



Understanding mixed phenotypic acute leukemia: A conundrum of six cases with review of literature

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Abstract:

BACKGROUND: Mixed phenotype acute leukemias (MPALs) are a heterogeneous group of rare leukemias, with an incidence of 2%–5% of all acute leukemias and varied clinical, cytogenetic, immunophenotypic, and molecular genetic features. They have been reported to have a poor prognosis, early relapse, and increased incidence of extramedullary infiltration.

MATERIALS AND METHODS: Retrospective analysis of all the acute leukemias (ALs) diagnosed in the department of pathology over the period of August 2017 to December 2019 was done. MPAL cases were identified in accordance with the World Health Organization (WHO) 2016 guidelines and European Group for the Immunological Classification of Leukemias Criterion. Beckman and Coulter FC500 flow cytometer was used using AL panel with CD3, cyCD3, CD5, CD1a, CD2, CD4, CD8, and CD7 as the markers for T-cell lymphoid lineage, along with CD19, CD 79a, CD22, and CD20 for B-cell lineage and CD13, CD33, CD 14, CD64, and myeloperoxidase for myeloid lineage/monocytic lineage. Molecular analysis was also done.

RESULTS: Of the 153 cases newly diagnosed cases of AL during this period, 6 fulfilled the European group for immunological characterization of acute leukemia/WHO criteria for MPAL (3.9%). The age ranged from 3 to 13 years with male-to-female ratio of 1:1. Four out of the six cases (66.6%) were assigned B-lymphoid/myeloid type and two (33.3%) were assigned B/T lymphoid. Cytogenetics was available in four out of six cases, among which three had a normal karyotype and one had Breakpoint Cluster Region-Abelson Murine Leukemia 1 (Philadelphia chromosome) (BCR-ABL) translocation (t [9;22] [q34; q11]).

CONCLUSION: Distinction at the outset is of paramount importance and the role of morphology, flow cytometry compounded with cytogenetic/molecular studies cannot be denied.

Keywords:

B/myeloid mixed phenotype acute leukemias, B/T mixed phenotypic acute leukemia, mixed phenotypic acute leukemia, pediatric leukemias

Introduction

Mixed phenotypic acute leukemia (MPAL) is a rare, heterogeneous group of disorders that constitute 2%–5% of all acute leukemias (ALs).^[1] They are thought to arise from a very early hematopoietic progenitor cell.^[2] MPALs are characterized by leukemias in which the blast population

cannot be assigned to a single lineage. Although they have heterogeneous clinical, cytogenetic, immunophenotypic, and molecular genetic features, broadly they can be classified into bilineal and biphenotypic leukemias according to the World Health Organization (WHO) 2008 guidelines. Biphenotypic MPALs have a single population of blasts that express antigens of more than one lineage. Bilineal MPALs are much rare and have distinct population

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of blasts of more than one lineage (both on morphology and flow cytometry [FCM]) where the sum of the two blast populations is more than or equal to 20% of all nucleated cells. The European group for immunological characterization of acute leukemia (EGIL) presented the guidelines for the classification of AL with biphenotypic marker expression in 1995. These criteria were subsequently integrated in the WHO 2001 guidelines for classifying ALs of ambiguous lineages. For many years, the EGIL guidelines were used for the diagnosis of MPAL. However, in 2008, new WHO criteria were proposed for the classification of MPALs.^[3] The WHO 2016 criteria added no new entities in this group.^[4] The WHO criteria are strict and simple but rely on specificity and sensitivity of immunophenotypic expression of antigens, and no thresholds are set up. They exclude ALs with certain recurrent cytogenetic aberrancies or clinical presentations from MPAL, i.e., ALs with t (8;21), t (15;17), or inv (16) despite their immunophenotypic marker expression. Furthermore, AL with FGFR1 mutations, chronic myeloid leukemia-blast crisis, AL with myelodysplastic syndromes-related change and therapy-related AL are separate entities. Aberrant antigen expression, which is quite commonly seen in cases of AL, also needs to be excluded before diagnosing a case as MPAL.

Materials and Methods

This was a retrospective analysis of all cases of MPALs diagnosed at the department of pathology of our center during the period of August 2017 to December 2019. Clinical details, morphological evaluation, and immunophenotyping details were retrieved for all patients. Karyotyping, cytogenetics, and treatment details were recorded where available. The diagnosis of MPALs was made according to EGIL 1998/WHO 2016 guidelines.

