Clinical characteristics and outcome of children with biphenotypic acute leukemia

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ABSTRACT

Background

Knowledge concerning the clinical and biological presentation, as well as the outcome of treatment, of biphenotypic acute leukemia in children is limited.

Design and Methods

This retrospective review analyzes the clinical features and outcome of children with biphenotypic acute leukemia diagnosed and treated over an 8-year period. According to the EGIL scoring system 24 (3.7%) of 633 patients with acute leukemia were classified as having biphenotypic acute leukemia. The diagnostic work-up and results were reviewed specifically for this study in the light of the newly published WHO criteria for the diagnosis of leukemia of ambiguous lineage. Based on these criteria, 11 (1.7%) patients were categorized according to the new nomenclature as having mixed phenotype acute leukemia. The majority of the patients (58.3%) had a B-lymphoid/myeloid phenotype, followed by the T-lymphoid/myeloid phenotype. The most frequent chromosomal abnormality involved the 14q32 locus. Patients received therapy based on a treatment regimen for acute lymphocytic leukemia regimen, which included myeloid-effective agents.

Results

At a median follow up of 4 years (range, 6 month – 7 years) the overall survival rate was 75.7% and the event-free survival rate was 73.5%. The survival of those patients who underwent hematopoietic stem cell transplantation in first complete remission was not different from that of the patients who were treated with chemotherapy alone (overall survival: 70.1% *versus* 81.1%, respectively, p=0.39; event-free survival: 70.1% *versus* 76.2%, respectively, p=0.75). The outcome of the 11 patients who were retrospectively classified as having mixed phenotype acute leukemia according to the new WHO criteria was excellent, with no relapses or deaths occurring among these patients.

Conclusions

An acute lymphocytic leukemia type of induction therapy, using agents that are active against lymphoid and myeloid leukemias, appears to be more effective in achieving and maintaining complete remissions regardless of whether the patients are classified according to EGIL criteria or the new WHO criteria. Hematopoietic stem cell transplantation may not be necessary for all patients in first complete remission.

Key words: biphenotypic acute leukemia, diagnosis, therapy, immunophenotyping, stem cell transplant.

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Introduction

Acute leukemias are broadly classified as myeloid or T/B lymphoid according to their morphological features as well as the surface and/or cytoplasmic antigen expression of proliferating blasts. These antigens have a varying degree of specificity to different lineages, with certain markers being more specific for either the myeloid or lymphoid phenotype. The European Group for Immunological Classification of Leukemias (EGIL) proposed a set of diagnostic criteria for biphenotypic acute leukemia (BAL).[1] This scoring system is based on the number and degree of the specificity of certain makers for myeloid or T/B lymphoid blasts. The World Health Organization (WHO) has recently released a revised version of the Classification of Haematopoietic and Lymphoid Malignancies, which includes significant modifications of the diagnostic criteria for acute leukemias of mixed phenotype. These criteria are more stringent than those of the EGIL and rely heavily on positivity for myeloperoxidase (MPO).[2]

Knowledge about BAL is limited, both in terms of clinical and biological presentation and also with regard to the outcome of treatment. BAL represents less than 5% of cases of acute leukemia.[3-6] Due to variations in the definitions used by different investigators for the diagnosis of BAL, it was not until the EGIL standards were published that incidence reports that could be compared between institutions became available.[6-8] While the overall incidence is reported to be about 3.5% of acute leukemias seen in all age groups, the exact incidence of BAL in childhood is unknown because pediatric populations have been reported within the adult studies. Two recently published studies, focusing only on pediatric patients, reported incidences of 2% and 4.4%.[9,10] The diagnostic criteria used in the two studies were, however, different.

More importantly, the optimal therapy for this subtype of leukemia is unclear. There is a lack of agreement regarding the treatment methodology, with proponents for myeloid and lymphoid strategies, and protocols incorporating both strategies. The need for hematopoietic stem cell transplantation (HSCT) in first remission also remains contentious.[7,9-14]

The Leukemia Team at our institution elected to treat these patients using a strategy based on the St. Jude Total XIII-B high risk protocol.[15] While this protocol was originally developed for the treatment of patients with high-risk acute lymphocytic leukemia (ALL), it incorporates several agents which are effective in the treatment of myeloid leukemias. This retrospective study reviews and analyzes the clinical features and treatment outcome of pediatric patients with BAL who were diagnosed in our institution, using the EGIL criteria, between January 2000 and December 2007, and were treated according to this strategy.

Design and Methods

The 27 patients of this retrospective study were among the 633 children (less than 14 years old) with leukemia referred to our hospital for investigation and treatment between January 2000 and December 2007. Clinical and pathological data including age at diagnosis, gender, white blood cell (WBC) counts, and results of bone marrow examination, immunophenotyping, cytogenetic/molecular studies, and cerebrospinal fluid analysis were obtained from both medical records and electronic data sources. These data were collected, analyzed and reported under the review and with the approval of the institutional Research Advisory Council which acts as the Institutional Review Board.

