Conclusions: These results show a higher prevalence of the Franco-American-British M3 subtype than that reported in the international literature, as well as a decreased OS compared with that of developed countries. Further multicenter Brazilian studies with a larger sample size are encouraged to better understand the characteristics of acute myeloid leukemia, and to improve the treatment and prognosis in this population.

Introduction

Acute myeloid leukemia (AML) is a rare neoplasm, accounting for 15–20% of all childhood acute leukemias. Approximately 500–600 children and adolescents are diagnosed with AML each year in the United States.[1] Despite the development of new drugs and the possibility of allogeneic hematopoietic stem cell transplantation (HSCT), treatment of this disease remains a challenge. Cure rates with current treatment protocols range from 60% to 70% in this age group.[2]

Recent studies have shown that cytogenetic and molecular abnormalities are involved in the pathogenesis of childhood AML, with clonal chromosome abnormalities in 70–85% of cases.[3] Great efforts have been made to improve characterization of the disease and prognostic stratification of patients. Several molecular and cytogenetic events have been identified that allow definition of the distinct subtypes of AML in childhood. Such changes can be used as markers and help better define targets for therapy, thereby reducing the toxicity of current treatment strategies.[4]

Currently, cytogenetic abnormalities, such as t(8;21) *RUNX1/RUNX1T1* and inv(16)/t(16;16) *CBFB/MYH11*, are considered the core-binding factor mutations of leukemic cells. Pediatric patients with these mutations have a favorable prognosis and do not benefit from matched related-donor HSCT.[5] Other genetic abnormalities, such as −5/del(5q), −7/del(7q) or complex karyotype, wild-type *NPM1*, and *FLT3*-ITD, are associated with poor prognosis.[6] The finding that patients with a normal karyotype may have cryptic mutations, and that these mutations may have prognostic value, provided new insight into these patients, who were previously considered at low risk in most protocols.[5]

It cannot be denied that, since the 1970s, childhood AML survival rates have improved; however, overall survival (OS) remains suboptimal. Despite improvements in risk stratification, tailoring of treatment to the individual characteristics of each patient and of the disease and better indications for HSCT, the 3-year OS still ranges from 63% to 75%.[6]

To date, little has been published about childhood AML in Brazil. Considering its relatively low incidence (seven occurrences per 1,000,000 children/year)[4] and prognosis, several international collaborative groups have been focused on analyzing epidemiological and prognostic factors in an attempt to improve treatment strategies and increase OS and event-free survival (EFS) rates.[4] The present study was therefore designed to describe the epidemiological features and survival rates of patients with childhood AML treated at hospitals of a South Brazil state (Rio Grande do Sul) and compare the findings with international data.

Materials and methods

The authors conducted a multicenter cohort study with retrospective data collection of all new AML cases in patients younger than 18 years treated at five public referral hospitals in a South Brazil state, references for AML treatment, from January 2005 to December 2015 (Hospital de Clínicas de Porto Alegre, Hospital São Vicente de Paula/Passo Fundo, Hospital da Criança Santo Antônio/Irmandade Santa Casa de Misericórdia de Porto Alegre, Hospital da Criança Conceição/Porto Alegre, Universidade Federal de Pelotas/Pelotas). The diagnosis was confirmed by morphological analysis and flow cytometric immunophenotyping of bone marrow aspirate and by anatomopathological study of bone marrow biopsy. HSCT indication was based on the occurrence of relapsed AML. Patients who reached the age of 18 during the study time period continued to be followed. There was no loss to follow-up and patients with secondary AML were excluded from the analysis and was no lost to follow-up by the patient. The study was approved by the ethics committee of the institution, with secondary approvals from the ethics committees of all participating centers. Informed consent was waived due to the non-interventional design of the study and the retrospective nature of data collection.

The patients' medical records were reviewed for data on demographics (sex, place of origin, and date of birth), presentation at diagnosis (date of diagnosis, French-American-British [FAB] classification, and karyotype), treatment (systemic chemotherapy regimen, autologous and/or allogeneic BMT), and follow-up after diagnosis (current status, date of recurrence, date of diagnosis of a second neoplasm, date of death, or date of last contact).

Statistical analysis

The number of patients aged <18 years with a diagnosis of AML who received care at the five participating centers during the study period determined the sample size. Continuous variables were expressed as standard deviation (SD) or median and range. Qualitative variables were expressed as absolute and relative frequencies. Survival curves were estimated by the Kaplan–Meier method. Cox regression models were used to evaluate survival assessment considering the following risk factors: more than 10 years of age, risk karyotype and leukometry of the diagnosis above 50,000 leukocytes/mm. The incidence rate was calculated from the number of new cases of a disease divided by the number of people at risk. Data were analyzed using SPSS, v. 20.0

Table 1 Baseline characteristics of the pediatric AML cohort.

