



Revista Brasileira de Hematologia e Hemoterapia Brazilian Journal of Hematology and Hemotherapy

www.rbhh.org



Original article

Frequency of p190 and p210 BCR-ABL rearrangements and survival in Brazilian adult patients with acute lymphoblastic leukemia

Ilana de França Azevedo*, Rui Milton Patrício da Silva Júnior,
Audrey Violeta Martins de Vasconcelos, Washington Batista das Neves,
Fárida Coeli de Barros Correia Melo, Raul Antônio Moraes Melo

Fundação de Hematologia e Hemoterapia de Pernambuco (HEMOPE), Recife, PE, Brazil

ARTICLE INFO

Article history:

Received 5 September 2013

Accepted 5 June 2014

Available online 18 July 2014

Keywords:

Acute biphenotypic leukemia

Adult

BCR-ABL

Survival analysis

ABSTRACT

Objective: This study investigated the occurrence of the p190 and p210 breakpoint cluster region-Abelson (BCR-ABL) rearrangements in adults with acute lymphoblastic leukemia and possible associations with clinical and laboratory characteristics and survival.

Methods: Forty-one over 18-year-old patients with acute lymphoblastic leukemia of both genders followed-up between January 2008 and May 2012 were included in this study. Clinical and laboratory data were obtained from the medical charts of the patients. Reverse transcription polymerase chain reaction (RT-PCR) using specific primers was employed to identify molecular rearrangements.

Results: At diagnosis, the median age was 33 years, and there was a predominance of males (61%). The most common immunophenotype was B lineage (76%). BCR-ABL rearrangements was detected in 14 (34%) patients with the following distribution: p190 (28%), p210 (50%) and double positive (22%). Overall survival of patients with a mean/median of 331/246 days of follow up was 39%, respectively, negative BCR-ABL (44%) and positive BCR-ABL (28%).

Conclusion: These results confirm the high frequency of BCR-ABL rearrangements and the low survival rate of adult Brazilian patients with acute lymphoblastic leukemia.

© 2014 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier Editora Ltda. All rights reserved.

Introduction

Acute lymphoblastic leukemia (ALL) in adults comprises a group of diseases with biological, clinical, laboratorial and prognostic heterogeneity characterized by abnormal proliferation and accumulation of immature lymphoid cells in the bone

marrow and lymphoid tissues.¹ Unlike ALL in children, the advances in the therapy in adults have been slow, with a mean survival of 35% in patients aged between 18 and 60 years.² As a result, considerable effort has been made to identify markers that can be translated to the clinic as new prognostic tools and therapeutic targets.³

* Corresponding author at: Laboratório de Biologia Molecular, Fundação de Hematologia e Hemoterapia de Pernambuco (HEMOPE), Rua Joaquim Nabuco, 171, Graças, 52011-000 Recife, PE, Brazil.

E-mail address: ilanafranca87@gmail.com (I. de França Azevedo).

<http://dx.doi.org/10.1016/j.bjhh.2014.07.016>

1516-8484/© 2014 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier Editora Ltda. All rights reserved.

At present, the diagnosis and classification of acute leukemia depend on cytomorphologic, immunophenotypic, cytogenetic and molecular analyses. Molecular tests are part of the criteria for the risk classification system of the World Health Organization (WHO) and are used to evaluate the prognosis correctly and define therapeutic strategies.⁴

Of the various genetic alterations observed in adult ALL, the breakpoint cluster region-Abelson (BCR-ABL) fusion gene is the most common and is associated with a particularly poor prognosis.^{1,5,6} This gene rearrangement can present two distinct isoforms, p190 and p210 due to different breakpoints.⁷ Recent studies indicate that these two isoforms may be associated with different clinical phenotypes in adult ALL patients.⁸

The aim of this study was to investigate the occurrence of the p190 and p210 BCR-ABL rearrangements in adult ALL patients and to investigate possible associations with clinical and laboratory features and survival.

Methods

The study group comprised 41 over 18-year-old patients of both genders diagnosed with ALL at the Fundação de Hematologia e Hemoterapia de Pernambuco (Hemope) from January 2008 to May 2012. The diagnosis was established by clinical, cytomorphological and immunophenotypic criteria. The standard treatment protocol used was the hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (HyperCVAD) regimen.⁹ This project was approved by the Research Ethics Committee of the institution (#17/2010) and the study was conducted in accordance with the Declaration of Helsinki 2008.

