Research Article

B+Myeloid MPAL: boys have it all?

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Abstract

Background: MPAL are rare, accounts 4% of acute leukemias .B+MYELOID are extremely rare. There is not much information on biologic features of these strange leukemias. Present study from Kidwai state cancer institute focused on rare types of MPAL, especially B+MYELOID MPAL. **Design and Methods**: We classified MPAL based on WHO 2008 classification and summarised diagnostic criteria, cytochemistry, cytogenetics clinical, immunophenotyping and molecular featuresofB+MYELOID MPAL. **Results**:Out of 27 MPAL cases reported in the present study 13 cases were B/Myeloid, followed by B+T MPAL, T+Myeloid .Very few cases of undifferentiated and unclassifiable leukemias were reported. 13 B/myeloid MPAL cases were reported majority of these, to our surprise were seen in children (8 cases) .Only 5 cases were seen in adults. Except a single Ph (+) case in a girl, rest all pediatric B/Myeloid cases, surprisingly were seen in Boys. **Conclusion**: Among MPAL high incidence of B+MYELOID 13/27 cases (48%) was noted. High incidence of B/myeloid MPAL was seen in children and majority were Boys (87.5%). All paediatric cases were treated by MCP 841 Protocol &attained remission at end of induction, 5/8 cases were referred for Bone marrow transplant.3 cases are on follow up after median of 6 months and are in remission.

Keywords: acute leukemias of ambiguous lineage, Mixed phenotypic acute leukemia,aberrant antigens,cytogenetics, flowimmunophenotyping.MCP841protocol, B/Myeloid .

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Introduction

WHO Classification of Tumors of Haematopoietic and Lymphoid tissue in 2008 " recognized "Acute leukemias of ambiguous lineage" (ALAL) as a distinct rare entity. When the blasts express more than one lineage antigens, assigning them to particular lineage certainly is not possible, these leukemias are termed MPAL. By definition MPALdoesn't fit into any category, either by clinical or genetic features [1,2]. MPAL accounts for 4% of acute leukemias [3]. Mixed phenotype acute leukemia can be classified as B/Myeloid, T/Myeloid, B+T or trilineage MPAL based on Immunophenotypic findings [1,4].

There is dearth of data regarding the biologic characterisation of MPAL, as well as established guidelines regarding the management and prognosis. This is because the formal criteria for diagnosis have been established recently& these leukemias are extremely rare [3,5].

Manuscript received: 2nd March 2016
Reviewed: 15th March 2016
Author Corrected: 25th March 2016
Accepted for Publication 7th April 2016

B+MYELOID are uncommon and comprise only 4% of MPAL.Till date, present study of 13 cases of B+MYELOID is the largest, exclusive study of B+MYELOID in literature till date, from a single institute. Study was concenterated on B/Myeloid MPAL in paediatric population.

Material &Methods

Our laboratory received 1855 acute leukemias, during a period of 4 yearsfrom January 2012 to march 2016, where diagnostic flow cytometric analysis was done and leukemias were classified based on WHO 2008 criterias ,along with, morphology, cytochemistry, cytogenetics,

clinical & immunophenotyping findings. ALAL was diagnosed in 32 patients. Out of these, 27 cases were MPAL. Study concenterated mainly on of B+MYELOID MPAL cases. Surprisingly these leukemia's were seen predominantly in paediatric population.We had 8 children in the study, whereas adults were only 5. Paediatric B/Myeloid (8 cases) MPAL were studied in detail. Majority of B/Myeloid MPAL were seen in boys, gender ratio was 7:1.

Treated Leukemias , that switch their lineage later were excluded from the study.Giemsa stain was used on air dried smears and morphologic examination of Bone marrow aspiration, biopsy and peripheral blood was done .Cytochemical Myeloperoxidase (MPO) and Periodic acid Schiff (PAS) stains was done in all cases. Nonspecific esterase and specific esterase accordingly if required.