All specimens were obtained and prepared for morphologic examination using the standard techniques, as per the protocol followed in the department.

Three milliliters of blood samples were received in ethylenediaminetetraacetic acid vials for complete hemogram including blood smears. Samples were run in automated hematology analyzers (Sysmex XT 2000i and XN 2000i). Total leukocyte count (TLC), differential count, hemoglobin, platelet count, and red blood cell parameters were studied.

Peripheral blood smears and bone marrow aspirate smears were air dried and stained with Giemsa stain. Myeloperoxidase (MPO) and periodic acid-schiff (PAS) was done routinely in all peripheral smears and bone marrow aspirate smears. Other cytochemical

stains (nonspecific esterase and specific esterase) were done according to the morphological details of the blasts.

Five color flow cytometric analysis was performed using Beckman and Coulter FC500 flow cytometer on Bone Marrow Aspirate (BMA) and peripheral blood samples. Standard lyse-wash procedure was used followed by incubation at the room temperature for 15 min. Permeabilization of cells was done for the cytoplasmic markers. The cells were stained with various combinations of fluorochrome labelled monoclonal antibodies such as fluorescein isothiocyanate, phycoerythrin, electron coupled dye, phycoerythrin-cyanin 5 (PC5) and PC7 and immunophenotyping was done. Gating of blasts was done using CD45/side scatter. The following markers were used in the panel: CD45, CD19, CD20, CD10, CD79a, CD22, CD123, CD38, CD56, HLADR, CD34, CD117, TDT, CD1a, CD2, cCD3, CD7, CD4, CD8, CD14, CD16, CD11c, CD64, CD13, CD33, and cMPO.

The MPAL cases were reviewed, and the clinical findings of each case were noted. The laboratory parameters including TLC, differential leukocyte count, percentage of blasts, and cytochemistry in peripheral smear and bone marrow were noted. The differential count was performed on 200 cells on bone marrow and peripheral blood and on 500 cells where the count was low. The flow cytometric analysis and the immunophenotypic lineage assignment of the MPAL cases were done and reviewed. For interpretation, 20% positivity was used as a cutoff value for surface markers and 10% for cytoplasmic markers as mentioned in the EGIL criteria. For MPO, cutoff of more than or equal to 3% was taken on cytochemistry or 10% by FCM.^[3]

Conventional cytogenetic analysis was carried out on the bone marrow samples according to standard techniques. The AL related mutations that were evaluated included: t (8, 21), t (15, 17), t (9, 22), t (12, 21), t (4, 11), and inv (16) in B/Myeloid MPALs and t (12, 21), t (9, 22), and t (4, 11) in B/T lymphoid MPALs.

Results

Of the 153 newly diagnosed cases of AL during this period, only 6 (3.9%) fulfilled the EGIL/WHO criteria for MPAL.

Clinical details

The age ranged from 3 to 13 years with male-to-female ratio of 1:1. Five of the cases had splenomegaly and three of them had hepatomegaly. One B/myeloid case had lymphadenopathy and both the B/T lymphoid cases presented with lymph nodes. Case 1 presented to the hospital with splenic rupture after a year-long history of splenomegaly. Case 2 had an atypical presentation

with proptosis of the left eye and Case 3 and 4 had tendency to bleed. TLC ranged from 3830 to 36,470/mm³ with a median of 16,770/mm³. There was leucocytosis in four cases, TLC normal in one and one case showed leukopenia (case 6). Platelet count ranged from 9000 to 170,000/mm³ with a median of 15,000 mm³. Five cases had thrombocytopenia and one case had normal platelet count (Case 6). Blast population ranged from 39% to 87% on peripheral blood with a median of 55% and between 50% and 88% with a median of 70% in the bone marrow. All the cases presented with low hemoglobin for age, ranging between 3.8 and 8.6 g/dl. The details are summarized in Table 1.

Morphology and immunophenotyping

Peripheral smear examination revealed monomorphic population of lymphoid looking blasts in all B/myeloid cases and undifferentiated blast population in B/T lymphoid cases [Figures 1 and 2]. Cytochemistry details are given in Table 1.