Diagnosis of biphenotypic acute leukemia

The diagnostic work-up of BAL was based on initial morphological and cytochemical evaluation of the bone marrow and supported with extensive immunophenotyping. In a minority of patients a peripheral blood sample was used for diagnosis when a bone marrow biopsy could not be performed and the patient had a high WBC count with significant circulating leukemic blasts. The final diagnosis of BAL was made utilizing the EGIL scoring system. The diagnostic work-up and results were reviewed specifically for this study in the light of the newly published WHO criteria for the diagnosis of leukemia of ambiguous lineage. Based on these criteria 11 patients were categorized according to the new nomenclature as having mixed phenotype acute leukemia (MPAL).

Detailed immunophenotyping using four-color flow cytometry (Becton-Dickinson FACSCalibur instruments; BD Biosciences, San Jose, CA, USA) was performed on blast cell populations identified by CD45 *versus* light side-scatter properties, using standard staining and analytic methods. All cases were characterized with a panel of antibodies to leukocyte-associated markers including surface CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD9, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD22, CD24, CD33, CD34, CD36, CD41a, CD42b, CD45, CD56, CD61, CD64, CD65, CD66c, CD71, CD117, cytoplasmic CD3, CD79a, MPO, terminal deoxynucleotidyl transferase (TdT), and surface and cytoplasmic immunoglobulins μ, κ, and λ. A marker was considered positive by this method when 20% or more of the blasts reacted with antibodies to that marker with a definite intensity shift greater than that of a corresponding negative control.

Cytogenetic analysis was performed on direct preparations or overnight unstimulated cultures of bone marrow and tissue samples, followed by banding with Wright-trypsin stain, as previously described.[6]

Treatment

All patients were treated uniformly, on a modified St. Jude TXIII-B high risk protocol.[15] (Table 1). Induction chemotherapy consisted of six agents effective in both lymphoid and myeloid leukemias. This was followed by consolidation therapy with two doses of high-dose methotraxate. After consolidation patients received weekly continuation therapy consisting of non-cross-resistant drug pairs for 120 weeks. Patient without involvement of the central nervous system (CNS)

received triple intrathecal therapy once every 8 weeks during the first 54 weeks of therapy, along with high-dose methotrexate. Patients with CNS2 and CNS3 disease received triple intrathecal therapy every 4 weeks during the first 54 weeks of continuation therapy and craniospinal radiation therapy (2400 cGy to the brain and 1200 cGy to the whole spine) at week 56 of continuation therapy. Patients with hyperleukocytosis (WBC >100×10^9/L) and those with Philadelphia chromosome-positive leukemia were considered at high risk of CNS relapse and also received triple intrathecal therapy on a similar schedule and prophylactic radiation therapy (1800 cGy) to the brain.

Following achievement of remission, all patients were eligible for allogeneic HSCT if a fully matched related donor was available. In 2003 cord blood from unrelated donors was also introduced as a potential source of hematopoietic stem cells.

Statistical Package for Social Sciences (SPSS) version 13.0 was used for all statistical analyses. Treatment outcome was evaluated using Kaplan–Meier analysis and the differences between outcomes were tested using the log-rank test.

Table 1. Chemotherapy based on the St. Jude Total Therapy XIII-B HR protocol (see Pui *et al.*[15] for further details).

Induction
Prednisone 40 mg/m^2/day for 28 days
Daunorubicin 25 mg/m^2 weekly for 2 doses
Vincristine 1.5 mg/m^2 weekly for 4 doses
L-asparaginase 10,000 IU/m^2 3 times/week for 6 doses; patients with >5% blasts on day 15 receive an additional 3 doses
Etoposide 300mg/m^2 for 3 doses on days 22, 25 and 29
Cytarabine 300 mg/m^2 for 3 doses on days 22, 25 and 29
Triple intrathecal therapy weekly for 2 doses for patients with CNS1 and 4 doses for CNS2/3
Consolidation
Methotrexate 2000 mg/m^2 IV over 24 hours weekly for 2 doses
Mercaptopurine 75 mg/m^2 PO daily for 14 days
Triple intrathecal therapy weekly for 2 doses
Continuation*
1. Etoposide 300 mg/m^2 IV + Cyclophosphamide 300 mg/m^2 IV
2. Methotrexate 40 mg/m^2 IV + Mercaptopurine 75 mg/m^2 PO daily for 7 days
3. Methotrexate 40 mg/m^2 IV + Cytarabine 300 mg/m^2 IV
4. Vincristine 1.5 mg/m^2 IV + Dexamethasone 8 mg/m^2/day PO for 7 days[a]
5. Etoposide 300 mg/m^2 IV + Cyclophosphamide 300 mg/m^2 IV
6. Methotrexate[b] 2000 mg/m^2 IV over 24 hours + Mercaptopurine 75 mg/m^2 PO daily for 7 days[c]
7. Etoposide 300 mg/m^2 IV + Cytarabine 300 mg/m^2 IV
8. Vincristine 1.5 mg/m^2 IV + Dexamethasone 8 mg/m^2/day PO for 7 days[a]
Delayed Intensification (administered between weeks 16-22)
Prednisone 40 mg/m^2/day for 28 days
Daunorubicin 25 mg/m^2 weekly for 2 doses
Vincristine 1.5 mg/m^2 weekly for 4 doses
L-asparaginase 10,000 IU/m^2 3 times/week for 6 doses
Etoposide 300mg/m^2 for 1 dose on day 22
Cytarabine 300 mg/m^2 for 1 dose on day 22
Methotrexate 2000 mg/m^2 IV over 24 hours weekly for 2 doses on days 36 and 43
Mercaptopurine 75 mg/m^2 PO daily for 14 days starting on day 36
Triple intrathecal therapy 1 dose on day 43

