HEMATOL TRANSFUS CELL THER. 2023;45(S2):S108–S112

HEMATOLOGY, TRANSFUSION AND CELL THERAPY

www.htct.com.br

Original article

Outcome of adolescents and young adults with acute lymphoblastic leukemia in a single center in Brazil

Thais Ferraz Aguiar [a,b,*], Thayana da Conceição Barbosa [a], Ana Luiza Tardem Maciel [a], Caroline Barbieri Blunck [a], João Sellos-Laclette [a], Andreia Cristina de Melo [a], Marcela B. Mansur [a,c,d], Mariana Emerenciano [a,*]

[a] Instituto Nacional de Câncer (INCA), Rio de Janeiro, RJ, Brazil
[b] Instituto Estadual de Hematologia Arthur Siqueira Cavalcanti, Rio de Janeiro, RJ, Brazil
[c] Children's Hospital, John Radcliffe Hospital, Oxford, UK
[d] Weatherall Institute of Molecular Medicine -WIMM, University of Oxford, Oxford, UK

ARTICLE INFO

Article history:
Received 17 February 2022
Accepted 24 June 2022
Available online 1 August 2022

Keywords:
Acute lymphoblastic leukemia
Adolescents and young adults
Survival
Latin America

Introduction: Acute lymphoblastic leukemia (ALL) presents a poor prognosis in adults. The adoption of pediatric protocols has been changing this scenario, especially for adolescents and young adults (AYA).
Objective and method: We aimed to evaluate a consecutive series of patients treated at the State Institute of Hematology of Rio de Janeiro between 2012 and 2020, focusing on the AYA subgroup.
Result: The B-ALL was the most frequent subtype (81.6%) and AYA, the predominant age group (57.7%). The median overall survival (OS) was 9.4 months. High early mortality was observed and sepsis was the main cause of death. Better OS results were noted in AYA, in comparison to over 39y (13.3 × 6.2 months, respectively), the Berlin-Frankfurt-Münster (BFM) being the protocol of choice in this group.
Conclusion: The use of the pediatric protocol seems to improve the OS of AYA, however, high rates of deaths from infection were observed, demonstrating the need for advances in the Brazilian public system clinical support.

Introduction

Despite being the most common neoplasm in children, accounting for nearly a quarter of all pediatric cancers, acute lymphoblastic leukemia (ALL) is a relatively rare type of cancer in adults and is associated with a worse prognosis in this age group, when compared to pediatric cases. These distinct results can be largely attributed to the higher incidence of high-risk cytogenetic-molecular signatures (*e.g.*, *BCR-ABL1*+, *BCR-ABL1*-like) in adults, resulting in higher rates of chemoresistance and disease relapse.[1–4] However, social factors, such as higher rates of treatment drop-out, structural limitations of the public health service and socioeconomic status also significantly contribute to outcome differences between adults and children, especially in low-income countries.[4–7]

* Corresponding authors at: Division of Clinical Research and Technological Development, Research Center - Instituto Nacional de Câncer. Rua André Cavalcanti, 37, 3° floor. CEP: 20231050 - Rio de Janeiro, RJ, Brazil.
E-mail address: memerenciano@inca.gov.br (M. Emerenciano).
https://doi.org/10.1016/j.htct.2022.06.006

The subgroup of patients aged between 15 to 39 years old, named adolescents and young adults (AYA), is historically associated with an unfavorable ALL prognosis with an event-free survival (EFS) of 30 to 45%.[6,7] The presence of specific genetic signatures and heterogeneity of the therapeutic approach (use of pediatric *versus* adult protocols) are some of the underlying causes for the low survival rates.

The use of more intensive protocols, *i.e.*, pediatric or based on pediatric schemes, in the AYA subgroup is associated with increased overall survival (OS) and EFS, when compared to the use of adult protocols. Moreover, although treatment-related toxicities are more prevalent in the AYA subgroup, especially in the older patients, overall, these regimens have demonstrated feasibility in this population.[6,7] Data on different treatment schemes, as well as on clinical-demographic characteristics of ALL in the AYA subgroup, are scarce in Brazil. Most of the current data come from collaborative efforts, mostly from European and North American groups.