Immunophenotyping details of the cases are given in Tables 2 and 3. Out of the 6 cases of MPAL, 4 (66.6%) were assigned B-lymphoid/myeloid type (three were females and 1 male) and 2 (33.3%) were assigned B/T lymphoid (both males). Three cases of B/myeloid type had MPO positivity by both cytochemistry and FCM (Case 1–3). As for Case 4, result for MPO cytochemistry was inconclusive which later showed bright MPO positivity on FCM. CD34 expression was seen in 3/6 cases (50%). HLADR expression was seen in 4/6 cases (66.6%).

B/myeloid mixed phenotypic acute leukemia

WHO specifies MPO positivity to be the most specific criteria for assignment of myeloid lineage by cytochemistry, immunohistochemistry (IHC), or immunophenotyping by FCM, more so on cytochemistry followed by IHC rather than immunophenotyping by FCM alone. Furthermore, single blast cell with the presence of Auer rod is given cognisance.^[1]

In the present study, four cases of B/myeloid cases were diagnosed. Three of the cases were diagnosed using the WHO criteria while one was diagnosed using the EGIL

guidelines. Three cases showed single blast population showing MPO positivity with Case 4 having MPO inconclusive on cytochemistry. Case 2 also had PAS positivity (dot+). On FCM, all four cases had bright cytoplasmic MPO (cMPO) positivity. Case 1–3 had both CD33 and CD13 positivity and Case 4 was CD13 positive and CD 33 negative. Furthermore, myeloid lineage can be assigned if blast population is positive for two of the following monocytic markers: NSE, CD11c, CD14, CD64, and lysozyme. However, the blast population in the present study showed no monocytic differentiation.

For assignment to B lymphoid lineage, multiple markers are required: Strong CD19 with strong CD79a/cCd22/CD10 or weak CD19 with strong expression of two of these.

One of our cases (Case 1) had strong expression of CD19 (95% blasts) with 44% of blast cells showing coexpression of CD10 and 19 and CD79a dim positive. It fulfilled the MPAL criteria laid down by EGIL, but falls short of the WHO criteria.

Other two cases (Case 2, 3) had weak positivity for CD19 with dim to moderate strong positivity for CD10 and CD79a, which fulfils the WHO criteria [Figure 3]. Case 4 had moderate positivity for CD19 with strong expression of cCD79a, CD10, and moderate expression of CD20; this fit into B/myeloid by the WHO criteria.

It is suggested that the WHO criteria are too stringent and if followed, may miss out on MPAL cases.

B/T lymphoid mixed phenotypic acute leukemia

Two cases were diagnosed as B/T lymphoid MPAL: A 3 and 5 year old children, both males child, one by EGIL and one by WHO. Case 5 had moderate positivity for CD19 and bright for CD10, CD20, and cCD79a. In Case 6, B lineage assignment was done using positivity of the blasts for moderate CD19, dim CD10, and cCD79a [Figure 4]. T lymphoid lineage was assigned using positivity for cytoplasmic CD3 in 71% blasts and 63.3%, respectively, in Case 5 and 6.

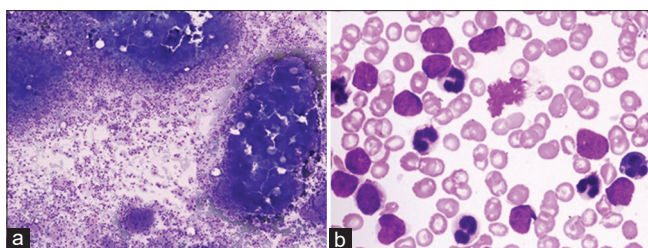


Figure 1: (a and b) May-Grunwald-Giemsa-stained bone marrow smears showing a population of large blasts ($\times 40$ and $\times 600$) (Case 2)

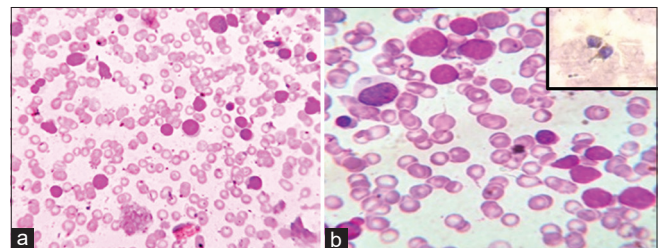


Figure 2: (a and b) May-Grunwald-Giemsa-stained peripheral smears showing a population of large blasts with inset showing myeloperoxidase positive blasts ($\times 400$ s and $\times 1000$) (Case 6)

