Content available at: https://www.ipinnovative.com/open-access-journals

Indian Journal of Pathology and Oncology

Journal homepage: www.ijpo.co.in

Case Report

Fatal hemorrhagic manifestations of B/T mixed phenotype acute leukemia mimicking acute promyelocytic leukemia: Case report and a review of literature

Priyanka Mishra[1*], Sanjeevan Sharma[2], Ashok Bhandari[3], Manu Chopra[4]

[1]Dept. of Pathology, Command Hospital, Lucknow, Uttar Pradesh, India
[2]Dept. of Medicine, Command Hospital, Lucknow, Uttar Pradesh, India
[3]Dept. of Pediatrics, Command Hospital, Lucknow, Uttar Pradesh, India
[4]Dept. of Respiratory Medicine, Command Hospital, Lucknow, Uttar Pradesh, India

Abstract

B/T Mixed phenotype acute leukemias (MPAL) presenting with Acute promyelocytic leukemia (APML) like catastrophic bleeding manifestations is extremely rare. Our patient was a six years male who presented with fever, bleeding gums and skin bleeds for three days. Physical examination revealed pallor, multiple ecchymotic patches and splenomegaly. He had pancytopenia with 2% blasts with folded nuclei on the peripheral smear and deranged coagulation parameters. In view of the above, APML was suspected and patient was administered ATRA and FISH for PML-RARA on peripheral blood was sent. Throat swab for RT-PCR was positive for COVID-19 and X-ray chest showed bilateral ground glass opacities. In view of worsening saturation, he was put on Bilevel Positive Airway Pressure and later mechanical ventilation. He was managed with antibiotics, blood components and inotropes after an episode of hematemesis induced hypovolemic shock. However, patient deteriorated and succumbed to his illness. Post-mortem bone marrow showed 88% blasts which were positive for CD19, cyto CD79a, CD10, cyto CD3, CD34 and TdT. RT-PCR for PML-RARA (Promyelocytic leukemia/ retinoic acid receptor alpha) and break apart FISH for RARA (retinoic acid receptor alpha) were negative. A final diagnosis of B/T MPAL, NOS (Not otherwise specified) was made post-mortem. The aim of this case report is to highlight the extremely rare presentation of a rare leukemia in children and blasts with convoluted nuclei leading to a diagnostic dilemma. Our patient had a tumultuous course aggravated by the COVID19 pneumonia leading to fatal outcome.

Keywords: Bleeding, Mixed phenotype acute leukemias, Acute promyelocytic leukemia.

Received: 17-04-2024; **Accepted:** 22-06-2024; **Available Online**: 15-03-2025

1. Introduction

Mixed phenotype acute leukemia (MPAL) accounts for 5% of all leukemias in pediatric population.[1] These leukemias are classified under Acute leukemias of ambiguous lineage (ALAL) and have more complex genetic and mutational landscape accounting for treatment resistance and worse prognosis.[2] We describe here a rare case of B/T MPAL that presented with florid bleeding manifestations arousing the clinical suspicion of Acute promyelocytic leukemia (APML). This is the first reported case of B/T MPAL in a child presenting with extensive bleeding manifestations.

2. Case Presentation

We present here the first case of B/T MPAL in a child with catastrophic bleeding manifestations and DIC reported in literature till date. A 6 years male child was brought to the outpatient department of a tertiary care centre with history of fever, skin bleeds and bleeding from gums for past 3 days. History from the mother elicited that the child had been lethargic for the past 15 days. A detailed physical examination revealed pallor, multiple ecchymotic patches over the body and spleen palpable 4 cms below the costal margin in splenic axis. Laboratory investigations revealed pancytopenia and 1-2% blasts with convoluted nuclei and intra-cytoplasmic inclusions which appeared like Auer rods as shown in **Figure 1**A. The prothrombin time (Test-= 23s,