**Drug pairs were administered in weekly rotation for a total of 120 weeks, interrupted by delayed intensification therapy from weeks 16 to 22. [a]L-asparaginase (10,000 IU/m^2 IM) administered until week 36, thereafter vincristine and dexamethasone alone. [b]The last high-dose methotrexate was given on week 53, after which it was replaced by regular-dose methotrexate. [c]Triple intrathecal therapy administered with week #6 of each cycle for all patients until week 54. Triple intrathecal therapy administered on week #3 of each cycle for patients with CNS leukemia or at high risk of CNS relapse.*

Results

Twenty-seven cases (4.3% of 633 patients with acute leukemia) were classified as having BAL according to the EGIL classification. Three of these patients had the *TEL-AML1* translocation and on review were felt to exhibit a phenotype typically associated with this cytogenetic subtype and were, therefore, reclassified as having ALL. These patients were excluded from further analysis. Of the remaining 24 patients with BAL, 14 (58.3%) had a B-lymphoid/myeloid phenotype, seven (29.2%) had a T-lymphoid/myeloid phenotype, two (8.3%) had B-lymphoid/ T-lymphoid disease and one patient (4.2%) had a T-lymphoid/B-lymphoid/myeloid assignment according to the EGIL system. The median age of the patients at diagnosis was 8.7 years (range, 7 months-14.4 years) and there were 11 females. The median WBC count was 26.9×10^9/L (range, 1-261×10^9/L) and five patients had WBC counts higher than 100×10^9/L. Five patients (20.8%) had CNS disease (CNS2/CNS3) at presentation.

Only 11 (1.7% of the total cohort) of these patients could be categorized as having MPAL according to the new WHO criteria. Ten of these patients had a lymphoid/myeloid phenotype (5 B-lymphoid/myeloid and 5 T-lymphoid/myeloid) and one patient had a bi-lymphoid phenotype. Two of the patients with the B-lymphoid/myeloid phenotype could be further sub-classified according to the WHO classification; one had MPAL with t(9;22) and the other had MPAL with t(v;11q23). The median age of this group was 10.0 years (range, 7 months – 13.8 years) and only three were female. The median WBC count was 9.0×10^9/L (range 1-242×10^9/L) and two patients had CNS disease at presentation.

Immunophenotyping

All cases had an EGIL score of more than 2 in each lineage. Table 2 summarizes the relevant immunophenotyping for the patients. For those with myeloid lineage disease, the most frequently observed positive markers were CD33 and CD13 in 21/25 (84%) patients, CD15 in 13/22 (59.1%) patients and CD117 in 17/24 (70.8%) patients. For the B-lymphoid phenotype, CD79a was tested in 15 patients and was positive in all of them. The other frequently encountered markers were CD19 (19/20; 95%), CD22 (17/20; 85%) and CD10 (17/27; 63%). The most frequently found positive T-lymphoid marker was cyCD3, which was positive in all the nine (100%) patients who had the T-phenotypic association.

CD7 was positive in 11 patients, including two patients who did not have T-cell-specific markers and in whom the marker was, therefore, aberrantly expressed. CD2 was also expressed aberrantly, in this case in one patient who did not have T-cell specific-markers, as well as being found in eight other patients. The stem cell markers CD34 and TdT were positive in 23/26 (88.5%) patients. CD11b was positive in eight cases (30%). MPO activity was evaluated by flow cytometry and/or cytochemistry in all cases and in eight cases by electron microscopy. MPO was positive in nine patients by flow cytometry and/or by cytochemistry while in three others it was positive only by electron microscopy. It was positive by all three methods only in one case (patient #7). These 11 patients fulfilled the criteria for MPAL according to the new WHO classification.[2]

Cytogenetics

Results of cytogenetic analysis were available for all patients. For four patients metaphases could not be seen, while 7/23 had normal karyotypes. Sixteen (69.6%) patients showed a clonal abnormality. Fluorescence *in situ* hybridization (FISH)/molecular studies were done in 20 patients. Details of cytogenetic analyses and molecular studies are given in Table 3. The 14q32 locus was the most common site of chromosomal abnormalities, being involved in four patients: two patients had add(14q32), one had t(8:14)(p21;q32), while the fourth was found to have rearrangement of the 14q32 locus involving the *IGH* gene by FISH examination. Abnormalities involving the *MLL* gene at the 11q23 locus were found in three cases: two had *MLL* rearrangements while the third had a deletion of the *MLL* gene. None of these children was less than 12 months old at diagnosis. Only one patient had t(9;22).