Table 1: Clinical and laboratory findings in mixed-phenotype acute leukemias

Case number	Age (years) and gender	Hb (g/dl)	TLC/mm ³	Platelets/mm ³	PB blast (%)	BM blast (%)	Spleen (%)	LN	Liver	Others	Cytochemistry	Cytogenetics and karyotyping	Diagnosis
1	13/female	6.7	15,650	9000	39	71	Present	Nil	Present	Splenic rupture	MPO+, PAS-	Not available	B/myeloid
2	6/female	5.7	26,850	14,000	70	70	Nil	Nil	Nil	Proptosis left eye	MPO+, PASdot+	Not available	B/myeloid
3	6/male	7.4	5320	16,000	35	50	Present	Nil	Nil	H/o multiple transfusions	MPO+, PAS-	46 XY	B/myeloid
4	4/female	7.7	36,470	14,000	87	ND	Present	Present	Present	Petechiae	MPO inconclusive, PAS-	46 XX	B/myeloid
5	5/male	3.8	17,890	22,000	77	88	Present	Present	Nil	Tachypnea, epistaxis	MPO-, PAS inconclusive	46 XY, BCR-ABL+	MPAL with BCR-ABL1
6	3/male	8.6	3830	170,000	40	60	Present	Present	Present	Cough	MPO-, PAS-	46 XY	B/T lymphoid

MPALs=Mixed-phenotype acute leukemias, Hb=Hemoglobin, TLC=Total leukocyte count, PB=Peripheral blood, BM=Bone marrow, LN=Lymph node, MPO=Myeloperoxidase, PAS=Periodic acid-Schiff, BCR-ABL=Breakpoint Cluster Region- Abelson Murine Leukemia 1 (Philadelphia chromosome), B/T=B lymphoid/T lymphoid

Discussion

MPALs are a heterogeneous group of leukemia in which the blasts cannot be assigned a single lineage. The blasts can have dual population with both populations belonging to different lineage or a single population with antigen expression of different lineages. Various lineage combinations are possible, out of which B/myeloid are the most common and B/T lymphoid are the most rarely found.^[5] Very rare cases of trilineage MPALs have also been documented in the literature.^[6,7]

Initial reports of MPAL were seen in the literature in 1980s when monoclonal antibodies were first used for immunophenotyping of ALs. With the availability of more antibodies, it became apparent that cross lineage aberrant antigen expression is also seen commonly which needs to be excluded before reporting a case as MPAL. Morphology can be helpful in suspecting a case of MPAL when there are two distinct populations of blasts on the peripheral smear. MPAL should also be suspected when lymphoid looking blasts (morphologically) show positivity for MPO on cytochemistry. However, MPAL blasts are seen to have an undifferentiated appearance more commonly. The diagnosis of MPAL eventually relies heavily on immunophenotyping by FCM.

There is a paucity of MPALs in the literature, especially in Indian children.

The present study reviewed 153 AL cases, of which 6 were diagnosed with MPAL (3.9%). This prevalence corroborates with the other existing literature.^[8,9] There was a female predominance in cases of B/myeloid ($n = 3/4$) which is consistent with a study by Yan *et al.*,^[8] however, Nair *et al.*^[9] and Matutes *et al.*^[6] showed male predominance in B/myeloid MPALs. Weinberg *et al.*^[10] showed no gender predilection. This might be explained due to the small sample size of the present study and ethnic/regional differences. Even though MPALs are more common in adults than in children, B/T lymphoid cases are more common in children.^[2,5] The present study shows all the cases of MPALs in only children which is in contrast to the other existing literature.^[6,9,11]

66.6% cases ($n = 4/6$) were diagnosed with B-Myeloid, whereas 33.3% cases were diagnosed with B/T lymphoid ($n = 2/6$). Higher incidence of B/myeloid cases is in accordance with other studies published.^[6,9,11,12] In the present study, four cases conformed to the WHO criteria while 2 fell short of it but satisfied the EGIL guidelines (Case 1 and 6). Detailed review of the existing literature was done [Table 4].