*Corresponding author: Priyanka Mishra
Email: priyanka9581@gmail.com

https://doi.org/10.18231/j.ijpo.2025.016

Control = 10s) and activated partial thromboplastin time (Test= 56s, Control=30s) were prolonged with low fibrinogen of 45mg/dL (normal range= 250-450mg/dL) and raised D-Dimer (9275ng/mL, normal <500ng/mL). Biochemical investigations including liver function tests were normal except for raised LDH levels (1250 IU/L). There were no biochemical features of tumour lysis syndrome and uric acid, calcium, phosphate and potassium levels were normal. Chest X-ray revealed non-homogenous opacities in lower lobes of both the lungs. During hospital stay, the patient developed hematemesis and features of hypovolemic shock. Patient was started on inotropes, transfusion support and antibiotics. In view of marked bleeding manifestations, pancytopenia, blasts with convoluted nuclei and intra-cytoplasmic Auer rod like inclusions, deranged coagulation parameters, a suspicion of APML was raised and patient was administered All-trans retinoic acid (ATRA). Throat swab sent for reverse transcriptase polymerase chain reaction (RT-PCR) was positive for COVID19. In view of low oxygen saturation (spO2=70%), patient was initially put on Bilevel Positive Airway Pressure (BiPAP) and later shifted to mechanical ventilation. However, the patient continued to remain critical and succumbed to his illness. Post-mortem bone marrow aspirate showed 88% blasts with round nuclei as shown in **Figure 1**. However, no auer rods or faggots were seen. On flow cytometry, the blasts were positive for CD19, cyto CD79a, CD10, cyto CD3, CD34 and TdT and negative for CD20, CD7, CD5, CD56, CD8, CD1a, MPO, CD13, CD33, HLA-DR, CD11c, CD14, CD64 and CD117 as shown in **Figure 2**. Cytogenetics and molecular studies were normal. RT-PCR for PML-RARA (Promyelocytic leukemia/ retinoic acid receptor alpha) and break apart FISH for RARA (retinoic acid receptor alpha) was negative. A final diagnosis of B/T MPAL was made as per WHO 2022 classification.

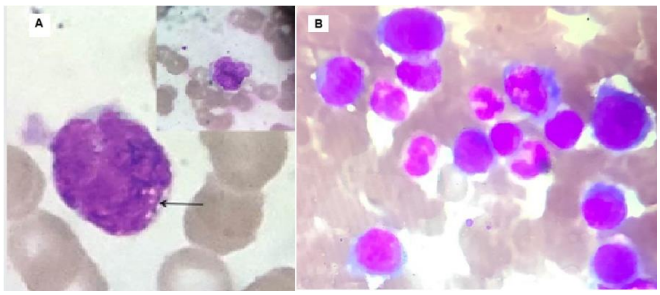

Figure 1: Peripheral smear (LG, 100X): Blast with convoluted nucleus and intra-cytoplasmic Auer rod like inclusions (shown by arrow); Inset- another blast with convoluted nucleus. 1B: Bone marrow aspirate (LG, 40X)- Numerous blasts with round nucleus and prominent nucleoli in few cells

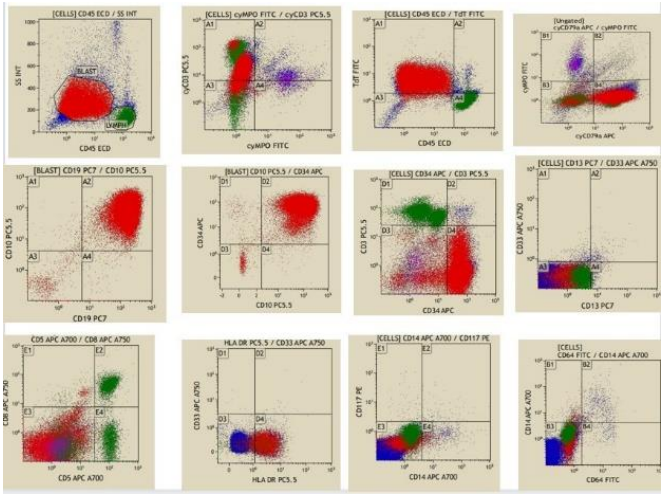

Figure 2: Blasts gated on CD45-SSC plot are positive for cyto CD3, TdT, cyto CD79a, CD19, CD10, CD34 and negative for MPO, surface CD3, CD13, CD33, CD5, CD8, HLA-DR, CD14, CD117 and CD64

3. Discussion

MPALs are heterogenous group of leukemias classified under ALAL along with acute undifferentiated leukemia. The diagnosis is made on the basis of expression of more than one lineage specific markers in the same population of blasts (biphenotypic) or the identification of blast populations of different lineages (bilineage). The 2022 revision of the WHO classification of hematopoietic neoplasms categories MPAL as MPAL with BCR-ABL1 rearrangement, MPAL with KMT2A rearrangement, B/Myeloid MPAL, T/Myeloid MPAL and MPAL NOS which includes even rarer subtypes such as B/T MPAL and B/T/Myeloid MPAL.[2]

