

**Original Research Article****Clinico-Haematological Profile of patients with Mixed Phenotype Acute Leukemia: In a Tertiary Care Centre**

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Abstract

Introduction: Mixed phenotype acute leukemia (MPAL) is a rare subset of acute leukemia where the blasts exhibit lineage specific antigens of more than one lineage. Flow cytometric immunophenotyping is essential for the diagnosis of MPAL and the accurate diagnosis highly depends on the panel of markers used. The aim of the study is to study the incidence, clinical presentation, haematological parameters, immunophenotypic, molecular features of MPAL and their correlation to the treatment and prognostic significance.

Materials and Methods: Cases of Acute Leukemia consisting of both the paediatric and adult age group admitted to the department of Haematology S C B Medical College and Hospital Cuttack, Odisha from September 2015 to November 2016 were analysed.

Results: During the study period, flow cytometric analysis of 680 cases was performed. B lymphoblastic leukemia was the most common subtype of acute leukemia. A diagnosis of MPAL was made in 24 cases, which accounted for 3.5% of all leukemias. 13 cases were diagnosed as B/myeloid, 10 cases as T/myeloid and 1 case as B/T/myeloid. 19 of 24 cases received induction chemotherapy and 10 cases achieved complete remission. The overall median survival of all patients in our study was 10 months and the survival at 15 months was 38 %.. The survival of pediatric patients at the end of 15 months was 62% and that of adults was 32%.

Conclusion: Mixed phenotype acute leukemia is a rare subset of acute leukemia. Flow cytometry is critical in establishing a diagnosis of MPAL. Outcome-related prognostic factors include age, HLA-DR, CD34 negativity. BCR-ABL fusion and MLL rearrangement are associated with poor prognosis. Complete remission is achieved more in cases of ALL directed therapy than AML regimen and more in cases of paediatric patients than in adult cases.

Keywords: B/myeloid, T/myeloid and B/T/myeloid.

Introduction

Most cases of acute leukemias can be classified based on the lineage of the leukemic cells as

myeloid, B cell acute lymphoblastic leukemia (B-ALL) or T cell acute lymphoblastic leukemia (T-ALL). However, there are uncommon cases in

which the blasts show differentiation towards more than one lineage. Historically the terms used to describe these cases as mixed lineage leukemia, hybrid acute leukemia, bilineal leukemia, undifferentiated leukemia, and biphenotypic acute leukemia (BAL). WHO classification of hematopoietic and lymphoid tumors (WHO-2008) modified the diagnostic criteria and introduced a new designation for this entity which is termed Mixed-phenotype acute leukemia (MPAL). MPAL encompasses leukemias containing separate populations of blasts of more than one lineage (bilineal or bilineage) or a single population of blasts co-expressing antigens of more than one lineage (biphenotypic).^{1,2}

Flowcytometry immunophenotyping for acute leukemia is important for classification into different cell lineages, assessing the response to treatment, detection of minimal residual disease and prognosis of disease.³

Mixed phenotype acute leukemia is a rare disease, representing only 3 - 5% of acute leukemias of all age groups, and 2.4 - 3.7% in children. It affects both adults and children, more frequently adults and has slight male preference. The prognosis for MPAL is poor comparing to other acute leukemias, with an overall survival of 18 months.^{1,4}

Our Study mainly focuses mainly on the incidence, clinical presentation, haematological parameters, immunophenotypic, molecular features of MPAL and their correlation to the treatment with prognostic significance.

Materials & Methods

Case Selection: In our longitudinal study 680 cases of acute leukemia consisting of both the pediatric and adult age group admitted to SCB Medical College and Hospital Cuttack, Odisha from September 2015 to November 2016 were analysed and 24 cases of MPAL were diagnosed according to the WHO 2008 criteria.

Cases of relapse and secondary leukemia, CML in blast crisis, AML after myelodysplasia and

therapy-related myeloid neoplasms are excluded from the study.

All the cases of Acute Leukemia were evaluated by detailed history taking and clinical examination. Haematological parameters were detected by fully automated 5 part cell counter (SYSMEX XT 2000-1). Bone marrow aspiration followed by morphological study, and immunophenotyping by flowcytometer (BD FACS CALIBUR) was done. Fluorescence in situ hybridization (FISH), using dual color translocation probes of BCR/ABL and LSI MLL (Vysis Inc) was done as per the standard procedure and methodology for detecting the BCR-ABL and MLL rearrangement only. Diagnosis and classification of all Acute Leukemia patients was done by correlating the haematological parameters, morphology, cytochemical staining, bone marrow study, immunophenotyping and molecular markers as per WHO 2008 criteria for lineage assessment.