All patients received induction chemotherapy according to the St. Jude TXIII-B high-risk protocol except two patients (#15 and 16) who received induction

Table 2. Clinical characteristics of the pediatric patients with biphenotypic acute leukemia.

Patient	Age (years)	Gender	WBC count ($x10^9$/L)	CNS status	Cytogenetics	Molecular	Blasts in BM on day 15	CR	Therapy	Relapse	Outcome
1	7	M	4.26	1	46XY	ND	<5%	Yes	Chemo	No	Alive
2	13	M	2.71	1	47-48XY, t(3;8), del(15), +19	*MLL* –	<5%	Yes	Chemo	No	Alive
3	12	M	2.42	2	48XY, +6, +6	ND	<5%	Yes	Chemo	No	Alive
4	4	F	3.10	1	52XX, +x,+4, del(7)(p11), +9,+14,+21	ND	<5%	Yes	Chemo	No	Alive
5	10	M	261.0	3	No metaphases seen	*MLL* –	>5%	Yes	Chemo	Yes	Dead
6	6	F	59.0	1	46XX, add(14)(q32)	*BCR-ABL* –, *CMYC* –	<5%	Yes	Chemo	No	Alive
7	13	M	40.6	1	46XY, t(8;14)(p21;q32)	*BCR-ABL* –	ND	Yes	Chemo	No	Alive
8	3	F	7.95	1	46XX	ND	<5%	Yes	Chemo	No	Alive
9	4	M	105.0	1	No metaphases seen	*MLL* gene deletion *BCR-ABL* –	<5%	Yes	Chemo	Yes	Dead
10	8	M	126.0	1	47XY, add(7)(p15), del(7)(q34), del(13)(q22),+mar	*MLL* – *BCR-ABL* –	≥5%	Yes	Chemo	No	Alive
11	6	F	1.96	1	47X, del(X)(q22), add 21(p11.2)	*MLL* –	<5%	Yes	Chemo	No	Alive
12	1	M	35.4	1	No metaphases seen	*MLL* –, *BCR-ABL* – *TEL-AML1* –	>5%	No	Chemo	Yes	Alive
13	9	M	2.47	1	47XY, +mar	*IgH*/14q32 rearrangement + *MLL* –, *BCR-ABL* –	<5%	Yes	Chemo	No	Alive
14	4	M	2.88	1	46 XY	ND	<5%	Yes	Chemo+SCT	No	Alive
15	9 months	M	242.0	1	46XY, del(1)(p32)	ND	>5%	Yes	Chemo+SCT	No	Alive
16	13	M	42.5	1	46-47XY, del(5)(q22), del(6)(q22), del(12)(p11.2), del(13)(q12q14) x 2[cp9] /46XY	*MLL* –	>5%	Yes	Chemo+SCT	No	Alive
17	14	F	98.2	2	47XX, +4 [11]/47, idem, add(14)(q32)[9]	ND	<5%	Yes	Chemo+SCT	No	Alive
18	10	F	33.6	1	46XX	*MLL* –	>5%	Yes	Chemo+SCT	No	Dead
19	7	F	33.1	3	46XX, ?der(7)add(7)(q22), t(7;11)(p22;q23), -21, +mar	*MLL* rearrangement + *BCR-ABL* –	>5%	Yes	Chemo+SCT	Yes	Dead
20	10	F	148.0	3	46XX,t(9;22)(q34;q11.2)	*BCR-ABL* +, MLL –	>5%	Yes	Chemo+SCT	No	Alive
21	3	F	20.8	1	46XX	*MLL* –, *BCR-ABL* –	<5%	Yes	Chemo+SCT	No	Alive
22	10	F	7.91	1	46XX, inv(9)(p12;q13)	*MLL* –, *BCR-ABL* –	>5%	Yes	Chemo+SCT	No	Dead
23	10	F	1.0	1	47XX, del(1)(p34), add(5)(?22), +8,add(16)(p13.3) /46XX, idem, -9	*MLL* rearrangement + *BCR-ABL* –, *TEL-AML1* –	<5%	Yes	Chemo+SCT	No	Alive
24	9	M	9.0	1	46XY	*MLL* –, *BCR-ABL* –	>5%	Yes	Chemo+SCT	No	Alive

M. male; F: female; BM: bone marrow; CR: complete remission; ND: not done; chemo: chemotherapy; SCT: stem cell transplantation.

chemotherapy according to our protocol for acute myeloid leukemia (AML), which consists of intravenous idarubicin 12 mg/m^2 for 3 days, cytarabine 100 mg/m^2/day by continuous intravenous infusion for 7 days and oral thioguanine 60 mg/m^2 for 7 days). Bone marrow was evaluated on day 15 in 23/24 patients. In 13 patients (56.5%), the day-15 bone marrow showed a good early response with less than 5% blasts, while ten patients (43.5%) had residual leukemia (>5% blasts) in the bone marrow at this evaluation. Four of the ten patients with MPAL in whom the bone marrow was evaluated on day 15 had more than 5% blasts; two of these were the patients who received AML induction therapy and were subsequently switched to the St. Jude protocol for remission induction.