Literature shows that B/myeloid MPALs show a monomorphic blast population more commonly rather

Table 2: Immunophenotypic details of the B/myeloid mixed-phenotype acute leukemias

Case	Myeloid markers (%)		B lymphoid markers (%)		Immaturity markers
	Specific	Others	Specific	Others	
1	cMPO (53)	CD33 (81.6) CD13 (13.6)	CD19 (94.8)	cCD79a (43.8) CD10 (42)	HLADR (64.3)
2	cMPO (87)	CD33 (67), CD13	CD19 (53)	cCD79a (26.1), CD10	CD34 (98.8), CD117, HLADR (99)
3	cMPO (82)	CD33 (81.1) CD13 (49.4)	CD19 (74)	cCD79a (51)	CD34 (52)
4	cMPO (67)	CD13 (14)	CD19 (99)	cCD79a (97.8) CD20 (28) CD10 (93)	HLADR (97.8)

cMPO=Cytoplasmic myeloperoxidase, HLADRs=Human leukocyte antigen-drug reactions

Table 3: Immunophenotypic details of the B/T lymphoid mixed-phenotype acute leukemias

Case	B lymphoid (%)		T lymphoid markers (%)		Immaturity markers
	Specific	Others	Specific	Others	
5	CD19 (81.3)	cCd79a (89.1) CD10 (80.4) CD20 (82.7)	cCD3 (71)		CD38 (85.4)
6	CD19 (56)	cCd79a (18.8) CD10 (26.4)	cCD3 (63.3)		HLADR (80) CD34 (81)

HLADRs=Human leukocyte antigen-drug reactions

Table 4: Review of literature

Study	n (%)	B/myeloid, n (%)	T/myeloid n (%)	B/T, n (%)	Trilineal (%)	Male/female	Age	Morphology
Laxminarayan (KMC Manipal)	9/218 (4.1)	33.3	33.3	33.3		8/1	5/9 pediatric	5 had dual morphology
Pawar	14/628 (2.2)	9/14 (64)	4/14 (28)	1/14 (7)		8/6	3/15 pediatric	All undifferentiated
Matutes	100 cases	59	35	4		62/38	32% pediatric	All undifferentiated
Sukumaran (Kerala)	15/506 (2.9)	33.3	60	6		NA	5/15 pediatric	6 had dual morphology
Yan (China)	117/4780 (2.4)	55	38	12	0.9	Female > male in B/myeloid	All adults	NA
Lee <i>et al.</i>	12/377 (3.1)	50	41.6			7/5	Pediatric	
Orgell <i>et al.</i>	54 cases	33/54 (61.1)	19/54 (35.2)	0		28/26	Paediatric	
Present study	6/153 (3.9)	4/6 (66.6)		2/6 (33.32)		Female > male in B/myeloid	All pediatric	All monomorphic

NA=Not applicable, B/T= lymphoid

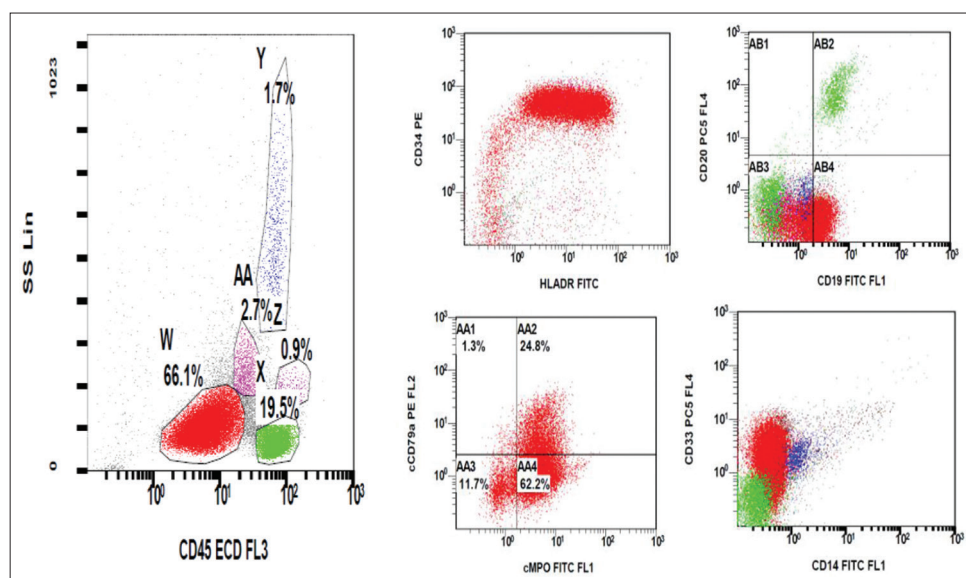


Figure 3: Dot plots with the blast population highlighted in red and lymphocyte population in green (Case 2)