For the morphological examination all the peripheral blood smears and bone marrow aspirates were air dried and stained with giemsa stain myeloperoxidase was done routinely in all peripheral blood smears and bone marrow aspirate smears. Other cytochemical stains like periodic acid Schiff and non specific esterase were done according to the morphological details of the cells.

Statistical Analysis

Statistical analysis was done using SPSS software version 20 (IBM) and InStat 3 software and Microsoft office excel 2007. Frequency Distribution, Chi Square tests, Fischer exact tests were used for the analysis of clinical and laboratory variables. Survival analysis and survival curves were obtained using Kaplan Meier method using SPSS software version 20. The median survival and the survival percentage were calculated as per the Kaplan Meier curve analysis. This study satisfies the ethical principles of medical research by World Medical Association Declaration of Helsinki and the ethical clearance is given by the Institutional ethics Committee of S C B Medical College, Cuttack.

Results

In the present study, out of 680 patients of Acute Leukemia, B Lymphoblastic leukemia (B ALL) was the most common subtype of acute leukemia with 294(43.2%) cases followed by T Lymphoblastic leukemia (T ALL) 152(22.3%) cases and acute myeloblastic leukemia (AML) in 210(30.9%) cases.

MPAL was found in 24 cases based on WHO 2008 criteria which accounted for 3.5 % of the total cases of acute leukemia between the age from 6 years to 56 years out of which 9(37.5%) cases were children (age<14 yrs) and 15(62.5%cases) were adults. Out of 28 cases 14(58.3%) were male and 10(41.6%) cases were female and the ratio was being 1.4:1

Fever was the most common presenting symptom among the patients in 21(87.5%) cases followed by generalised weakness 17(70.8%)cases, bleeding manifestations 13(54.2%)cases, weight loss 13(54.2%)cases, bone pain 9(37.5%)cases, dyspnoea 5(20.8%)cases and pain abdomen in 2(8.3%)cases were observed.

Pallor was the most common sign present among the patients in 23(95.8%) cases followed by hepatosplenomegaly in 16(66.6%) cases, lymphadenopathy in 11(45.8%) cases, sternal tenderness in 11(45.8%) cases, gum hypertrophy in 9 (37.5%) and easy bruisability 8(33.5%) were seen. Baseline investigations revealed leucocytosis in 20 (83.3%) cases and leucopenia in 2 cases. Low haemoglobin levels (< 11 g/dl) noted in 22 (91.7%) cases .Thrombocytopenia seen in 22 cases (platelet count<1 lakh/cmm). Serum lactate dehydrogenase was raised in 13 cases and serum alkaline phosphatase in 8 cases and raised liver enzymes in 6 cases of MPAL.

Most of the patients 17(70.8%) cases could be diagnosed as acute leukemia from the peripheral smear. 3 cases presented as pancytopenia, 2 cases as bicytopenia. Only one case (4.1%) was presenting with anaemia and thrombocytopenia. Leukemic blast cells were present in the peripheral blood in 17(70.8 %) cases of MPAL.

The bone marrow smears were studied for morphology and cytochemistry in all 24 cases. According to the FAB classification ALL Morphology L1 or L2 was found in 11 cases (45.8%) and AML Morphology mainly M1 OR M5 was found in 13 cases (54.2%) respectively. Cytochemical myeloperoxidase was positive in 8 cases.

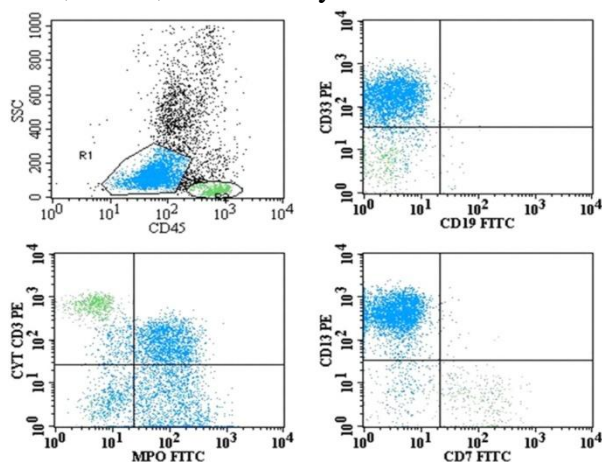
Flowcytometric Immunophenotyping

In the present study out of 24 cases of MPAL, 13(54.2%) cases had B lymphoid + myeloid immunophenotype (B/myeloid), 10(41.6%) cases had T lymphoid + myeloid immunophenotype (T/myeloid) and one case had trilineage immunophenotype (B/T/myeloid) based on WHO guidelines for lineage specificity. All cases showed co- expression of markers from at least two different lineages.