The present study aimed to characterize a consecutive series of patients treated in a reference institution for ALL management in the state of Rio de Janeiro, Brazil, from 2012 to 2020, focusing particularly on the AYA subgroup. Moreover, we aimed to understand the impact of demographic and clinical variables on patient survival after the adoption of high-intensity chemotherapy protocols.

Methods

Design of the study

An observational, longitudinal and retrospective study was conducted that included patients aged 15 or over, diagnosed with ALL at the *Instituto Estadual de Hematologia Arthur Siqueira Cavalcanti* in Rio de Janeiro (*HemoRio*), from January 2012 to December 2020. The *HemoRio* is a public institution and the main reference for treating hematological diseases in the state of Rio de Janeiro. Patients were diagnosed after morphological analysis and immunophenotyping of bone marrow and/or peripheral blood samples, as recommended by the World Health Organization classification[8] and the European Group for Immunological Characterization of Acute Leukemias (EGIL) criteria.[9]

To perform the cytogenetic-molecular characterization of patients with available samples, conventional cytogenetics and Real-Time PCR (RT-PCR) were performed to verify aneuploidy and common gene fusions in ALL (high hyperdiploidy, hypodiploid, *TCF3-PBX1*, *BCR-ABL1* and *KMT2A-AFF1*). Less frequent cytogenetic-molecular changes (*e.g.*, *CRLF2*, *JAK2*, and *ABL1* rearrangements) were evaluated by fluorescence *in situ* hybridization (FISH). The status of *IKZF1* deletions and other copy number alterations (CNAs), including *CDKN2A/2B*, *PAX5*, *EBF1*, *ETV6*, *BTG1*, *RB1* and the PAR1 region was determined by combining two techniques: Multiplex PCR (MP-PCR) and multiplex ligation-dependent probe amplification (MLPA), using the SALSA MLPA P202 and P335 kits.[10,11]

For analysis purposes, patients were grouped into 3 age strata, following the criteria already established by the institution for treatment decision, based on the National Cancer Institute Classification[12]: 15 to 39 years (a.k.a. AYA), 40 to 60 years or over 60 years old. Patients were classified as presenting hyperleukocytosis at diagnosis if their white blood cell (WBC) count was greater than 30,000 leukocytes/mm^3 for B-ALL or more than 100,000 leukocytes/mm^3 for T-ALL cases, following the criteria established by most protocols.[13-14] The central nervous system (CNS) infiltration was defined as the presence of at least 1 lymphoblast in the cerebrospinal fluid sample or clinical or imaging alteration compatible with disease involvement.

Data collection and laboratory procedures of this project were approved by the Research Ethics Committee of the institutions involved (#33709814.7.1001.5274 and #33709814.7.3001.5267).

Statistical analysis

Statistical analyses were performed in the R environment, ref.: R Core Team (2020), a language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria). The variables were described by their absolute and relative frequencies. The frequency graphs were generated using the Ggplot2 package, version 3.5.1.

The overall survival (OS) was calculated by the Kaplan-Meier method in months from the date of diagnosis to the outcome (dead or alive) and the curves were compared by the Log-rank method. Patients with loss of follow-up were censored at the date of the last contact. The crude Hazard Ratios (HR) and respective 95% Confidence Intervals (CIs) were individually estimated by the Cox's semiparametric regression. The HR for the 'age group' was adjusted by the leukemia subtype by multiple COX regression and $p < 0.05$ values were considered significant.

Results

The study included 104 patients, with equal distribution between men and women, and the majority of them were aged 15 to 39 years (AYA, 57.7%). The B-cell lineage was the most frequent subtype (81.6%), CNS infiltration was present at diagnosis in only 2.9% of patients and elevated WBC, in 35.3% of B-ALL and 42.1% of T-ALL cases. Population characteristics are shown in Table 1. Moreover, socioeconomic data was available for 35 patients. Our results showed that 57.5% of them presented a monthly income of up to one minimum wage. The assessment of schooling was possible in 100 patients, and 50.0% of them had only primary school.