Clinical and laboratory data were obtained from the patients' records. Samples of peripheral blood and bone marrow were collected after informed consent had been given. The identification of the p190 and p210 BCR-ABL gene rearrangements was performed by reverse transcription polymerase chain reaction (RT-PCR) according to the international BIOMED-1 protocol.¹⁰ The following controls were used in the RT-PCR reactions: positive, negative, endogenous and contamination.

Statistical analysis was performed using the Bioestat 5.0 and Stata 9.1 programs. The t-test was used to compare the groups regarding age, leukocyte count, blasts, platelet count and hemoglobin. The Fisher exact test was used for the categorical variables (gender and immunophenotype). Overall survival was calculated using the Kaplan-Meier *Log rank* method. *p*-values <0.05 were considered statistically significant.

Results

Of the 41 patients analyzed, ALL was more prevalent in young adults and men and the most common immunophenotype was B lineage (Table 1).

No statistically significant differences were found between the groups of BCR-ABL positive and negative patients in respect to the clinical and laboratory variables. However, the p210 BCR-ABL patients had higher leukocyte counts and all p190 BCR-ABL patients had the B immunophenotype (Table 2).

Table 1 – Clinical features at diagnosis of 41 adult patients with acute lymphoblastic leukemia from 2008 to 2012 at HEMOPE.

Clinical features	
Age (Years) – n (%)	
18–34	23 (56)
35–60	14 (34)
>60	4 (10)
Median (range)	33 (19–72)
Gender – n (%)	
Male	25 (61)
Female	16 (39)
Leucocytes ($\times 10^9/L$)	
Median (range)	47 (0.3–570.5)
Lymphoblasts PB (%)	
Median (range)	78 (0–98)
Platelets ($\times 10^9/L$)	
Median (range)	47 (8–571)
Hemoglobin (g/dL)	
Median (range)	8.5 (2.7–12.9)
Immunophenotype – n (%)	
B-ALL	31 (76)
T-ALL	9 (22)
Not done	1 (2)

The overall survival was 39% with a mean follow-up of 331 days (median 246 days). Survival was lower for BCR-ABL positive (28%) than for BCR-ABL negative (44%) patients. The Log-rank test, however, showed no statistically significant difference (*p*-value = 0.2297) between the survival curves of the two groups (Fig. 1). The mortality rate of BCR-ABL positive patients is 1.94 times greater [95% Confidence Interval (CI): 0.80–4.26] than the BCR-ABL negative individuals, but again the difference is not statistically significant (*p*-value = 0.148).

Discussion

The median age of the patients was 33 years, which is similar to several published series.^{11–17} Males predominated in the sample, which is in accordance with the main multicenter studies.^{11–15,18–21} The results of several studies have shown similar numbers of leucocytes^{11,12,20} at diagnosis, including the percentage of blasts in the peripheral blood¹³ and platelet count.¹⁷ The 76% frequency of B cell phenotype is in accordance with various published studies.^{11,12,17,21–23}