Bone marrow aspirate was analysedbyBecton Dickinson's FACS caliber,a multi colour flow cytometer . Strategy followed for gating was CD45 vs side scattering. Immunophenotype features were analysed. G banding was done on overnight-unstimulated cultures of bone marrow &were analysed.

Statistical methods: Event free survival was estimated using Kaplan –Meier method. Petoetal, Method was used to determine SEs. R software was used for survival comparison.

Results

MPAL was diagnosed using WHO criteria in 27 patients (1.4 %).5 cases were acute undifferentiated leukemias/unclassifiable leukemias. Among 27 MPAL cases, 13 cases were B+MYELOID MPAL(48%).Majority of B/myeloid MPAL(8/13) were seen in children(61.5% of B/Myeloid MPAL).
Paediatric B/myeloid MPAL were studied in detail .

Surprisingly most B+Myeloid MPAL ,7/8 cases (87.5%) were seen in boys,except for a single case of Ph(+) B/Myeloid MPAL seen in a 14 year old girl.Youngest patient was 2 year old boy.6 cases were younger than 10 years of age(75%). On ultrasonograghy in 2/13 cases revealed hepatosplenomegaly .Lymphadenopathy was noted in 2 cases on clinical examination. Single case which relapsed early developed cutaneous nodule and testicular involvement. Baseline investigations revealed leucopenia in 6 cases (75%), 3 cases had increased total count (TC), out of 3 cases,single case had Total count > 1 lakh, she incidentally was Ph positive. 5 patients (50%), low hemoglobin(<10g/dl) in 4 cases(50%), and low platelet count was seen in all patients. Mean serum lactate dehydrogenase (LDH) was 741 IU/L, serum alkaline phosphatase was with in normal range in all cases and elevated liver enzymes was noted in a single case. Serological studies for HIV, HBsAg and HCV were negative in all cases. Blasts percentage varied from 20% to 90%.