Bone marrow was also examined in all patients following the 6-week induction phase and prior to proceeding to consolidation therapy. Only one patient (#12) failed to achieve a complete remission, giving a remission induction rate of 95.8%. This patient, who initially had a poor response at day 15, was re-induced with multiple induction chemotherapy regimens including AML therapy, FLAG, and mitoxantrone/etoposide but failed to achieve complete remission and was subsequently placed on palliative care. All the remaining patients who achieved complete remission continued on chemotherapy following induction of the remission. Thirteen of these 23 patients were treated with chemotherapy alone according to the protocol outlined in Table 1, while 11 patients underwent allogeneic HSCT at a median of 12 weeks after achieving remission. All 11 patients diagnosed as having MPAL achieved complete remission; six of these patients subsequently underwent allogeneic HSCT while the remaining five continued on chemotherapy alone.

Chemotherapy was well tolerated by all patients and there was no non-leukemia mortality on chemotherapy. Four patients developed documented or presumed invasive fungal infections during induction, but were able to continue with chemotherapy without any subsequent problems.

The overall and event-free survival rates for all 24 patients are shown in Figure 1. At a median follow up of 4 years (range, 6 months – 7 years) the overall survival rate was 75.7% and the event-free survival rate was 73.5%. The survival figures for those patients who underwent HSCT in first complete remission were no different from those for patients who were treated with chemotherapy alone (overall survival: 70.1% *versus* 81.1%, respectively, p=0.39; event-free survival 70.1%

Table 3. Details of the immunophenotype of the leukemic blast cells from all the patients.

CD Patient	Anti TCR	1a	2	3	5	7	8	10	19	20	22	79a	TdT	13	14	15	33	64	65	117	MPO	34	HLADR	EGIL Scores T	B	M	Phenotype	WHO Diagnosis
1	0	–	–	–	–	–	–	+	+	–	+	+	0	+	–	0	+	0	0	0	+	+	+	0	6	4	B/M	MPAL
2	0	0	–	–	0	–	–	+	+	-	+	0	+	–	–	0	+	–	–	–	+	+	+	0	4.5	3	B/M	MPAL
3	0	–	+	+	–	+	–	+	+	+	+	+	+	–	–	0	–	–	–	–	–	–	0	4	7.5	0	B/T	MPAL
4	0	0	–	–	0	–	–	+	+	–	+	+	+	+	–	0	+	–	–	–	+EM	+	+	0	6.5	4	B/M	ALL*
5	0	–	–	–	0	–	–	+	+	–	–	0	+	+	–	–	+	–	–	–	+ EM	+	+	0	2.5	4	B/M	ALL*
6	0	–	+	+	–	+	–	–	+	–	–	0	+	+	–	+	+	–	–	+	+	+	+	3.5	0	5.5	T/M	MPAL
7	0	–	+	+	–	+	–	–	–	–	–	0	+	+	–	+	-	–	+	+	+	+	+	4	0	5.5	T/M	MPAL
8	0	0	-	-	0	-	-	+	+	-	+	+	+	+	–	–	+	–	–	+	-	+	+	0	6.5	3	B/M	ALL
9	0	0	-	-	0	-	-	+	+	-	+	+	+	+	-	-	+	-	0	+	-	+	+	0	6.5	3	B/M	ALL
10	0	-	+	+	+	+	-	-	-	-	-	0	-	+	-	+	+	-	+	-	-	+	-	4.5	0	3.5	T/M	ALL
11	0	0	-	-	0	-	-	+	+	-	+	0	+	+	-	-	+	-	-	+	-	+	+	0	4.5	3	B/M	ALL
12	0	0	-	-	0	-	0	-	+	-	+	+	+	-	-	+	-	-	+	+	-	+	+	0	5.5	2.5	B/M	ALL
13	0	0	-	-	0	-	-	+	+	-	+	+	+	+	-	-	+	-	-	+	-	+	+	0	6.5	3	B/M	ALL
14	0	0	+	+	+	+	-	+	-	-	-	0	+	-	+	-	+	-	+	+	+	+	+	6.5	1	5.5	T/M	MPAL
15	0	0	-	-	0	-	-	+	+	-	+	+	+	+	-	+	+	+	0	-	+	0	+	0	6.5	5	B/M	MPAL
16	0	-	-	+	+	+	-	-	-	-	-	0	+	+	-	+	+	+	-	+	+	+	+	4	0	6	T/M	MPAL
17	0	-	+	+	-	+	-	-	-	-	+	+	+	+	-	+	+	-	+	+	-	+	+	3.5	4.5	4.5	T/B/M	ALL
18	+	-	+	+	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	6.5	3	1	B/T	ALL
19	0	0	-	-	0	-	-	+	+	+	+	+	+	+	-	+	+	-	-	-	+EM	+	+	0	7.5	4.5	B/M	ALL*
20	0	0	-	-	0	-	-	+	+	-	+	+	+	+	-	-	+	-	-	-	+	+	+	0	8.5	3	B/M	MPAL
21	0	0	-	-	0	-	-	+	+	-	+	+	+	+	-	-	+	-	-	+	-	+	+	0	6.5	3	B/M	ALL
22	0	+	+	+	+	+	-	-	-	-	-	0	-	+	-	+	-	-	-	+	-	+	+	5	0	2.5	T/M	ALL
23	0	-	-	-	-	+	-	-	+	-	-	+	+	-	-	+	+	-	-	+	+	+	+	0.5	3.5	4.5	B/M	MPAL
24	0	-	+	+	-	+	-	-	-	-	-	0	+	+	-	+	-	-	+	+	+	+	+	4	0	5.5	T/M	MPAL