The WHO 2022 emphasizes the use of immunophenotyping, cytochemistry and immunohistochemistry to demonstrate the lineage specific markers before embarking on the diagnosis of acute undifferentiated leukemia. The lineage defining markers are- MPO for myeloid, two or more of CD11c, CD14, CD64, CD36 and lysozyme for evidence of monocytic differentiation, cyto or surface CD3 for T-lineage and strong cyto CD19 with one or more of CD10, CD22 or cyto CD79a or dim CD19 with strongly expressed two or more of these markers as evidence of B-lineage blasts. Immunohistochemistry with MPO, lysozyme, PAX-5 and CD3 can be used to confirm different populations in bone marrow biopsy.

The threshold for MPO varies according to the methodology used. The cut-off for cytochemistry was established at 3% by the French American British (FAB) classification.[3] A threshold of 10% on flow cytometry recommended by EGIL has been found to be less sensitive by various studies.[4] It is emphasized that MPO expression in blasts should be compared with the internal controls. Various studies have advocated different cut-offs by using isotype

control (13%) and normal lymphocytes as the reference (28%).[5]

If the criteria for MPAL is met, no definite threshold for the size of blast population is required. It is reiterated however that the expression of CD19 in blasts should be considered bright if it is comparable to residual normal B lymphocytes. Immunohistochemistry with anti-PAX5 antibodies helps in confirming B-lineage on bone marrow biopsy.[2] MPO positivity in bonafide cases of B-ALL have been reported in literature. These patients should be treated as B-ALL as the treatment outcomes are similar to other B-ALL patients.[6]

The monoclonal antibodies for CD3 should be directed against the anti-CD3e chain and ideally be conjugated with a bright fluorochrome such as allophycocyanin or phycoerythrin. The CD3 antibody for IHC is anti-zeta which detects both the T-lymphocytes and the NK cells. The T-lymphoblasts are usually positive for cyto CD3 and negative for surface CD3.[2]

In a large series of 100 cases of MPAL, B/myeloid MPAL (59%) was commonest followed by T/myeloid type (35%). B/T MPAL was the second most rare type constituting 4% of all mixed phenotype leukemias with the rarest being B/T/myeloid MPAL (2%). There is limited data in the pediatric population and paucity of guidelines on the management of such patients. In a study of 42 pediatric patients, only one case of B/T MPAL was reported.[7] In another study of 15 cases of MPAL from India, no case of B/T MPAL was described.[8]

These cases are diagnosed either during routine immunophenotyping or present as treatment resistant cases. In a large subset of patients, the smaller clone of blasts may manifest at relapse. Peripheral smear may rarely show two separate population of blasts or the blasts may be undifferentiated, more myeloblastic or lymphoblastic in majority of cases. Our patient presented with pancytopenia, extensive bleeding manifestations and coagulopathy raising the suspicion of APML. COVID 19 associated coagulopathy (CAC) presents as a prothrombotic state with macro- and microthrombi causing damage to vital organs like heart, liver, kidneys and brain. The underlying mechanism is a complex interaction between immune cells and vascular endothelial cells leading to endothelial dysfunction and 'immuno-thrombosis, defective coagulation, fibrinolytic and kallikrien-kinnin system causing a procoagulant state. Immune thrombocytopenia and Thrombotic Thrombocytopenic purpura have also been reported in COVID19 patients.[9] A previous case of T/Myeloid MPAL (M5) with PML-RARA rearrangement described by Zheng et al had similar clinical and coagulation profile.[10] Our patient also had monocytic morphology of blasts on peripheral smear similar to their case, however, no karyotypic or molecular abnormality was detected.

The complex genetic and molecular abnormalities in MPALs are attributed to their origin from either primitive cells which acquire multiple founder aberrations that are transferred to the subclones or the acquisition of multiple mutations in leukemic cell that leads to cross-lineage antigen expression. Studies by Matute et al and Yan et al revealed that complex karyotype was the commonest cytogenetic abnormality. The other patients had hyper-diploidy, normal karyotype, BCR-ABL1 fusion transcript and t (10;11) (p15; q21).[11,12] A study by Mi et al on nine cases of B/T Myeloid MPAL had genomic landscape similar to T-ALL particularly Early thymic precursor- T lineage acute lymphoblastic leukemia (ETP-ALL). These cases showed recurrent aberrations in PHF6 (plant homeodomain finger 6), JAK-STAT and Ras signaling pathways. There were mutations or deletions in genes encoding hematopoietic transcription factors, tumour suppressors, cell cycle regulators and chromatin modifiers. Genetic alterations seen in B-ALL like BCR-ABL1, ETV6-RUNX1 and TCF3-PBX1 fusion transcripts are rarely seen in B/T MPAL. All the patients were exhibited ALL-type chemotherapy with or without allogeneic stem cell transplant and majority of the patients achieved long term complete remission.[13]