Cytogenetic and molecular analyses were conducted in 77 patients with available material, however, not all cases were tested for all the abnormalities due to limited material availability. The *BCR-ABL1* was the most common primary abnormality found among B-ALL cases (42.8%). The CNA analysis was performed in 44 patients and 40.0% of them harbored deletions in at least one of the following genes: *IKZF1*, *CDKN2A/2B*, *PAX5*, *EBF1*, *ETV6*, *BTG1*, *RB1* and the PAR1 region. The *IKZF1* and *CDKN2A/B* were the most frequently detected gene deletions in our series of cases (50.0% and 45.4%, respectively). In general, the gene deletions studied have a different distribution across age groups, but no significant difference was observed (Figure 1). Comparing the patients according to the *BCR-ABL1* status, we observed that *IKZF1* deletions were

Table 1 – Demographic, clinical and genetic characterization of all patients.

Variables	Total n (%)	Age group (years) 15 - 39 (AYA) n (%)	40 - 60 n (%)	>60 n (%)
Sex				
Male	53 (51.0)	34 (56.7)	16 (55.2)	3 (20.0)
Female	51 (49.0)	26 (43.3)	13 (44.8)	12 (80.0)
ALL Subtype				
B-ALL	85 (81.7)	47 (78.3)	23 (79.3)	15 (100.0)
T-ALL	19 (18.3)	13 (21.7)	6 (20.7)	-
WBC (B-ALL)				
Hyperleukocytosis	30 (35.3)	13 (27.7)	11 (47.8)	6 (40.0)
Non-hyperleukocytosis	55 (64.7)	34 (72.3)	12 (52.2)	9 (60.0)
WBC (T-ALL)				
Hyperleukocytosis	8 (42.1)	6 (46.1)	2 (33.3)	-
Non-hyperleukocytosis	11 (57.9)	7 (53.9)	4 (66.7)	-
Recurrent gene fusions (B-ALL)				
High hiperdiploidy	1 (2.1)	1 (3.6)	-	-
BCR-ABL1	33 (67.3)	14 (50.0)	14 (87.5)	5 (100.0)
KMT2A-r	2 (4.1)	2 (7.1)	-	-
TCF3-PBX1	5 (10.2)	5 (17.9)	-	-
B-other	8 (16.3)	6 (21.4)	2 (12.5)	-
Total	104 (100.0)	60 (57.7)	29 (27.9)	15 (14.4)

*Abbreviations: AYA, adolescents and young adults; B-ALL, B cell acute lymphoblastic leukemia; CNS, central nervous system; NA, not applicable; T-ALL, T cell acute lymphoblastic leukemia; WBC, white blood cell count.*In 36 B-ALL cases, it was not possible to define the molecular group.

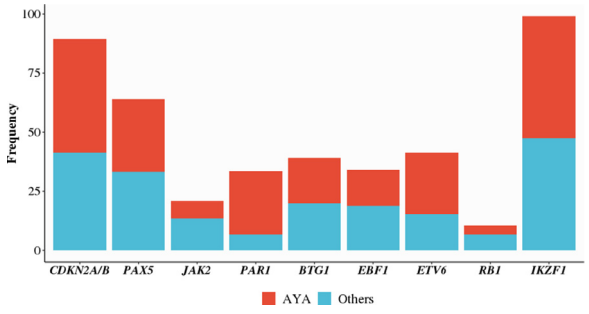

Figure 1 – Frequency of the most common copy number alterations according to age. Forty-seven patients were investigated for the presence of CNAs by MLPA assays P335 and P202 and Multiplex PCR. The genes analyzed in these techniques are plotted on the X-axis, *CDKN2A/B*, *PAX5*, *JAK2*, PAR1 region, *BTG1*, *EBF1*, *ETV6*, *RB1*, and *IKZF1*. Here, PAR1 was considered altered if two or more genes in this region were affected: *CRLF2*, *IL3RA*, *CSF2RA*, *P2RY8* and *SHOX*. The percentage of alteration frequency, loss or amplification in each gene is plotted on the Y-axis. The light gray bars represent the AYA group (age 15 - 39 years) and the dark gray, patients aged over 39 years.

significantly more frequent in patients aged over 39 years with Ph+ than Ph- ($p = 0.015$) (Supplementary Table 1).