0: test not performed; +: positive expression; – : negative expression; EM: positive only by electron microscopy; B: B-lymphoid; T:Tlymphoid; M: myeloid; MPAL: mixed phenotype acute leukemia; ALL: acute lymphoblastic leukemia; ALL:ALL with MPO positive by EM.*

versus 76.2%, respectively, p=0.75). These survival curves are depicted in Figure 2. Patients who had more than 5% blasts in the day-15 bone marrow evaluation had a significantly worse outcome than those who showed a brisker response. The event-free survival rate in these slow responders was 48% as compared to 90.9% among those who had less than 5% blasts on day 15 (p=0.015).

The survival outcome for the 11 patients who were retrospectively classified as having MPAL according to the new WHO criteria was excellent, with no relapses or deaths. Six of these patients were transplanted at a median of 11 weeks after achieving remission. These transplanted patients included four patients with a poor treatment response mid-induction, one of whom also had t(9;22). The patient with the *MLL* gene rearrangement was transplanted. All of the five patients who were treated with chemotherapy alone had good early responses to induction chemotherapy and had no known adverse cytogenetic abnormalities. Patients with MPAL did significantly better than the remaining patients who were classified as having ALL with myeloid co-expression (overall survival: 100% *versus* 54%, respectively, p=0.02). This difference was more marked for patients who received a transplant compared to those who were treated with chemotherapy alone (overall survival 100% *versus* 40%, p=0.045 and 100% *versus* 66.7%, p=0.18, respectively).

Discussion

Being an uncommon type of acute leukemia, BAL has only recently gained some significance especially with the availability of objective diagnostic criteria. Earlier reports on BAL showed variability in the incidence; however, due to this objectivity in definition, the incidence of BAL has recently shown some consistency (2–5%). In this pediatric study, the incidence of BAL was 3.8%. Two other studies that specifically evaluated pediatric patients used different immunophenotypic diagnostic criteria: Park *et al.* used the EGIL system,[10] while Rubnitz *et al.* employed criteria that are more

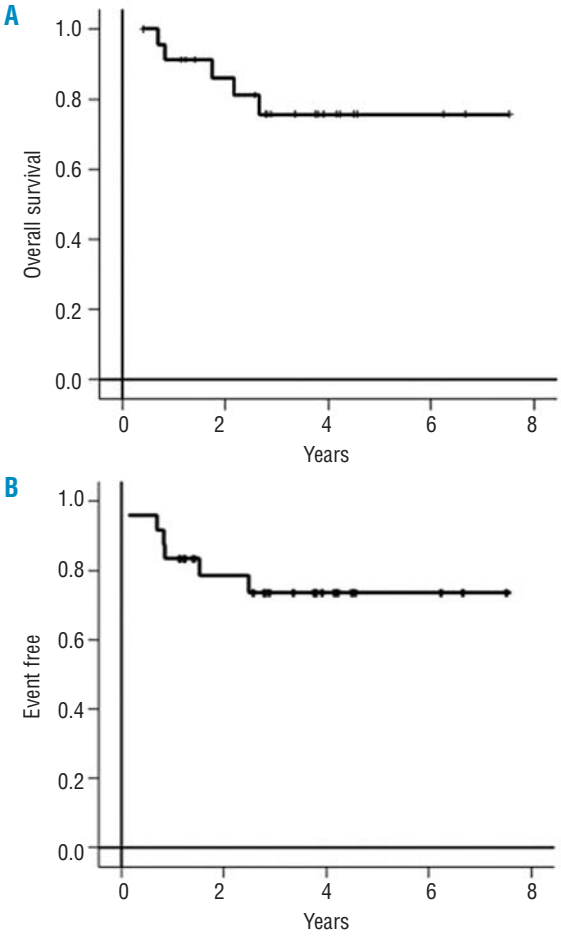

Figure 1. Kaplan-Meier plot for (A) overall survival (73.6%) and (B) event-free survival (72.4%) for all biphenotypic acute leukemia patients (n=27).