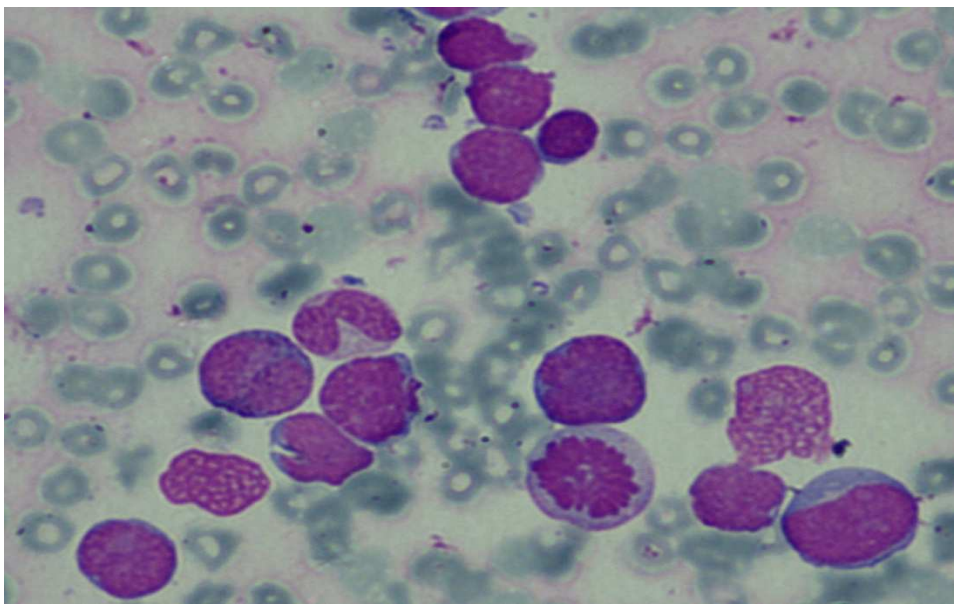


Fig-1: Blasts with Myeloid morphology

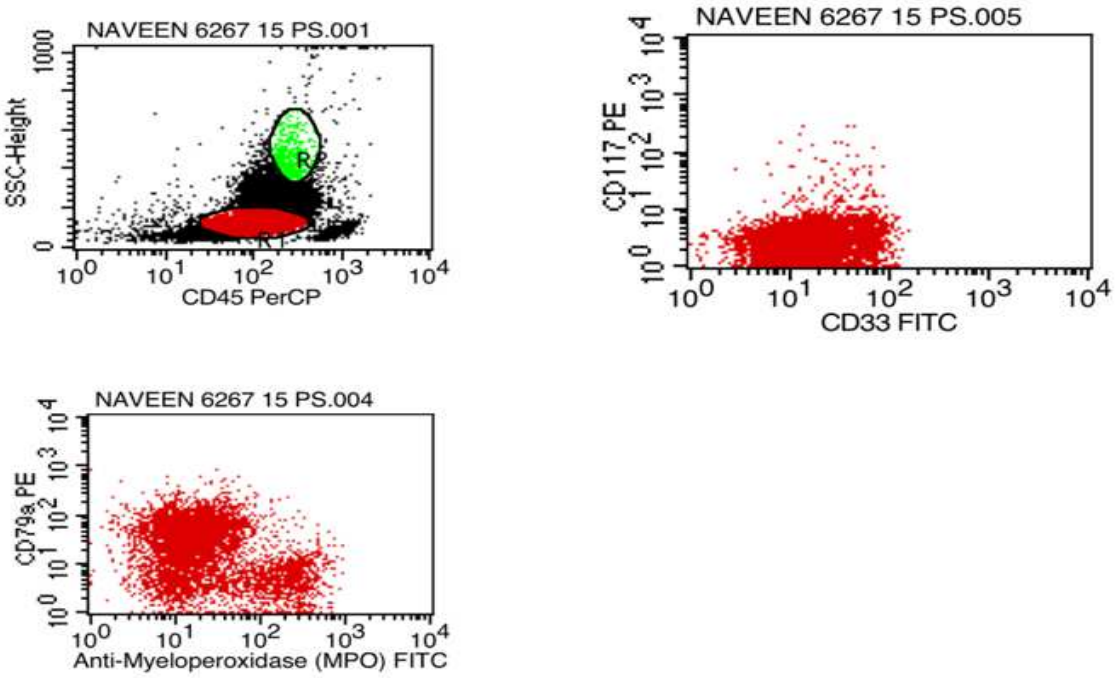


Fig-2: Flow cytometry scattergrams

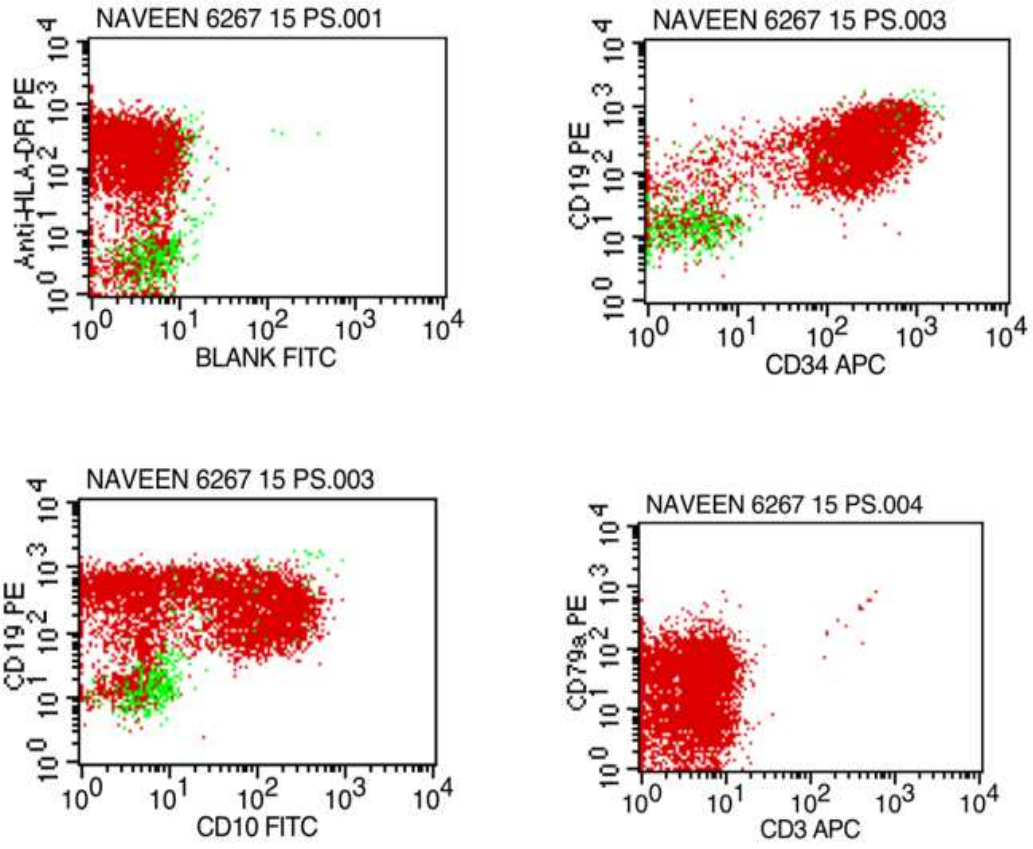
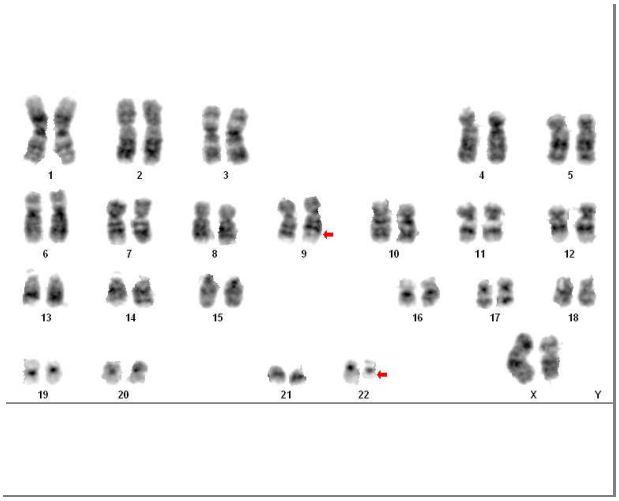
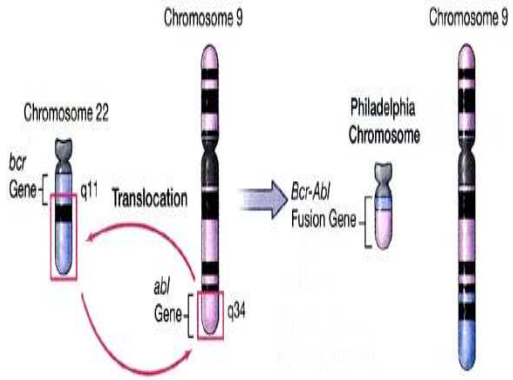


Fig 3: Flow cytometryscattergrams





46,XX, t(9;22)(q34;q11.2)

Fig-4: Phildelphia chromosome

Majority Cases of B+MYELOID MPAL revealed Myeloid morphology(fig1), 2 cases even showed auerrods,single case had lymphoid morphology but had coarse cytoplasmic granules. Flow cytometry scattergrams (fig 2&3) reveals the positive Immuno phenotypic markers. Myeloperioxidase was positive in all cases by FIC, however cytochemical MPO was negative in 2 cases.

Marker of early hematopoietic cells, CD34, was strongly positive in all 100% cases (50%). Tdt was done in 5 cases only, but was positive in 100% tested cases. All cases were positive for MPO, CD19 and CD 79a. CD13 &CD 33 was positive in 62% of cases.CD117 was positive in 50% of cases.CD 10 was positive in 75 % of cases.

All 8 paediatric cases cytogenetic data was available ,3 case showed abnormal karyotype , one was hyperdiploid ,other cases had Ph chromosome (fig 4) & a variant Phildelphia chromosome,t(7;9;22)(q11 ;q34 ;q11),RT PCR showed increased ABL/BCR,P210 fusion protein ,however patient did not have spleen nor antecedent history of CML.