Assignment of myeloid commitment in MPAL was demonstrated in all 24 cases by myeloperoxidase positivity. Flow cytometry demonstrated myeloperoxidase positivity in all 24 cases whereas cytochemistry showed myeloperoxidase positivity in 8 out of 24 cases., CD 13 was positive in 19 cases , CD 33 was positive in 19 cases and CD 117 were positive in 14 cases were observed in the present study T-cell lineage was assigned by positive cytoplasmic CD3 (cyt CD3) expression by flowcytometry and was demonstrated in 11 cases (10 cases of T/myeloid and 1 case of B/T/myeloid).The proportion of positive blasts ranged from 26% to 99% for cyt CD3. CD 5 was positive in 7 cases of T/myeloid and CD 7 was positive in 8 cases of T /myeloid respectively. (Figure -1).

Figure 1 Flowcytometry showing MPAL (T/myeloid) in the study group

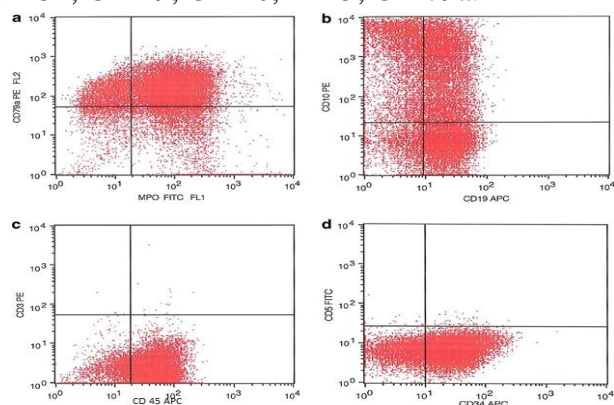
Gated population of cells are positive for CD 45, CD 33, CD13, MPO and cyt CD3.



B cell lineage was assigned by CD19 expression associated with strong expression of at least one other B cell markers (CD10, cytoplasmic CD79a) in 14 (13 B/myeloid and 1 B/T/myeloid) cases. The proportion of positive blasts ranged from 42% to 96% for CD 19. CD 10 was positive in 11 (10 B/myeloid and 1 B/T/myeloid) cases and CD 79a was positive in 6 cases of B /myeloid (Figure -2).

Figure 2- Flowcytometry showing MPAL (B/myeloid) in the study group

Gated population of cells are positive for CD 45, CD 34, CD 19, CD 10, MPO, CD 79a.



HLA DR was expressed in 14 (7 B/myeloid, 6 T/myeloid and 1 B/T/myeloid) cases, CD 34 was expressed in 13 (8 B/myeloid, 5 T/myeloid) cases and CD 45 was expressed in 15 (8 B/myeloid, 6 T/myeloid and 1 B/T/myeloid) cases respectively. Fluorescence in situ hybridization (FISH) for detecting only the BCR-ABL and MLL

rearrangement were carried out in cases of MPAL. FISH analysis were done in 16 cases out of 24 cases of MPAL .Only 2 cases each were positive for BCR-ABL(2 B/myeloid cases) and MLL rearrangement (2 T/ myeloid cases) respectively.

Response to therapy and outcome

In our study we had treated the cases of MPAL by standard AML regimen ie daunorubicin and cytarabine (3+7 protocol) and ALL regimen (MCP 841 protocol). Out of 24 patients of MPAL only 19 patients received induction chemotherapy and 5 patients were given palliative treatment, considering financial constraints and poor treatment response .Out of 19 cases who received induction chemotherapy, ALL protocol (MCP 841) was given to10 cases (6 B/myeloid , 4 T/myeloid cases) and AML regimen (3+7 protocol) was given to 7 cases (4 B/myeloid and 3 T/myeloid). Two cases of MPAL (BCR-ABL) received ALL directed therapy plus Tyrosine kinase inhibitor oral dasatinib as inducing agent . Palliative treatment was considered in 5 (2 MPAL (MLL), 1 B/MYELOID, 1 T/MYELOID, 1 B/T/myeloid) cases.