Variables	$n = 149$
Age, years, median (range)	10.5 (0–18)
Male sex, n (%)	94 (63.1)
White blood cell count (/mm³), n (%)	
<10,000	3 1(20.8)
10,000–50,000	29 (19.5)
>50,000–100,000	12 (8.1)
>100,000	11 (7.4)
Not reported	66 (44.3)
CNS+, n (%)	9 (6.0)
HSCT frequency	33 (22.2)
FAB subtype, n (%)	
M0	11 (7.4)
M1	15 (10.1)
M2	15 (10.1)
M3	43 (28.9)
M4	14 (9.4)
M5	12 (8.1)
M6	5 (3.4)
M7	11 (7.4)
Non-M3	23 (15.4)

Baseline characteristics of the AML cohort.
AML, acute myeloid leukemia; FAB, French-American-British classification of AML; CNS, central nervous system.

for Windows. The level of significance was set at 5% for all analyses.

Results

A total of 149 patients with a diagnosis of childhood AML treated at five pediatric hematology-oncology referral hospitals in southern Brazil over a 10-year period were analyzed. Most patients were male ($n = 94$, 63.0%), and 60 (40.3%) had a white blood cell (WBC) count below 50,000/mm³. The most common FAB subtype was M3 in 28.9% of patients ($n = 43$). Table 1 shows the main characteristics of the patient cohort.

Nine (6.0%) patients had central nervous system (CNS) disease, and one (0.6%) patient had pulmonary disease. Ten (6.7%) patients had extramedullary leukemia (EML). Of these, four were classified as M4/M5, one as M1, one as M3, one as M6, one as M7, and two as unspecified non-M3 FAB subtypes.

Regarding karyotype, only 102 (68.4%) patients had available information. Of these, 26 (25.5%) had a normal karyotype.

Fifteen patients (10.0%) were <1 year of age at diagnosis, 23 (15.4%) were 1–4 years of age, 22 (14.8%) were 4–9 years of age, 57 (38.3%) were 9–15 years of age, and 32 (21.5%) were >15 years of age. At diagnosis, Cox regression analysis showed no association between WBC count, age, and risk of death or relapse ($p > 0.1$).

Eight patients (5.4%) had associated syndromes: six had Down syndrome, one had trisomy 13 syndrome, and one had Fanconi anemia. Among patients with Down syndrome, four were male; five patients were classified as M7 subtype and one as M0 subtype. None of these patients had EML, and no radiotherapy or transplant was performed. Only one patient with Down syndrome relapsed; this patient died 17 months after the initial diagnosis. Two other patients with Down syndrome died, one due to refractory disease and the other due to infection. The patient with trisomy 13 syndrome underwent HSCT at first remission and is still alive after 75 months of follow-up. The patient with Fanconi anemia had two bone marrow relapses and died 9 months after the diagnosis due to disease progression.

This study found an incidence of 13.2 occurrences of AML per 1,000,000 children aged <18 years in the state of Rio Grande do Sul. In the United States, the incidence is approximately 8.9 occurrences per 1,000,000 children.[1] A possible explanation is race based. It is known that race can influence the incidence of childhood AML, with data showing a higher prevalence of the disease in patients of Latin-American origin.[7] Also, there was a higher prevalence of M3 over other FAB subtypes of AML. The Brazilian Collaborative Study Group of Infant Acute Leukemia found a prevalence of 11% (5/45) of acute promyelocytic leukemia (APL) in their AML sample.[7] This is consistent with the present finding of 28% (43/106) of cases classified as FAB M3. Although the diagnosis in some patients was molecularly confirmed, in most cases the diagnosis was confirmed by immunophenotype or karyotype.

Treatment

Most patients with APL (31/43 patients) were treated with the GIMEMA-AIEOP AIDA protocol, which consists of all-trans retinoic acid (ATRA) and idarubicin as induction, followed by three polychemotherapy consolidation courses.[8] The PETHEMA LPA-99 protocol was used in three other patients (also including idarubicin and ATRA as the induction regimen),[9] and two patients received treatment similar to that of the North American Leukemia Intergroup Protocol C9710 (ATRA + daunorubicin + cytarabine).[10] The other seven patients had no available information on the treatment protocol used.

Information on treatment protocol was accurately recorded in 79 of 106 patients with non-promyelocytic leukemia (74.5%). Most treatment strategies ($n = 44$, 55.7%) were based on the Berlin-Frankfurt-Münster (BFM) group protocols (1983, 1993, 1998, and 2004), in which there is a period of maintenance therapy and etoposide induction.[11] Fourteen patients (17.7%) received treatment based on the St. Jude AML02 multicenter trial, 13 (16.4%) were treated with a Brazilian protocol,[12] five (6.3%) were treated with the '7 + 3' regimen, and three (3.8%) were treated with other protocols.

Relapse and causes of death

A total of 48 (32.2%) patients relapsed, six of them with APL. Forty-five (93.7%) had a bone marrow relapse and three (6.3%) had a CNS relapse. The most frequently used regimens in second-line therapy were fludarabine, cytarabine, and granulocyte-colony stimulating factor (G-CSF), with or without idarubicin (FLAG/IDA-FLAG). In some patients,