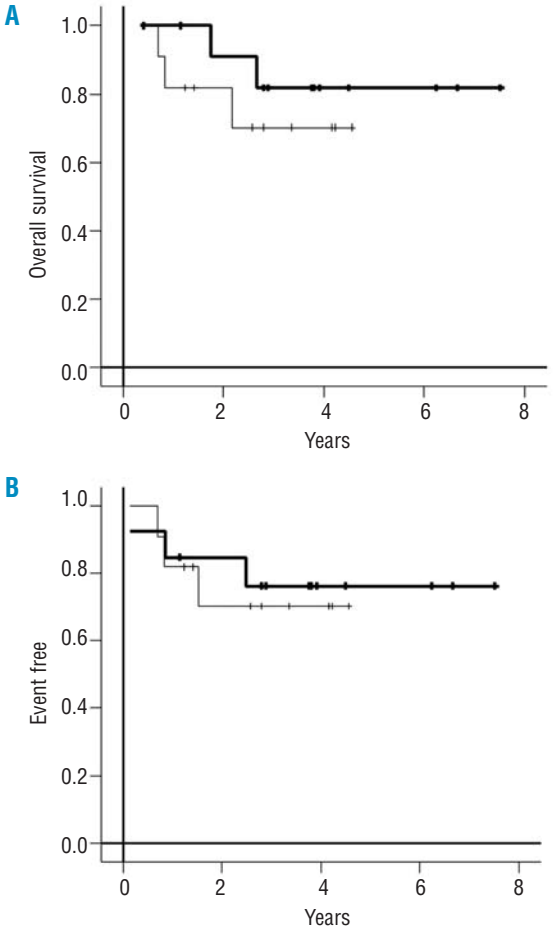

Figure 2. Kaplan-Meier plots for (A) overall survival (77.1% *versus* 70.1%; p= 0.5) and (B) event-free survival (73.1% *versus* 70.1%; p=0.8) for biphenotypic acute leukemia patients treated with chemotherapy alone (heavy line) and those treated with HSCT in first remission (fine line).

stringent than those proposed by the EGIL.[9] While our overall incidence rate is in agreement with the rates in previously published series which included both adult and pediatric patients[6,7] and with the rate in the pediatric series in which the EGIL diagnostic system was used,[10] when we utilized the more stringent, new WHO diagnostic criteria we found an incidence similar to that reported by Rubnitz *et al.*[9]

The phenotype distribution in our patients was no different from previously published distributions, with the majority of patients having a B-lymphoid/myeloid phenotype and the T-lymphoid/myeloid phenotype being the next common phenotype. Only two of our patients had a B-lymphoid/T-lymphoid phenotype. This bi-lymphoid BAL has previously been reported to have favorable presenting features and, generally, to have a good outcome.[16] Interestingly, there was a greater representation of the T-lymphoid/myeloid phenotype within the cohort classified as having MPAL by the WHO criteria. Rubnitz *et al.* also found a higher proportion of such patients in their study.[9]

Electron microscopic detection of MPO has been used in the diagnosis of patients with BAL, based on studies showing the higher sensitivity of electron microscopy in determining the presence of MPO and assignment of myeloid lineage.[6,17] However, during the reclassification of our patients according to the WHO criteria, we did not use the results derived from this methodology and, based on the specifications, used only the results of MPO determined by flow cytometry or immunohistochemistry. The three patients who were positive for MPO only by electron microscopy were not, therefore, included in the cohort of patients with MPAL. Two of these patients, one of whom had the *MLL* gene rearrangement, have relapsed. The issue of whether to include electron microscopy-determined positivity for MPO as a supplementary criterion, when available, among the other WHO criteria for classification of acute leukemia should be reconsidered after further studies in larger cohorts of patients.

There is no single chromosomal abnormality that is unique to BAL. However, our data and those of others have shown that structural abnormalities are common. In several previous studies the incidence of t(9;22) was high, ranging from 28 to 35%.[7,11,18] However, these were studies involving mixed populations of adults and children, and Philadelphia chromosome-positive leukemias are more common in older age groups. The incidence in our cohort was much lower (3%), since only one patient was found to have this translocation, which is not surprising as our population consisted of children under the age of 14 years old.

This incidence of *MLL* gene rearrangement in our cohort appears somewhat higher than previously reported,[6,7,10] but is consistent with the incidence found in studies confined to the pediatric age group,[9,10] and may in fact be a reflection of the younger overall populations in these studies.

Four patients had abnormalities of chromosome 14 involving the q32 locus. Translocations including the 14q32 locus have previously been reported as a non-random lesion in mixed lineage leukemias, particularly those involving T-lymphoid- and myeloid-associated antigen expression.[9,19] Only one of our patients had an evident t(8;14)(p21;q32) translocation (patient #7), while in the second patient (patient #13) only an *IGH* rearrangement could be documented by FISH without determination of the translocation partner. The other two patients (patients #6 and 17) had additions of the 14q32 chromosomal region. Only one of these was classified as having T-lymphoid/myeloid leukemia. Two of these patients (#6 and #7) could be classified as having MPAL.