Out of 10 cases who received ALL treatment 7 (4 B/myeloid and 3 T/myeloid) cases achieved complete remission (CR) (as defined by < 5% of blast cells in the bone marrow and complete absence of blast cells from the peripheral blood) after induction chemotherapy . 2 of the 10 cases who received ALL treatment died during the induction phase and one patient lost to follow up. Consolidation chemotherapy was given according to the MCP 841 protocol to the patients depending upon the performance status, tolerability and recovery of the blood counts and maintainance of the complete remission phase. Seven patients who achieved CR during the induction treatment were given consolidation treatment. During the last follow up 2 patients had died during the consolidation phase, 1 due to relapse of the disease and 1 due to sustained neutropenia and severe infection and 1 case lost to follow up. 4 pediatric cases (2 T/myeloid and 2 B/myeloid).

who achieved CR are still continuing treatment and had not relapsed .

Out of 7 cases who received AML regimen (3+7 protocol) as induction, complete remission (CR) was achieved only in 3 (1 B/myeloid and 2 T/myeloid) cases . 3 cases died during induction phase and 1 case lost to follow up. All the 3 cases that achieved complete remission were treated by consolidation therapy by high dose cytarabine as per the standard AML regimen. During the last follow up, 1 case had died during the consolidation phase due to the relapse of disease. 2 cases who achieved CR are still in continuing treatment and had not relapsed.

The 2 MPAL (BCR-ABL) patients who had received ALL plus dasatinib as induction agent, died during the induction phase of treatment.. Out of 5 patients who received palliative treatment, 2 cases of MPAL (MLL) expired early during treatment and rest 3 patients lost to follow up.

Survival analysis by Kaplan Meier method: As shown in Fig 3,4 and 5

Figure 3- Survival Analysis of All the Patients OF MPAL in the study group

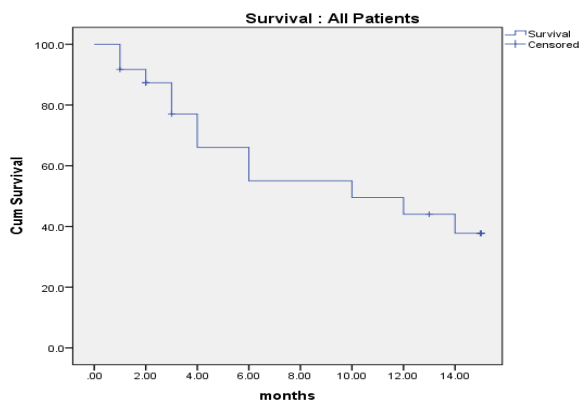


Figure 4 Relationship between Survival and Age of patients with MPAL in the study group

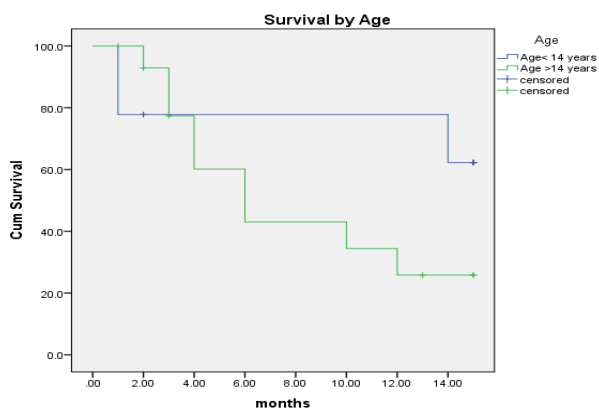
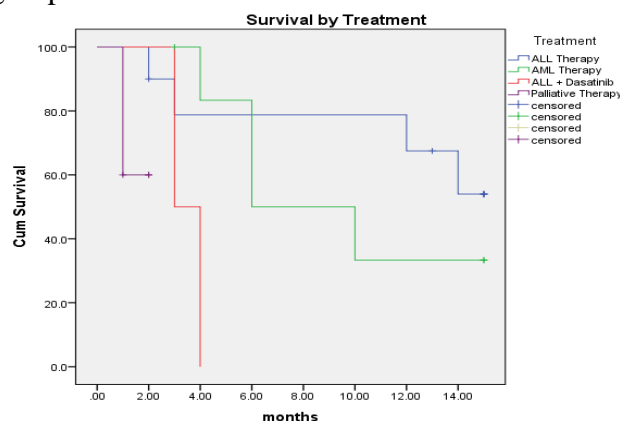