Various NGS based studies have reported mutations in RAS NRAS, PTPN11, KMD6A, MLLT3, CREBBP and EP300 in B/Myeloid MPAL and mutations in JAK/STAT pathway, ETV6, FLT3, NOTCH1, WT-1 and Ikaros in T/Myeloid MPAL.[14,15] A recent study of pediatric MPAL of more than hundred cases elucidated higher FLT3 expression in B/Myeloid MPAL due to ZNF384 rearrangements in contrast to higher prevalence of mutations of JAK/STAT signaling proteins in T/Myeloid MPAL.[15] MPAL with MLL rearrangement associated with inferior survival are more common in children and have two separate lineages of lymphoblasts and myeloblasts.[16,17] Lineage switch is seen in MPALs (commonest in MLL rearranged) due to origin from multipotent progenitor cell, reprogramming by transcription factors, cytokine and tumour microenvironment and selection pressure induced by treatment.[18]

MPALs are associated with higher rates of induction failure and inferior survival. The adverse risk factors include older age, high TLC at diagnosis, adverse cytogenetics, MLL rearrangement, MRD positivity, extramedullary disease and T/myeloid phenotype.[16] Various studies have reiterated that pediatric MPAL has higher survival rates than adult MPAL and treatment with ALL induction regimens lead to superior induction remission rates and overall survival than hybrid ALL/AML or AML induction chemotherapy.[19,20]

Patients with MLL rearrangement should be transplanted upfront in case of failure of induction or at relapse. Philadelphia positive MPALs (MPAL with BCR-ABL1 rearrangement) commonly associated with B/Myeloid phenotype constitute 25% of all MPALs and are commoner

in adults. Like MLL rearranged MPALs, they have higher propensity of CNS involvement and require additional CNS directed chemotherapy. The addition of TKIs to ALL induction regimens have dramatically improved their induction remission and survival rates. A diagnostic dilemma however in these patients is blast crisis of Chronic Myeloid leukemia presenting de novo. There are various case reports of use of novel agents like Bispecific T-cell engagers and chimeric antigen receptor directed targeted therapy (CART) for refractory MPAL.[19]

Unlike adult MPALs, the role of transplant in pediatric cases is only in induction failure, MRD more than 5% after induction, persistent MRD positivity at consolidation and lineage switch. In the absence of the aforementioned criteria, no survival benefit was found after transplant in patients treated with ALL regimens.[21] The challenges of MRD assessment in MPAL lie due to the non-validation of the available techniques, paucity of data on the cut-off to be considered actionable, lineage switch, variable and cross-lineage antigen expression. A multi-centric study of 100 pediatric MPALs showed that end of induction MRD positivity was associated with poor event free survival and overall survival.[22] Various centers are using flow based and NGS based protocols for assessing MRD in these patients.

4. Strength of Study

The strength and peculiarity of our case lies in the rarity of B/T MPAL in children. To the best of our knowledge, this is the first case of B/T MPAL in a child presenting with extensive bleeding manifestations at presentation mimicking APML. The question whether these fatal hemorrhagic manifestations can be attributed to underlying COVID19 is an enigma in this case.

5. Limitation

The limitation of our case is that NGS could not be done due to resource constraints. An insight into the molecular profile of the case could have probably explained the accelerated course and the unusual clinical presentation of the disease.

6. Conclusion

B/T MPAL is rare pediatric hematological malignancy and multi-centric studies are required to comprehend the clinical spectrum and molecular landscape of the disease.

7. Source of Funding

No financial support received.

8. Conflict of Interest

The authors declare no conflicts of interest.

9. Patient Consent

Informed patient consent was taken.