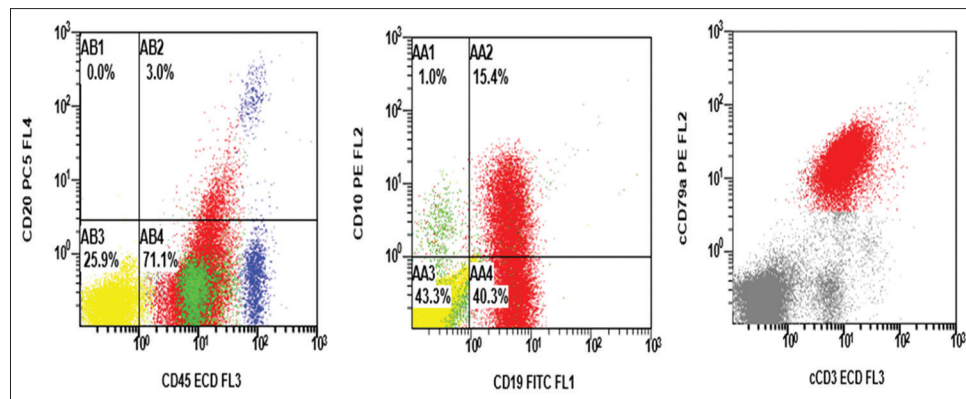


Figure 4: Dot plots with the blast population highlighted in red and lymphocyte population in green (Case 6)

than dual.^[9,11] All the cases in the present study had monomorphic population with lymphoid looking blasts. As for B/T lymphoid MPALs, the morphology was undifferentiated which is in accordance with the other existing literature. However, only few cases of B/T lymphoid MPAL have been reported in the literature.^[15,7]

On the basis of associated cytogenetic abnormalities, MPALs can be divided into MPAL with Philadelphia chromosome (t [9, 22]/BCR-ABL1; MPAL with t [v; 11q23]/MLL), and not otherwise specified. MPAL with t (9, 22) show breakpoint p210 most commonly. In the present study, cytogenetics information was available in four out of six cases. Two B/Myeloid cases (Case 3 and 4) showed normal karyotype with no clonal abnormality on FISH for the tested translocations. Among B/T lymphoid cases, Case 5 had translocation t (9, 22) positive by FISH while Case 6 had normal karyotype. Owaidah *et al.*^[7] showed 68% to have a clonal abnormality and 32% to have normal karyotype. Additional abnormalities include deletion of 6q, 5q, and 12p. While Pawar *et al.*^[11] showed a normal karyotype in 33% of the cases and Philadelphia in 25%, Laxminarayan showed a normal karyotype in four out of six cases Philadelphia in two.

Because of the rarity of MPALs, a standard therapeutic approach has not been set up. While majority of the studies show ALL therapeutic regimen to have a better outcome than adult acute myeloid leukemia (AML) type, a study by Rubio *et al.*^[13] reported that T/Myeloid responded better to AML regimen. However, overall literature favors ALL therapy over AML. In the present study, two of the six children did not follow-up for treatment initiation postdiagnosis. Two of the B/myeloid cases (Case 3 and 4) were started on B-ALL induction protocol with corticosteroids (ICICLE high risk [HR] protocol). Case 3 did not show morphological remission at the end of induction and succumbed to progressive illness in 3rd month of illness. Case 4 died on day 7 of illness due to febrile neutropenia [Table 1]. Both the B/T lymphoid cases (Case 5 and 6) were initially treated with

B-ALL MCP-849 protocol, however neither achieved remission at the end of induction. Thereafter, both were shifted to the Berlin-Frankfurt-Munster-HR protocol with additional Imatinib for Case 5. After this shift, Case 5 achieved remission and is on maintenance therapy at the time of follow-up. Case 6 did not achieve remission despite the intensified protocol and succumbed to progressive disease after 6 months of treatment. A study by Matutes *et al.*^[6] with 100 cases (32% paediatric) showed that complete remission was achieved in 85% patients with ALL treatment and 41% patients treated with AML treatment. The overall median survival was 18 months with the survival at 5 years being 37%. A study by Nair *et al.*^[9] showed similar findings. Orgel *et al.*^[14] assessed the relative efficacy of various chemotherapy regimens in pediatric MPALs and found that ALL induction regimens achieved remission in 72% (28/39) of cases versus 69% (9/13) for AML regimens.