Figure 5 Relationship between Treatment and Survival of patients with MPAL in the study group



Survival analysis was done at the end of 15 months and survival curves were obtained using Kaplan Meier method .As shown in Figure-3, the overall median survival of all the cases of MPAL included in the study was 10 months and the median survival at 15 months was 38 % . As depicted in Figure-4, the median survival of patients aged >14 years with MPAL was 6 months and the survival at the end of 15 months was 25%. The survival of paediatric patients (aged < 14 years) at the end of 15 months was 62% and the median survival has not yet reached. So it interprets that the prognosis and survival of paediatric patients was better as compared to the adults. As shown in Figure-5, the median survival of patients treated with AML directed therapy was 6 months and the survival at the end of 15 months was only 32%. The median survival of patients treated with ALL directed therapy has not yet reached and the survival at the end of 15 months was 56%.

Discussion

Majority of cases of acute leukemias are classified as ALL or AML depending on the specific lineage origin, either lymphoid or myeloid, exhibited by blasts.⁵ A minority of cases of acute leukemias will show no evidence of differentiation along a single lineage. These leukemias are termed acute leukemias of ambiguous lineage and consist of two separate entities. When the blasts exhibit no

lineage-specific antigens, it is termed acute undifferentiated leukemia. When the blasts express antigens of more than one lineage, to such a degree that it is not possible to assign to any particular lineage with certainty, it is called MPAL.⁶ The first published reports on Biphenotypic acute leukemia (BAL) occurred in the 1980s, when monoclonal antibodies were first being used to characterize leukemic cells. The frequency and significance of acute leukemia was displaying both lymphoid and myeloid characteristics in 123 children Mirro et al. In this study, the definition of acute mixed lineage leukemia included having individual blasts with more than one lineage. This was determined using lymphoid-associated antibodies such as anti-CALLA (CD10), T-11 (CD2) or T101 (CD5), and myeloid associated antibodies were composed of MY1(CD15), MCS.2 (CD13) and Mo1 (CD11b); however, none of these markers are now considered to be lineage specific. On the basis of these markers, acute mixed lineage leukemia comprised 20% of the total number of 123 cases.⁷ As more monoclonal antibodies became available, It became clear that a significant number of AML and ALL cases demonstrated aberrant immunophenotypes and specific criteria were needed to diagnose a true mixed phenotype leukemia.⁸ In 1991 *Catovsky et al* proposed a defined scoring system for biphenotypic acute leukemia. In Catovsky's scoring criteria most specific markers included cytoplasmic CD3, cytoplasmic CD22 and myeloperoxidase (MPO). Two or more points from two separate lineages were needed to classify a case as biphenotypic acute leukemia.⁹

Scoring criteria by European Group for Immunological classification of Acute Leukemia (EGIL), aimed at distinguishing cases of MPAL from those with aberrant antigen expressions.¹⁰ It allowed a better definition of biphenotypic acute leukemia. In EGIL scoring system, the various markers were assigned with a score of 2, 1 or 0.5 and cases with a score of >2 for at least two

lineages were classified as biphenotypic acute leukemia.^{10,11}

The 2008 WHO classification has suggested strict criteria for the diagnosis of MPAL. AML with recurrent cytogenetic abnormalities, chronic myelogenous leukemia (CML) in blast crisis are excluded. Presence of a complex karyotype, multi lineage dysplasia or any other MDS-related cytogenetic changes without a history of MDS should be considered as AML with myelodysplasia related changes.^{6,12}

Two genetic lesions previously reported under MPAL were now being considered as separate entities. The first is MPAL with t(9;22) (q34;q11.2) or BCR-ABL1 rearrangement and the second is MPAL with translocations involving MLL gene¹². These are associated with poor prognosis and decreased overall survival. The remaining cases were designated as MPAL NOS (not otherwise specified)^{12,13}.

In our study, out of 680 patients of Acute Leukemia, diagnosis of MPAL was made in 24(3.5 %) cases as per the WHO 2008 criteria . Out of 24 cases of MPAL, 11 B/myeloid, 8 T/myeloid, 2 cases of MPAL with BCR-ABL fusion, 2 cases of MPAL with MLL rearrangement and one MPAL had trilineage expression. Nine (37.5%) cases were children (age<14 yrs) and 15(62.5%cases) were adults. 14(58.3%) cases of MPAL were male and 10(41.6%) cases were female similar as studied by *Matutes E et al (2011)*. B-myeloid (59%), T-myeloid (35%), B-T (4%) and trilineage (2%) combinations were found¹. *Yan L et al (2012)* studied 4,780 Acute leukemia patients, and identified 117 (2.4%) patients with MPAL fulfilling WHO criteria; classified as B/myeloid (n=64), T/myeloid (n=38), B + T lymphoid (n=14) and trilineage (n=1) respectively.¹⁴

The Bone Marrow revealed total 11 (45.8%) cases with ALL morphology and 13 (54.2%) cases with AML morphology according to the FAB Classification. Our finding had more cases of AML morphology than of ALL morphology which is in contrast to the previous literatures.