References

1. Steensma DP. Oddballs: acute leukemias of mixed phenotype and ambiguous origin. *Hematol Oncol Clin North Am*. 2011;25(6):1235–53.
2. Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka HM, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. *Blood*. 2022;140(11):1200–28.
3. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposal for the recognition of minimally differentiated acute myeloid leukaemia (AML-MO). *Br J Haematol*. 1991;78(3):325–9.
4. Craig FE, Foon KA. Flow cytometric immunophenotyping for hematologic neoplasms. *Blood*. 2008;111(8):3941–67.
5. Guy J, Antony-Debré I, Benayoun E, Arnoux I, Fossat C, Le Garff-Tavernier M, et al. GEIL (Groupe d'Etude Immunologique des Leucémies). Flow cytometry thresholds of myeloperoxidase detection to discriminate between acute lymphoblastic or myeloblastic leukaemia. *Br J Haematol*. 2013;161(4):551–5.
6. Borowitz MJ. Mixed phenotype acute leukemia. *Cytometry B Clin Cytom.* 2014;86(3):152–3.
7. Salama M, Ahmed S, Soliman S, El-Sharkawy N, Salem S, El-Nashar A, et al. Characteristics, Treatment Complexity, and Outcome of Mixed-Phenotype Acute Leukemia in Children in a Low-Middle-Income Country. *Front Oncol*. 2022;12:941885.
8. Sharma M, Sachdeva MUS, Bose P, Varma N, Varma S, Marwaha RK, et al. Haematological profile of patients with mixed-phenotype acute leukaemia from a tertiary care centre of north India. *Indian J Med Res*. 2017;145(2):215–21.
9. Conway EM, Mackman N, Warren RQ, Wolberg AS, Mosnier LO, Campbell RA, et al. Understanding COVID-19-associated coagulopathy. *Nat Rev Immunol*. 2022 Oct;22(10):639–49.
10. Zheng X, Shen H, Zhu M, Shi Y, Wang H, Chen Z, et al. Mixed phenotype acute leukemia with PML-RARα positive: a case report and literature review. *Mol Cytogenet*. 2021;14(1):10.
11. Matutes E, Pickl WF, Van't Veer M, Morilla R, Swansbury J, Strobl H, 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(11):3163–71.
12. 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(11):1708–12.
13. Mi X, Griffin G, Lee W, Patel S, Ohgami R, Ok CY, et al. Genomic and clinical characterization of B/T mixed phenotype acute leukemia reveals recurrent features and T-ALL like mutations. *Am J Hematol*. 2018;93(11):1358–67.
14. 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(7727):373–9.
15. Manola KN, Panitsas F, Polychronopoulou S, Daraki A, Karakosta M, Stavropoulou C, et al. Cytogenetic abnormalities and monosomal karyotypes in children and adolescents with acute myeloid leukemia: correlations with clinical characteristics and outcome. *Cancer Genet*. 2013;206(3):63–72.
16. Rayes A, McMasters RL, O'Brien MM. Lineage Switch in MLL-Rearranged Infant Leukemia Following CD19-Directed Therapy. *Pediatr Blood Cancer*. 2016;63(6):1113–5.
17. Monma F, Nishii K, Ezuki S, Miyazaki T, Yamamori S, Usui E, et al. Molecular and phenotypic analysis of Philadelphia chromosome-positive bilineage leukemia: possibility of a lineage switch from T-lymphoid leukemic progenitor to myeloid cells. *Cancer Genet Cytogenet*. 2006;164(2):118–21.
18. Hu T, Murdaugh R, Nakada D. Transcriptional and Microenvironmental Regulation of Lineage Ambiguity in Leukemia. *Front Oncol*. 2017;7:268.
19. Mejstrikova E, Volejnikova J, Fronkova E, Zdrahalova K, Kalina T, Sterba J, et al. Prognosis of children with mixed phenotype acute

leukemia treated on the basis of consistent immunophenotypic criteria. *Haematologica*. 2010;95(6):928–35.

20. Shi R, Munker R. Survival of patients with mixed phenotype acute leukemias: A large population-based study. *Leuk Res*. 2015;39(6):606–16.
21. Batra S, Ross AJ. Pediatric Mixed-Phenotype Acute Leukemia: What's New? *Cancers (Basel)*. 2021 Sep 16;13(18):4658.
22. Oberley MJ, Raikar SS, Wertheim GB, Malvar J, Sposto R, Rabin KR, et al. Significance of minimal residual disease in pediatric mixed phenotype acute leukemia: a multicenter cohort study. *Leukemia.* 2020;34(7):1741–50.

Cite this article: Mishra P, Sharma S, Bhandari A, Chopra M. Fatal hemorrhagic manifestations of B/T mixed phenotype acute leukemia mimicking acute promyelocytic leukemia: Case report and a review of literature. *Indian J Pathol Oncol*. 2025;12(1):74-78.