The patients with follow-up information had a median OS of 9.4 months (95%CI 6.4 - 14.0), as shown in Figure 2A. The OS analysis revealed that patients aged over 39 years had poorer outcomes, compared to AYA patients ($p = 0.004$, 6.2 months, 95%CI 3.1 - 9.8 *vs.* 13.3 months, 95%CI 10.2 - 22.5, respectively) (Figure 2B). Moreover, T-ALL patients displayed a median OS of 4.2 months (95%CI 1.9 - NA) and 10.2 months for the B-ALL ($p = 0.097$, 95%CI 7.0-15.0) (Figure 2C). No significant difference in OS was observed according to sex, organomegaly, CNS infiltration, WBC count, treatment and molecular subgroups (Supplementary Tables 2 and 3). A multivariate Cox analysis including the variables that had significant effects revealed that age ($p = 0.050$, HR 1.8, 95%CI 1.0 - 3.3) and leukemia subtype ($p = 0.032$, HR = 1.9, 95%CI 1.1 - 3.4) independently impacted the prognosis in this study.

The two main treatment protocols used were the BFM (41.7%) and HyperCvad (37.9%) and 71.2% of the patients in the AYA group were treated with the BFM protocol, while the HyperCvad was more frequently used in patients aged 40 years or older (65.9%). All patients with Ph+ ALL received a combination of the tyrosine kinase inhibitor and chemotherapy protocol, with imatinib being the medication currently provided.

Overall, 74.7% of the patients died, being 20.7% of the T-ALL and 79.2%% of the B-ALL patients. The AYA group presented a mortality rate of 64.4%, while for patients aged over 39 years it was 88.6%. The main cause of death was sepsis, which occurred in 67.6% of the cases. Nineteen patients (26.8%) died in the first 30 days of treatment after diagnosis and sepsis was also identified as the leading cause of mortality (68.4%), occurring mainly in patients over 39 years old (13 patients).

Discussion

Despite the advances made in recent decades in the treatment of ALL, especially in pediatrics, with adult outcomes still

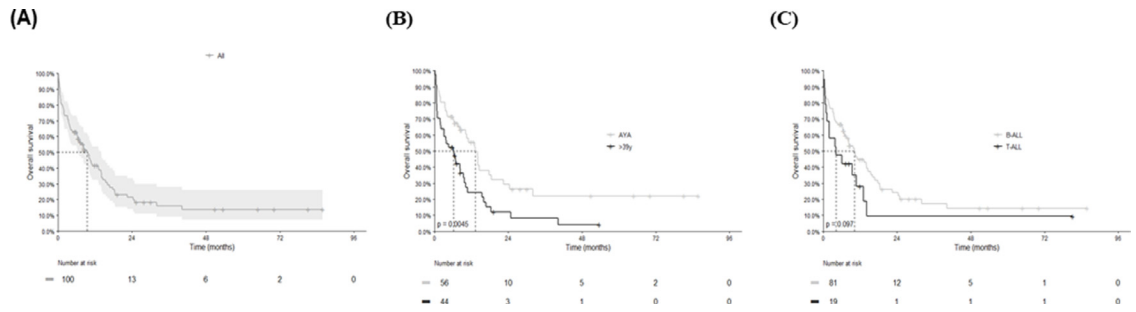

Figure 2 – Overall survival analysis of ALL patients. (A) Overall survival (OS) of all patients included in this study. (B) Comparison of OS according to age group and (C) leukemia subtype. *P*-values were calculated by the Log-rank method.

being much lower, with EFS and OS rates at 3 years below 45%. This difference is largely due to the higher frequency of molecular cytogenetic alterations of poor prognosis in the adult population, with the *BCR-ABL1* being the most common, present in approximately 25% of the patients and reaching 60% in the elderly cases.[4,14] The AYA subgroup shows a disproportionately unfavorable prognosis, compared to younger patients. Data from the US SEER program show a reduction in survival from 75% at 17 years to 45% at 20 years,[14] also partly related to a higher incidence of poor prognostic markers, such as the *BCR-ABL1* and Ph-like in this age group. The adoption of pediatric protocols or pediatric-based regimens showed survival improvements in this subgroup with acceptable toxicity and is currently the recommended treatment for this group.