Studies by Yan L *et al* 2012. The remaining 26 (22%) cases were categorized as acute undifferentiated leukemia¹⁴.

Mixed phenotype acute leukemia is thought to arise from a multipotential hemopoietic stem cell that has the potential to differentiate into any lineage. Most of the reported cases of MPAL express early hematopoietic markers - CD34 and HLADR suggesting an early precursor stem cell origin.¹⁵ In our series, 13/24 cases of MPAL demonstrated CD34 positivity. HLA DR was positive in 14/24 cases.

FISH analysis were done in 16/24 cases of MPAL. Only 2 cases were positive for BCR-ABL fusion and MLL rearrangement. Studies had shown that Philadelphia (Ph) positivity and MLL rearrangement cases are associated with poor survival and overall bad prognosis. In our study the 2 BCR-ABL patients who had received ALL plus dasatinib as induction agent, died during the induction phase of treatment. But studies have shown that administration of imatinib in addition to induction and consolidation chemotherapy for newly diagnosed Ph positive ALL enhances efficacy and prevents the development of secondary resistance.^{16,17,18}

The optimal therapeutic approach to MPAL case has not been clearly defined whether these patients to be treated with ALL regimen or AML regimen and whether it should be followed by hematopoietic stem cell transplantation is still unclear.¹⁹ In our study 19 /24 patients received induction chemotherapy and 5 patients were given palliative care. Out of 19 cases ALL protocol (MCP 841) was given to 10 cases (6 B/myeloid, 4 T/myeloid cases) and AML regimen (3+7 protocol) was given to 7 cases (4 B/myeloid and 3 T/myeloid). Two cases of MPAL (BCR-ABL) received ALL directed therapy plus dasatinib as inducing agent. Palliative treatment was considered in 5 (2 MPAL (MLL), one B/myeloid, one T/myeloid and one B/T/myeloid) cases. Seven (4 B/myeloid and 3 T/myeloid) cases who received ALL treatment achieved complete remission (CR) and 3 (1 B/myeloid and 2

T/myeloid) cases who received AML regimen achieved complete remission. The 2 MPAL (BCR-ABL) patients who had received ALL plus dasatinib as induction agent died during the induction phase of treatment. Out of 5 patients who received palliative treatment, 2 cases of MPA (MLL) had early deaths. The overall median survival of all patients in our study was 10 months and the survival at 15 months was 38 %. The survival of pediatric patients at the end of 15 months was 62% and that of adults was only 32%. The median survival of patients treated with ALL directed therapy was 6 months and the survival at the end of 15 months was only 32%. The median survival of patients treated with ALL directed therapy has not yet reached and the survival at the end of 15 months was 56%.

In the present study complete remission was achieved more in cases of ALL directed therapy than AML regimen and more in cases of paediatric patients than in adult cases^{20,21}.

In the present study Immunophenotyping has definite prognostic implications in patients with MPAL and paediatric MPAL patients and ALL directed therapy have better prognosis. Outcome-related prognostic factors include age, HLA-DR, CD34 negativity. BCR-ABL fusion and MLL rearrangement cases are associated with poor survival and overall bad prognosis.

Conclusion

Mixed phenotype acute leukemia are rare leukemias. Strict diagnostic criteria should be followed in the diagnosis. Accurate diagnosis is important because of the worse clinical outcome. It should be distinguished from acute myeloid and lymphoid leukemias with cross-lineage antigen expression. Immunophenotyping is absolutely essential in the diagnosis. The lineage specific markers including the cytoplasmic markers should be included in the primary flow cytometry panel for the identification and proper categorization of MPAL. The patients should be considered for consolidation with intensive chemotherapy and stem cell transplantation at first remission.

Prospective studies analysing gene expression of MPAL are warranted as they will shed insights into the pathogenesis of the disease, by providing relevant information of dysregulated genes which may in turn be putative therapeutic target.

Conflict of interest: None

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