Discussion

Acute undifferentiated leukemia is diagnosed when the blasts exhibit no lineage-specific antigens. When the blasts express more than one lineage antigens it is not possible to assign to any particular lineage, it is called MPAL[2,6].

In the majority of patients with acute leukemia, blast cells can be assigned to specific lineage, Myeloid, B- or T-lymphoid. However, in approximately 2-5% of patients, after immunophenotyping by flow cytometry (FCM), lineage of leukemias remains ambiguous. Historically various terminologies were used, such as mixed lineage leukemia, hybrid acute leukemia, bilineal leukemia, and biphenotypic leukemia [2,6]. Diagnostic criteria were modified by WHO classification of hematopoietic and lymphoid tumors in 2008 & introduced a new designation as mixed-phenotype acute leukemia [2,6] As the years passed more antibodies became available, it was found that expression of antigens was aberrant, and was common in acute

leukemias. Many researchers tried to address this issue by allotting a score. Scoring criteria by European Group for Immunological classification of Acute Leukemia (EGIL), tried to address this issue and to define MPAL accurately. Various markers were allotted a score of 0.5.1 or 2, Score of >2 for at least two lineages were classified as biphenotypic acute leukemia. However, this approach was questioned , due to the lack of lineage specificity with available markers.

Thus in 2008,WHO laid down strict criteria and defined the lineage specific markers for each lineage[6].

B cell lineage can be chosen from cCD79a, cCD22, CD24, intracytoplasmic mu chains, CD20 or CD21. The expression of CD10 is also considered as B lineage marker..So far cCD3 is the strongest marker indicating T cell lineage [4] .

Immunophenotypically Mixed phenotype acute leukemia can be further subclassified as B/Myeloid, T/Myeloid, B/T, or trilineage based on immunophenotypic findings[4] .

There is no single marker, which is sufficiently specific to indicate B-cell differentiation with certainty [2]. In addition to strong CD19 strong CD79a was present in all cases, whereas expression of CD10 was noted in 75% of cases in present study (fig 2&3).

Assigning Myeloid lineage depends on the expression of positive myeloperoxidase or by the presence monocyticmarkers. Myeloid lineage was established in 100% of cases B/myeloid MPAL by the presence of myeloperoxidase positivity on flowcytometry. In 2 cases cytochemistry could not detect myeloperoxidase positivity. This high lights the importance of doing both cytochemistry and flow cytometry for myeloperoxidase. There are many studies comparing the sensitivity of flow cytometry and enzyme cytochemistry in detecting myeloperoxidase. we again reconfirm in the present study that concomitant use of both Flow cytometry and cytochemistry increases the sensitivity[6].

The mixed phenotype in B/myeloid MPAL can occur in three ways:
1. Two distinct blast populations, one showing immunophenotype of AML and the other showing immunophenotype of lymphoid blast population.

2. Single population of blasts meeting criteria of B-ALL/T-ALL with expression of myeloperoxidase.

3. Single population of blasts meeting criteria of B-ALL/T-ALL with evidence of monocytic differentiation [2,6].

Present study 1 &2 immunophenotype patterns were noted, however monocytic differentiation was not seen. Morphology renders some clue to the presence of two types of blasts, but immunophenotyping is absolute requirement in the identification of this rare subset of leukemias [6].

CD19 detection in tissue sections is technically critical and difficult to evaluate , however, a suspicion of MPAL, can be made by immunohistochemical staining of a bone marrow biopsy if blasts express MPO and B cell-associated marker such as CD79a, CD22,PAX-5 and CD10[1].

Rarity of B+MYELOID is established by following facts. The largest study so far is by HeidrunnGerr (2010) et al, he has analysed 92 children, involving multiple centers, over a period of 8 years. 45cases of B/Myeloid were reported [7] .