Currently, there is no universal treatment approach for patients with BAL. Our data demonstrate that an ALL type of induction utilizing agents that have activity against lymphoid and myeloid leukemias appears to be more effective than using AML type therapy for achieving complete remission. The myeloid-active agents were used at much lower doses than customary in the treatment of AML. This induction chemotherapy was well tolerated and there were no early deaths. Our results compare very favorably with other published results of studies in which patients were treated with either ALL therapy or AML therapy or a combination using higher doses of cytarabine. Killick *et al*. reported treatment outcome of 20 patients with *de novo* BAL; five were treated with ALL induction, five with AML induction and ten with a combined strategy. The reported remission rate was 70%, with an induction death rate of 25%.[7] Another study utilizing either ALL-type therapy or AML-type therapy for induction also reported lower remission rates (ALL-type therapy 78%; AML-type therapy 57%) than those achieved in our study.[13] In their pediatric study, Rubnitz *et al.* likewise found that the complete remission rate was higher among patients undergoing induction with ALL-type therapy than in those receiving AML-type induction therapy (83% *versus* 52%, respectively).[9] Furthermore, eight of the ten patients in their study who failed to achieve complete remission with AML-type therapy subsequently achieved it with ALL-type induction therapy.[9] In their pediatric patients, Park *et al*. also reported a significantly lower complete remission rate of 52% with a predominantly ALL-type therapy.[10]

We feel that our post-induction strategy has also been successful. Treatment during this period involved rotational drug pairs which included agents such as cytarabine, etoposide and cyclophosphamide which have activity against myeloid leukemia, in addition to antimetabolites, corticosteroids and vincristine which are the mainstay of therapy for lymphoblastic leukemia. The survival of our patients was better than that reported for other series (8.1-60%).[9] Killick *et al.* compared the outcomes of their adult patients and their patients under 15 years of age, finding a significantly higher overall survival for the younger patients (75% *versus* 17% at 2 years; p=0.01).[7] The proportion of patients with Philadelphia chromosome-positive leukemia was higher in the older group than in the younger patients (5/12 *versus* 2/8) and this may have contributed to the difference in outcomes. This overall survival of 75% in the younger group is very similar to the rate in our pediatric cohort. The study from St. Jude Children's Research Hospital also indicated that patients may benefit from a treat-

ment strategy based on ALL-type therapy utilizing agents that are effective against both myeloid and lymphoid leukemias.[9] We have shown that this strategy is highly effective even for those patients diagnosed using the more stringent, new WHO criteria.

The issue of HSCT remains contentious. Park *et al.* reported no benefit from HSCT over chemotherapy alone in patients with BAL.[10] However their overall survival results were fairly poor and may have been indicative of sub-optimal pre-transplant chemotherapy. Our decision to transplant was based primarily on the availability of a source of HLA-matched stem cells. However, with the current availability of stem cells (either from a matched, related donor or in the form of an umbilical cord blood unit) for almost all patients and the nearly identical outcomes in transplanted and not transplanted patients, other risk-determining variables need to be included in this decision process. Based on our results and those published elsewhere we recommend that patients with Philadelphia chromosome-positive BAL [MPAL with t(9;22)], infants (particularly those with 11q23 abnormalities) and those patients who have a poor response to early therapy (>5% blasts in the bone marrow on day 15 or >1% minimal residual disease at the end of induction therapy) should probably undergo allogeneic HSCT. Whether older children with 11q23 abnormalities should also be transplanted remains unclear and requires assessment in larger, possibly multi-institutional, studies.

Those patients who were originally classified as having BAL according to the EGIL criteria but subsequently diagnosed with ALL according to the revised WHO classification seem to have done very poorly, even with this intensive protocol, with an overall survival of only 54%. Myeloid antigen co-expression in ALL *per se* has not been found to have prognostic value in the context of current modern therapy.[19,20] However, our patients seemed to have significant chemoresistance, with six of these 13 patients showing a poor bone marrow response at mid induction and one failing to achieve remission. There were five deaths in this group of patients: three died following relapse and disease progression and two died in complete remission following complications of HSCT. Both of the patients who died in complete remission had more than 5% blasts in their bone marrow at the mid-induction evaluation. Only one of these patients (#19) had a known adverse cytogenetic abnormality, t(7;11)(p22;q23). The reasons for this poor response remain unknown and further evaluation of this group of patients is needed in larger cohorts of patients.

Our study shows that pediatric BAL may be distinct from its adult counterpart in terms of clinical and genetic characteristics, and response to chemotherapy. Although our study is limited by relatively small numbers, important conclusions can still be drawn; the prognosis of children with BAL is good, with their survival being comparable to that of patients with high-risk ALL and better than that of patients with AML. This good prognosis was retained even when the diagnostic criteria for BAL were those of the more stringent WHO classification. Patients with BAL should be stratified according to their risk and receive treatment tailored to their prognostic factors. HSCT in first complete remission may not be necessary for all patients and our strategy of chemotherapy was effective and safe in the treatment of low-risk BAL. Certainly, larger numbers of patients are needed to identify subsets of patients with distinct risk levels; given the rarity of BAL, multicenter collaboration will be required for such studies.

Authorship and Disclosures

ASA and AFB contributed equally to this study. They designed and performed the research, collected, analyzed and interpreted data, and wrote the manuscript. TMO performed the hematopathology and flow cytometric evaluations and interpreted the cytogenetic data. MA, HES, MAM and AA interpreted data.

All authors critically reviewed and approved the manuscript.

The authors declare no competing financial conflicts of interest.

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