Most of the patients in the current study were part of the AYA group (57.7%), which is in agreement with the age distribution for the state of Rio de Janeiro according to data from the IBGE (Brazilian Institute of Geography and Statistics),[15] and also with other literature reports from developing countries.[16,17] They received high-intensity treatment protocols, according to the current guidelines. Nevertheless, despite having higher OS when compared to the other age groups, a high number of deaths from infectious causes was observed. When the deaths of the entire series were evaluated, 26.8% of the patients died in the first 30 days of treatment, which is much higher than the reports from collaborative groups (3 - 8%).[17] Patients diagnosed with T-ALL presented a lower survival rate than that of the B-ALL patients, being sepsis the main cause of death in this group.

The cytogenetic analyses in the present study were impaired by the low index of mitosis, however, molecular analyses performed of the available samples showed no increase in the incidence of high-risk alterations in relation to what was expected according to the age groups. Despite that, the survival rates differed from those currently reported by multicenter studies conducted in countries with a high Human Development Index (HDI) – and the main reason for the difference lies in the high mortality rates due to sepsis. Similar results were found in reports from other centers in Latin America. Additionally, the low socioeconomic status of the population and the deficiency in clinical support commonly observed in low- and middle-income countries and in the public health setting are well established factors associated with inferior outcomes. Despite the limitations present in our study related to the establishment of the cytogenetic-molecular profile due to the unavailability or inadequate quality of the material, this is as far as we know, the first study to characterize the AYA subgroup in a Latin American population.

Since the study population had molecular cytogenetic characteristics expected for the age groups, without observing a higher incidence of high-risk alterations, the increase in mortality rates can be related to the deficiency in clinical support commonly observed in low- and middle-income countries and in the public health setting.

The high rates of infection by multiresistant gram-negative bacteria, as well as high infection rates associated with deep catheters, are the main causes of deaths from sepsis. The treatment in shared wards, usual in the public Brazilian health system, and the vulnerable socio-economic situation of the population attended at the study institution, with a high number of patients living far from the hospital and having a low family income and low level of education, generating prolonged hospitalizations, explain the high incidence of infection by multidrug-resistant bacteria. The high number of deaths due to treatment-related complications (infections) leads to a reduction in OS rates, when compared to the curves of developed countries, urging the need for discussions on adjustments of the protocols, due to the impossibility of reproduction of the same clinical conditions, as well as the improvement of supportive care.

Conclusion

In conclusion, our study showed a cohort of patients with low income and schooling levels, especially in the AYA group that, although preferentially treated with the pediatric protocol, still presented a very poor survival rate.

Authorship

TFA: Conceptualization, Methodology, Validation, Formal analysis, Investigation and Writing of the Original Draft; ALTM, CBB and TCB: Methodology, Formal analysis, Investigation and Writing of the Review and Editing; JSL: Methodology and Formal analysis; ACM: Resources and Writing of the Review and Editing; ME and MBM: Conceptualization, Methodology, Investigation, Resources, Writing of the Original

Draft and Review and Editing, Project administration and Funding acquisition.

Conflicts of interest

None.

Acknowledgments

The authors would like to thank the patients and their families who agreed to be involved in the study. ALTM has been supported by the CAPES Foundation. CBB and TCB are supported by the Ministry of Health (INCA-Brazil). ME is supported by the Brazilian National Council of Technological and Scientific Development-CNPq [PQ-311220/2020-7] and the *Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro-FAPERJ* [E_26/203.214-2017; E-26-010.101072-2018 and E-26/010.002187-2019] research grants. MBM was supported by the Ministry of Health (INCA-Brazil) and is currently funded by a John Goldman Fellowship from Leukaemia UK (University of Oxford-UK).