Zhang et al has reported 16 cases of B+Myeloid in his series [8]. Owadiah et al reported 15 cases of B+myeloid MPAL[9],Weir et al reported 9 cases,most of these were in adults and over a period of many years[10].Rubinetz et al one of the largest studies in children from St Judes children hospital studied 12 B/myeloid MPAL cases in 20 years[11].

Yadong zhang et al in 2011 reported 8 cases of B/Myeloid MPAL over a period of 10 years[12].Study period was over a decade in most of these studies.

Present study comprised 13 cases of B+Myeloid, the largest exclusive study of B/Myeloid MPAL till date from a single center in a short period of 4 years. Surprisingly Majority 8 cases were seen in children, boys were affected commonly.

This study involved clinical features, diagnostic criterias,immunophenotyping by flow cytometry, cytogenetics&molecular methods like RT PCR.
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 positivity & HLADR suggesting an early precursor stem cell origin[13]. In our series,100% cases demonstrated CD34 Positivity, along with Tdt.

Commonly described cytogenetic groups in MPAL are , compelx karyotypes, t(9;22), (q34;q11) and t(v;11q23)[2,7]. Phildelphia chromosome has been associated with poor prognosis in MPAL.The great majority of, t(9;22) (q34;q11) positive MPAL are B+Myeloid.Even though Ph + is reported in other MPAL subcategories[14], in the present study, of pediatric MPAL , Ph (+) were exclusively seen in B/Myeloid MPAL.

Analysis of cytogenetics is becoming increasingly important as potential targets for therapy in future Presence of the Phildelphia chromosome should be checked always as it affects the treatment [2,5].

The lineage specific cytoplasmic markers should be included in the primary panel for categorization of MPAL accurately.

There are no set treatment protocols for patients with MPAL ,thus evaluation of outcome of treatment becomes difficult[15,16].The optimal therapeutic approach to B+MYELOID, has not been defined.In 41% patients treated with AML protocol and 85% patients with ALL treatment ,achieved complete remission ,In a recently published larger analysis of 100 MPAL patients. The overall median survival was 18 months,in this series.

Patients were treated with ALL regimens based protocol,MCP841 protocol in children ,at the end of induction period morphological complete remission (CR) (<5% blasts in marrow) was achieved in all cases .5 paediatric patients were Referred for BMT after remission &3 cases continue to be in remission for median 6 months. Our data suggests that children, who received ALL directed treatment MCP 841 protocol had a better response.

chromosome deletions which are Submicroscopic , amplifications, and unbalanced chromosome rearrangements are recognized frequently by Genomic microarrays in recent years and are widely used to identify cryptic leukemia-related genetic alterations,especially in hematologic malignancies[16,17,18] .Study by Lingzhi yanetal is the first molecular genomic study of MPAL[17].

Our data suggests that B+Myeloid MPAL frequently affects Boys,and with ALL directed treatment, have a better response and Ph+ was exclusively seen in B/Myeloid MPAL in paediatric population.More studies in Paediatric B/Myeloid MPAL might further clarify our findings.

Novel genomic technologies, such as next-generation sequencing, may help to define the leukemogenic mechanisms in B/Myeloid MPAL cases and to determine a standardized treatment for these rare leukemias.

Molecular explorations, will be of great help in understanding of these strange leukemias, however due to financial constraints Gene sequencing was not done in these cases. B+MYELOID are relatively frequent in India ,especially in paediatric age group .Molecular studies and multi-center studies to determine optimal regimens approach in Indian scenario will be of immense help in understanding B/Myeloid MPAL .

Funding: Nil,
Conflict of interest: None.
Permission of IRB: Yes

References

1. Béné MC, Porwit A. Acute leukemias of ambiguous lineage. Semin Diagn Pathol. 2012 Feb;29(1):12-8.

2. Borowitz M J BeneMC,HarrisNL,PorwitA,Matutes E Acute leukemia of ambiguous lineage : WHO classification of hematopoietic and lymphoid tissue 4th ed.IARC, Lyon, France:2008.pp 150-155.