In our series, only one of the four cases attained remission on the intensified ALL protocol. In one of these cases, death occurred at day 7 of diagnosis due to febrile neutropenia. For two cases, no follow-up for treatment initiation was there at all. Although it is seen that children with MPALs respond better to ALL-directed treatment than adults, in our series only one child survived. This may be attributed to poor underlying nutritional status, low socioeconomic and hygiene conditions predisposing to infections, poor treatment compliance, and undiagnosed additional cytogenetic abnormalities.

Limitations of the study

A small sample size.

Conclusion

MPALs need to be diagnosed based on flow cytometric immunophenotyping and following a uniform standard diagnostic criteria. They portend a poor prognosis for the patient and indicate a need for a different treatment protocol than the classical leukemias. Meticulous

attention on morphology is of paramount importance when dealing with MPALs along with the ancillary techniques to not underdiagnose this entity.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Borowitz MJ, Bene MC, Harris NL, Porwit A, Matutes E. Acute leukemias of ambiguous lineage. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, *et al.*, editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press; 2017. p. 179-87.
2. Alexander TB, Gu Z, Iacobucci I, Dickerson K, Choi JK, Xu B, *et al.* The genetic basis and cell of origin of mixed phenotype acute leukaemia. *Nature* 2018;562:373-9.
3. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, *et al.* The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: Rationale and important changes. *Blood* 2009;114:937-51.
4. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, *et al.* The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood* 2016;127:2391-405.
5. Porwit A, Bene MC. Acute leukemias of ambiguous origin. *Am J Clin Pathol* 2015;144:361-76.
6. Matutes E, Pickl WF, Attarbaschi A, Hopfinger G, Ashley S, Bene MC, *et al.* Mixed-phenotype acute leukemia: Clinical and laboratory features and outcome in 100 patients defined according to the WHO 2008 classification. *Blood* 2011;117:10.
7. Owaidah T, Beihany AA, Iqbal M, Elkum N, Roberts G. Cytogenetics, molecular and ultrastructural characteristics of biphenotypic acute leukemia identified by the EGIL scoring system. *Leukemia* 2006;20:620-6.
8. Yan L, Ping N, Zhu M, Sun A, Xue Y, Ruan C, *et al.* Clinical, immunophenotypic, cytogenetic, and molecular genetic features in 117 adult patients with mixed-phenotype acute leukemia defined by WHO-2008 classification. *Haematologica* 2012;97:1708-12.
9. Nair R, Jacob P, Nair Anila KA, Prem S, Binitha R, Kusumakumary P, *et al.* Flow cytometric analysis of mixed phenotype acute leukemia: Experience from a tertiary oncology center. *Indian J Pathol Microbiol* 2015;58:181.
10. Weinberg OK, Seetharam M, Ren L, Alizadeh A, Arber DA. Mixed phenotype acute leukemia: A study of 61 cases using World Health Organization and European Group for the Immunological Classification of Leukaemias criteria. *Am J Clin Pathol* 2014;142:803-8.
11. Pawar RN, Banerjee S, Bramha S, Krishnan S, Bhattacharya A, Saha V, *et al.* Mixed-phenotypic acute leukemia series from tertiary care center. *Indian J Pathol Microbiol* 2017;60:7.
12. Lee HG, Baek HJ, Kim HS, Park SM, Hwang TJ, Kook H. Biphenotypic acute leukemia or acute leukemia of ambiguous lineage in childhood: Clinical characteristics and outcome. *Blood Res* 2019;54:63-73.
13. Rubio MT, Dhedin N, Boucheix C, Bourhis JH, Reman O, Boiron JM, *et al.* Adult T-biphenotypic acute leukaemia: Clinical and biological features and outcome. *Br J Haematol* 2003;123:842-9.
14. Orgel E, Alexander TB, Wood BL, Kahwash SB, Devidas M, Dai Y, *et al.* Mixed-phenotype acute leukemia: A cohort and consensus research strategy from the Children's Oncology Group Acute Leukemia of Ambiguous Lineage Task Force. *Cancer* 2020;126:593-601.