ABL1* transcript e14a2 with thrombocytosis and the *BCR-ABL1* transcript e13a2 with higher leukocytosis in patients with chronic myeloid leukemia.

Key words: Chronic myeloid leukemia; *BCR-ABL1* transcripts; Imatinib; Prognosis

INTRODUCTION

The influence of the genomic breakpoint location and its association with specific clinical-biological characteristics and response to therapy remains controversial (De Braekeleer, 2016). In this regard, some authors found no significant association but others suggested a possible prognostic value of the transcript types in relation to better response to treatment with Imatinib (de Lemos et al., 2005; Lucas et al., 2009; Sharma et al., 2010).

Although various breakpoints within the *BCR* and *ABL* genes have been described, more than 95% of patients with chronic myeloid leukemia (CML) produce an mRNA in which either the *BCR* exon 13 (e13) or *BCR* exon 14 (e14) is fused to the *ABL* exon 2 (a2), yielding fusion forms e13a2 and e14a2, respectively (Apperley, 2015). The e13a2 and e14a2 transcript types produce a 210-kDa fusion protein (p210), an abnormal tyrosine kinase known to be critical for the clinical and pathologic features of CML (van Dongen et al., 1999). The production of fusion proteins increases the diversity of protein-protein binding domains associated with tyrosine kinase activity (Hai et al., 2014). Agents that block the tyrosine kinase activity, such as Imatinib, have been successfully used for treatment (Hughes et al., 2003).

The identification of prognostic molecular markers may be important to characterize the response of CML patients subjected to current treatments. The aim of this study was to detect the different transcript types of p210 *BCR-ABL1* gene rearrangement in patients with CML and identify possible associations with variables of prognostic importance.

MATERIAL AND METHODS

The study included 203 patients over 18 years old of both genders diagnosed with CML from January 2009 to May 2015 at Hemope Hospital in Recife, Brazil. It was approved by the Institutional Research Ethics Committee (No. 13/2013) with clinical and laboratory data obtained from patients' records and database. Peripheral blood samples were obtained for molecular analysis after informed consent and Ethics Committee approval.

The identification of the transcript p210 *BCR-ABL1* gene rearrangement was performed by reverse transcription-polymerase chain reaction (RT-PCR) according to the international BIOMED-1 protocol (van Dongen et al., 1999). The LeukemiaNet 2013 criteria were used to

establish the molecular response (Baccarani et al., 2013). Statistical analysis was done using the Stata 12.0 software. The Student *t*-test and the Mann-Whitney U-test were used and $P \leq 0.05$ was considered statistically significant.

RESULTS

The male:female ratio was 1.4:1 and the median age at diagnosis was 48 years old, range between 18 and 92 years. The majority of patients were in chronic phase of the disease (95%). At diagnosis, the blood count showed leukocytosis above 50 x 10^9/L in 89% of cases, anemia in 72%, and thrombocytosis in 40%. The *BCR-ABL1* rearrangement was found in all samples with the following distribution of the transcripts: e14a2 (N = 130; 64%), e13a2 (N = 68; 34%), and both types (N = 5; 2%). The comparison between variables at diagnosis and the two main transcript types is shown in Table 1.

Table 1. Clinical and laboratory data of 203 adult patients with chronic myeloid leukemia according to *BCR-ABL1* transcript types.

Variable	e14a2 (N = 130)	e13a2 (N = 68)	P value
Age			0.5180
<60 years	90	44	
≥60 years	40	24	
Gender			0.2890
Male	72	43	
Female	58	25	
Disease phase			0.3280
Chronic	122	66	
Accelerated	4	0	
Blastic	4	2	
Hemoglobin (g/dL)*	10.5 (± 2.0)	10.5 (± 2.3)	0.9314
Leukocytes (x10^9/L)**	129 (84;222)	147 (90;290)	0.0491
Blasts in peripheral blood (%)**	2.0 (1;5)	2.0 (1;4)	0.7299
Basophils (%)**	3.0 (2;7)	3.0 (2;5)	0.2488
Platelets (x10^9/L)**	429.0 (262;646)	287.0 (193;443)	0.0005
Molecular response***			0.6540
Optimal	21	11	
Warning/Failure	7	5	

Data are reported as *means (± SD) or as **median (P_{25}; P_{75}). ***N = 40.

DISCUSSION

The clinical value of the transcript types in CML remains controversial (Table 2). Meissner et al. (1999), Cruz et al. (2014), and Al-Achkar et al. (2016) found no significant association between the transcript type and various clinical and laboratory parameters. However, the e14a2 transcript has been associated with high platelet count (Inokuchi et al., 1991; Perego et al., 2000; Rosas-Cabral et al., 2003; Bennour et al., 2013) and age at diagnosis (Bennour et al., 2013).

Regarding the main variables at diagnosis, results in our patient group are similar to those already published (Barboza et al., 2000; Bennour et al., 2013; Smith et al., 2014). In agreement with published data, our study showed a statistically significant association between the e14a2 transcript and high platelet count (Bennour et al., 2013), but also between the e13a2 transcript with high leukocyte count (Hanfstein et al., 2014). The statistical analysis for the e13a2/e14a2 was not possible, due to the small number of patients with this transcript (N = 5).

Table 2. Main published studies about the association between p210 *BCR-ABL1* transcript types and laboratory data or prognosis in patients with chronic myeloid leukemia.

Reference	N	Type of study	e14a2 (%)	e13a2 (%)	e13a2/e14a2 (%)	Reported associations
Inokuchi et al., 1991	57	Single Center	60	30	10	BCC
Perego et al., 2000	88	Single Center	61	39	-	BCC
Rosas-Cabral et al., 2003	97	Single Center	28	59	13	BCC
de Lemos et al., 2005	22	Single Center	59	32	9	MR
Vega-Ruiz et al., 2007	480	Single Center	49	39	12	MR
Lucas et al., 2009	74	Single Center	53	43	4	CR
Sharma et al., 2010	87	Single Center	53	39	8	CR
Balatzenko et al., 2011	98	Multicenter	54	45	1	BCC
Bennour et al., 2013	44	Single Center	64	36	-	BCC
Tabassum et al., 2014	24	Single Center	63	33	4	-
Hanfstein et al., 2014	1105	Multicenter	45	41	14	BCC and MR
Lin et al., 2016	166	Single Center	50	37	13	MR
Jain et al., 2016	481	Single Center	41	42	18	MR
Present study	203	Single Center	64	34	2	BCC

BCC = blood cell count; MR = molecular response; CR = cytogenetic response.

Some authors have found a better molecular response with the e14a2 transcript (Hanfstein et al., 2014; Lin et al., 2016; Jain et al., 2016) or e13a2 (de Lemos et al., 2005; Sharma et al., 2010). Lin et al. (2016) showed that males with e13a2 were a less favorable group in their response to imatinib treatment. In our study, it was not possible to demonstrate association between the transcript type and molecular response.

The identification of the type of transcript is important not only for minimal residual disease monitoring as different transcript types seem to represent distinct biological entities (Hanfstein et al., 2014), but also because the transcript type could be used to select treatment regimen for patients with CML (Jain et al., 2016).

CONCLUSION

Our data show an association between the *BCR-ABL1* transcript type e14a2 with thrombocytosis and the *BCR-ABL1* e13a2 with leukocytosis in patients with chronic myeloid leukemia.

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