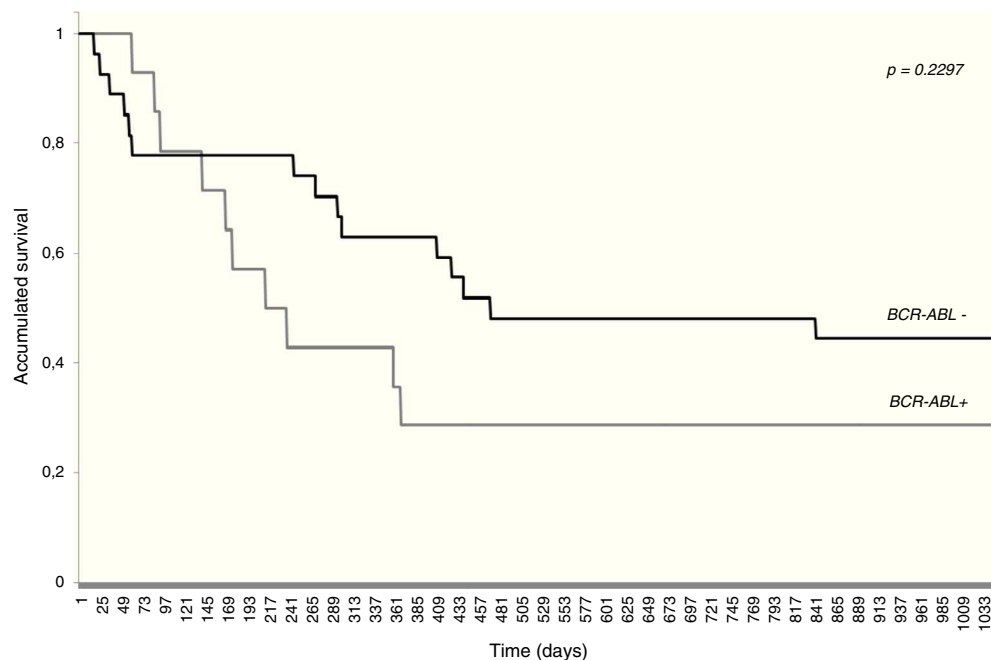
The 34% frequency of the BCR-ABL rearrangement is similar to that found in several studies with values ranging from 17% to 37%,^{1,11–14,17–22} including in elderly patients, as reported by Larson.²⁴ No published Brazilian studies with data regarding the molecular analysis of BCR-ABL in adult ALL patients were found for comparison. A case series of 42 adult Brazilian patients showed 7% of Ph⁺ samples.²⁵

The results presented in this study confirm the high frequency of BCR-ABL rearrangements in adult ALL patients, but, differ from other studies regarding the type of isoforms found. Gleißer et al.²¹ showed a 37% positivity for the BCR-ABL fusion gene in 478 adult ALL patients including the p190 (77%) and p210 (20%) rearrangements and both isoforms (3%). Dombret et al.²² found the following frequencies among 154 adult ALL patients: p190 (68%), p210 (28%) and both isoforms (4%). The explanation for our results is not clear, including the

Table 2 – Summary of clinical and laboratory parameters of 41 adult patients diagnosed with acute lymphoblastic leukemia at HEMOPE.

Variable	BCR-ABL ⁻ (n = 27)	BCR-ABL ⁺ (n = 14)	BCR-ABL ⁺ p190 (n = 4)	BCR-ABL ⁺ p210 (n = 7)	BCR-ABL ⁺ p190/p210 (n = 3)
Age (years)					
Median (range)	35 (19–72)	38 (20–72)	45 (22–61)	35 (20–72)	34 (20–48)
Leucocytes ($\times 10^9/L$)					
Median (range)	30.4 (1–492)	77.1 (0.3–570)	76.6 (0.3–378)	96.0 (6.7–570)	7.7 (4.2–298.8)
Platelets ($\times 10^9/L$)					
Median (range)	41 (8–571)	69 (18–213)	175.5 (18–213)	60 (27–155)	70 (60–112)
Lymphoblasts (%)					
Median (range)	78 (0–97)	80 (0–98)	75 (0–93)	85 (31–98)	36 (3–94)
Immunophenotype ^a – n (%)					
B-ALL	21 (78.0)	10 (71.5)	4 (100.0)	3 (43.0)	3 (100.0)
T-ALL	5 (22.0)	4 (28.5)	0 (0)	4 (57.0)	0 (0)

–: negative; +: positive.

^a n = 40.**Figure 1 – Overall survival curves of 41 adult patients with acute lymphoblastic leukemia at Fundação HEMOPE according to the presence of BCR-ABL rearrangements.**

occurrence of BCR-ABL positivity in T-ALL cases¹⁹, but may be due to sample size or characteristics of the population studied, as well as patients with chronic myeloid leukemia in acute phase.²⁶

The analysis of the survival curves, in addition to confirming the low rate of overall survival for adult patients diagnosed with ALL,^{9,14,20,21,23} also suggests an increased adverse prognosis conferred by the presence of BCR-ABL rearrangements,^{2,18,21–23,27} and therefore a need for other therapeutic modalities, including targeted therapies and bone marrow transplantation.²⁸ The range of the confidence interval of the mortality rate suggests that the sample size was too small to show a difference and that increasing it would make it more evident. Phenotypic differences between p190 and p210 BCR-ABL patients is controversial.^{8,21} Further studies with a

larger sample size, including elderly patients, are needed to better characterize the association between these rearrangements and different phenotypic expressions and survival. The detection of the BCR-ABL fusion gene is important for the classification of risk groups of ALL patients and the correct targeting of therapy.^{2,3} Moreover, in addition to the BCR-ABL fusion gene, other rearrangements, such as E2A-PBX1, TEL-AML1, MLL-AF4, should be screened, because they also have prognostic significance.²⁹