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.htct.2022.06.006.

REFERENCES

1. Jabbour E, O'Brien S, Konopleva M, Kantarjian H. New insights into the pathophysiology and therapy of adult acute lymphoblastic leukemia. Cancer. 2015;121(15):2517–28.
2. Bassan R, Bourquin J-P, DeAngelo DJ, Chiaretti S. New approaches to the management of adult acute lymphoblastic leukemia. J Clin Oncol. 2018:JCO2017773648.
3. Aldoss I, Stein AS. Advances in adult acute lymphoblastic leukemia therapy. Leuk Lymphoma. 2018;59(5):1033–50.
4. Aldoss IT, Marcucci G, Pullarkat V. Treatment of acute lymphoblastic leukemia in adults: applying lessons learned in children. Oncology. 2016;30(12):1080–91.
5. Fagundes EM, Rocha V, Glória ABF, Clementino NCD, Quintão JS, Guimarães JPO, et al. De novo acute myeloid leukemia in adults younger than 60 years of age: socioeconomic aspects and treatment results in a Brazilian university center. Leuk Lymphoma. 2006;47(8):1557–64.
6. Curran E, Stock W. How I treat acute lymphoblastic leukemia in older adolescents and young adults. Blood. 2015;125(24):3702–10.
7. Boissel N, Baruchel A. Acute lymphoblastic leukemia in adolescent and young adults: treat as adults or as children? Blood. 2018;132(4):351–61.
8. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Beau MML, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
9. Bene MC, Castoldi G, Knapp W, Ludwing WD, Matutes E, Orfao A, et al. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia. 1995;9(10):1783–6.
10. Barbosa TC, Terra-Granado E, Quezado Magalhães IM, Neves GR, Gadelha A, Filho GEG, et al. Frequency of copy number abnormalities in common genes associated with B-cell precursor acute lymphoblastic leukemia cytogenetic subtypes in Brazilian children. Cancer Genet. 2015;208(10):492–501.
11. Meyer C, Zur Stadt U, Escherich G, Hofmann J, Binato R, Barbosa TC, et al. Refinement of IKZF1 recombination hotspots in pediatric BCP-ALL patients. Am J Blood Res. 2013;3(2):165–73.
12. Coccia PF, Pappo AS, Beaupin L, Borges VF, Borinstein SC, Chugh R, et al. Adolescent and young adult oncology, version 2.2018, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw. 2018;16(1):66–97.
13. SEER*Explorer [Internet]. [cited 2021 Sep 3];Available from: https://seer.cancer.gov/explorer/
14. DeAngelo DJ, Advani AS, Marks DI, Stelljes M, Liedtke M, Stock W, et al. Inotuzumab ozogamicin for relapsed/refractory acute lymphoblastic leukemia: outcomes by disease burden. Blood Cancer J. 2020;10(8):81.
15. IBGE | Biblioteca | Detalhes | Sinopse do censo demográfico : 2010 /IBGE. - [Internet]. [cited 2021 Sep 3];Available from: https://biblioteca.ibge.gov.br/index.php/biblioteca-catalogo?view=detalhes&id=249230
16. Jaime-Pérez JC, Jiménez-Castillo RA, Pinzón-Uresti MA, Cantú-Rodríguez OG, Herrera-Garza JL, Marfil-Rivera LJ, et al. Real-world outcomes of treatment for acute lymphoblastic leukemia during adolescence in a financially restricted environment: results at a single center in Latin America. Pediatr Blood Cancer. 2017;64(7).
17. Fernandes da Silva, Junior W, Medina AB, Yamakawa PE, Buccheri V, Velloso EDRP, Rocha V. Treating adult acute lymphoblastic leukemia in Brazil-increased early mortality using a German multicenter acute lymphoblastic leukemia-based regimen. Clin Lymphoma Myeloma Leuk. 2018;18(6):e255–9.