Conclusion

Our results provide the first published evidence of the high frequency of BCR-ABL and poor survival in adult Brazilian ALL

patients. The study confirms the importance of detecting BCR-ABL rearrangements for the treatment and prognosis of these patients.

Authors' contributions and declaration of conflicts of interest

The authors Ilana de França Azevedo and Rui Milton Patrício da Silva Júnior contributed equally to the development of the work. The authors declare no conflicts of interest.

Acknowledgements

The authors thank the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (#APQ-1343-4.00-08) for funding the study and Prof. Ricardo de Alencar Ximenes Arraes for his support with the statistics.

REFERENCES

1. Foá R, Chiaretti S, Guarini A, Vitale A. Adult acute lymphoblastic leukemia. *Rev Bras Hematol Hemoter.* 2009;31 (Suppl 2):41-7.
2. Bassan R, Hoelzer D. Modern therapy of acute lymphoblastic leukemia. *J Clin Oncol.* 2011;29(5):532-43.
3. Mullighan CG. New strategies in acute lymphoblastic leukemia: translating advances in genomics into clinical practice. *Clin Cancer Res.* 2011;17(3):396-400.
4. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. World Health Organization classification of tumors of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2008.
5. Juric D, Lacayo NJ, Ramsey MC, Racevskis J, Wiernik PH, Rowe JM, et al. Differential gene expression patterns and interaction networks in BCR-ABL – positive and – negative adult acute lymphoblastic leukemias. *J Clin Oncol.* 2007;25(11):1341-9.
6. Schaffel R, Simões BP. Philadelphia positive acute lymphoblastic leukemia. *Rev Bras Hematol Hemoter.* 2008;30(1):52-8.
7. Advani AS, Pendergast AM. BCR-ABL variants: biological and clinical aspects. *Leuk Res.* 2002;26(8):713-20.
8. Jaso J, Thomas DA, Cunningham K, Jorgensen JL, Kantarjian HM, Medeiros LJ, et al. Prognostic significance of immunophenotypic and karyotypic features of Philadelphia positive b-lymphoblastic leukemia in the era of tyrosine kinase inhibitors. *Cancer.* 2011;117(17):4009-17.
9. Kantarjian H, Thomas D, O'Brien S, Cortes J, Giles F, Jeha S, et al. Long-term follow-up results of hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (Hyper-CVAD), a dose-intensive regimen, in adult acute lymphocytic leukemia. *Cancer.* 2004;101(12):2788-801.
10. Van Dongen JJ, Macintyre EA, Gabert JA, Delabesse E, Rossi V, Saglio G, et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. *Leukemia.* 1999;13(12):1901-28.
11. Gabert J, Beillard E, van der Velden VH, Bi W, Grimwade D, Pallisaard N, et al. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia – a Europe Against Cancer Program. *Leukemia.* 2003;17(12):2318-57.
12. Takeuchi J, Kyo T, Naito K, Sao H, Takahashi M, Miyawaki S, et al. Induction therapy by frequent administration of doxorubicin with four other drugs, followed by intensive consolidation and maintenance therapy for adult acute lymphoblastic leukemia: the JALSG-ALL93 study. *Leukemia.* 2002;16(7):1259-66.
13. Hunault M, Harousseau JL, Delain M, Truchan-Graczyk M, Cahn JY, Witz F, et al. Better outcome of adult acute lymphoblastic leukemia after early genoidentical allogeneic bone marrow transplantation (BMT) than after late high-dose therapy and autologous BMT: a GOELAMS trial. *Blood.* 2004;104(10):3028-37.
14. Thomas X, Boiron JM, Huguet F, Dombret H, Bradstock K, Vey N, et al. Outcome of treatment in adults with acute lymphoblastic leukemia: analysis of the LALA-94 trial. *J Clin Oncol.* 2004;22:4075-86.
15. Huguet F, Leguay T, Raffoux E, Thomas X, Beldjord K, Delabesse E, et al. Pediatric-inspired therapy in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia: the GRAALL-2003 study. *J Clin Oncol.* 2009;27(6):911-8.
16. Gokbuget N, Arnold R, Bohme A, Fietkau R, Freund M, Ganser A, et al. Improved outcome in high risk and very high risk ALL by risk adapted SCT and in standard risk ALL by intensive chemotherapy in 713 adult ALL patients treated according to the prospective GMALL study 07/2003. *Blood.* 2007; 110.
17. Brüggemann M, Raff T, Flohr T, Gökbuget N, Nakao M, Droese J, et al. Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukemia. *Blood.* 2006;107(3):1116-23.
18. Bassan R, Spinelli O, Oldani E, Intermeosoli T, Tosi M, Peruta B, et al. Improved risk classification for risk-specific therapy based on the molecular study of minimal residual disease (MRD) in adult acute lymphoblastic leukemia (ALL). *Blood.* 2009;113(18):4153-62.
19. Annino L, Vegna ML, Camera A, Specchia G, Visani G, Fioritoni G, et al. Treatment of adult acute lymphoblastic leukemia (ALL): long-term follow-up of the GIMEMA ALL 0288 randomized study. *Blood.* 2002;99(3):863-71.
20. Ribeira JM, Oriol A, Bethencourt C, Parody R, Hernández-Rivas JM, Moreno MJ, et al. Comparison of intensive chemotherapy, allogeneic or autologous stem cell transplantation as post-remission treatment for adult patients with high-risk acute lymphoblastic leukemia: results of the PETHEMA ALL 93 trial. *Haematologica.* 2005;90(10):1346-56.
21. Gleibner B, Gokbuget N, Bartram CR, Janssen B, Rieder H, Janssen JW, et al. Leading prognostic relevance of the BCR-ABL translocation in adult acute B-lineage lymphoblastic leukemia: a prospective study of the German Multicenter Trial Group and confirmed polymerase chain reaction analysis. *Blood.* 2002;99(5):1536-43.
22. Dombret H, Gabert J, Boiron JM, Huguet FR, Blaise D, Thomas X, et al. Outcome of treatment in adults with Philadelphia chromosome-positive acute lymphoblastic leukemia – results of the prospective multicenter LALA-94 trial. *Blood.* 2002;100(7):2357-66.
23. Rowe JM, Buck G, Burnett AK, Chopra R, Wiernik PH, Richards SM, et al. Induction therapy for adults with acute lymphoblastic leukemia: results of more than 1500 patients from the international ALL trial: MRC UKALL XII/ECOG E2993. *Blood.* 2005;106(12):3760-7.
24. Larson RA. Management of acute lymphoblastic leukemia in older patients. *Semin Hematol.* 2006;43(2):126-33.
25. Fogliatto L, Bittencourt H, Nunes AS, Salenave PR, Silva GS, Daudt LE, et al. Outcome of treatment in adult acute lymphoblastic leukemia in southern Brazil using a modified German multicenter acute lymphoblastic leukemia protocol. *Acta Haematol.* 2002;107(4):203-7.

26. Raanani P, Trakhtenbrot L, Rechavi G, Rosenthal E, Avigdor A, Brok-Simoni F, et al. Philadelphia-chromosome-positive T-lymphoblastic leukemia: acute leukemia or chronic myelogenous leukemia blastic crisis. *Acta Haematol.* 2005;113(3):181-9.
27. Rego MF, Pinheiro GS, Metze K, Lorand-Metze I. Acute leukemias in Piauí: comparison with features observed in other regions of Brazil. *Braz J Med Biol Res.* 2003;36(3):331-7.
28. Alvarado Y, Apostolidou E, Swords R, Giles FJ. Emerging therapeutic options for Philadelphia-positive acute lymphocytic leucemia. *Expert Opin Emerg Drugs.* 2007;12(1):165-79.
29. Zerbini MC, Soares FA, Velloso ED, Chauffaille ML, Paes RP. Classificação da Organização Mundial da Saúde para tumores dos tecidos hematopoético e linfóide, 4th edition, 2008 – principais modificações introduzidas em relação à 3th edition, 2001. *Rev Assoc Med Bras.* 2011;57(1):66-73.