3. EstellaMatutes, RicardoMorilla, NahlaFarahat, FelixCarbonell, JohnSwansbury, MartinDyer, DanielCatovsky. Recent Advances in the cyto biology of Leukemias, Definition of Acute BiphenotypicLeukemia. Haematologica 1997; 82(1):64-66.

4. Tseih Sun .Mixed phenotypic acute leukemia: In Flow cytometry, immunohistochemistry and molecular genetics for hematologic neoplasms: 2nd edition. Wolterskluwer/Lippincott Williams & wilkins, Phildelphia. 2012;chapter 16:168-173.

5. Sameer kulkarni,MichaelLill and DimitriosTzachanis .HSOA Journal of Hematology.Blood transfusion and Disorders.JHematolBloodTransfusDisord2014;vol1:issue1.

6. Renu Sukumaran, Rekha ANair, PriyaMaryJacob, Kunjulekshmi Ammaraveendrannair Anila, ShruthyPrem, Rajeshwary Binitha et al .Flowcytometry analysis of Mixed phenotype acute

leukemia: Experience from a tertiary oncology center.Indian Journal of Pathology and Microbiology.June 2015;58,181-186.DOI 10.4103/0377-4929.155309.

7. Gerr H, Zimmermann M, Schrappe M, Dworzak M, Ludwig WD, Bradtke J, Moericke A, Schabath R, Creutzig U, Reinhardt D. Acute leukaemias of ambiguous lineage in children: characterization, prognosis and therapy recommendations. Br J Haematol. 2010 Apr;149(1):84-92. doi: 10.1111/j.1365-2141.2009.08058.x. Epub 2010 Jan 18.

8. Zhang YM, Wu DP, Sun AN, Qiu HY, Sun YM, He GS, Jin ZM, Tang XW, Miao M, Fu ZZ, Han Y, Chen SN, Zhu MQ. [Study on the clinical characteristics of 32 patients with mixed phenotype acute leukemia]. Zhonghua Xue Ye Xue Za Zhi. 2011 Jan;32(1):12-6.

9. Owaidah TM, Al Beihany A, Iqbal MA, Elkum N, Roberts GT. Cytogenetics, molecular and ultrastructural characteristics of biphenotypic acute leukemia identified by the EGIL scoring system. Leukemia. 2006 Apr;20(4):620-6.

10.Weir EG, Ali Ansari-Lari M, Batista DA, Griffin CA, Fuller S, Smith BD, Borowitz MJ. Acute bilineal leukemia: a rare disease with poor outcome. Leukemia. 2007 Nov;21(11):2264-70. Epub 2007 Jul 5.

11. JeffreyE.Rubnitz,MihaelaOnciu,Stanley Pounds ,Sheila shurt ,Xueyancao,suiana c Raimondi et al ,Acute mixed lineage leukemia in children: the experience of St Jude Children's Research Hospital.Blood 2009;113:5083-5089.

12. Zhang Y, Tan L, Zhang X, Wei H, Hu Q. Clinical Study of Acute Mixed-lineage Leukemia in 14 Children. Iran J Pediatr. 2011 Dec;21(4):521-5.

13. Gujral S, Polampalli S, BadrinathY, Kumar A, SubramanianPG,Raje G, et al.Clinico-hematological profile in biphenotypic acute leukemia.Indian Journal of Cancer, April-June, 2009; Vol. 46: No. 2pp. 160-168.Doi 10.4103/0019-509x49156

14. Kohla SA, Sabbah A A,Omri H E, Ibrahim FA,Otazu I B, Alhajri H Yassin M A.Mixed Phenotype Acute Leukemia with Two Immunophenotypically Distinct B and T Blasts Populations,Double Ph(+) Chromosome and complex Karyotype.Clin Med Insights Blood Disord.2015; sept21.doi:10.4137/CMBDs24631.c collection2015.

15. Wolach O, Stone RM. How I treat mixed-phenotype acute leukemia. Blood. 2015 Apr 16;125(16):2477-85. doi: 10.1182/blood-2014-10-551465. Epub 2015 Jan 20.

16. Weinberg OK, Arber DA. Mixed-phenotype acute leukemia: historical overview and a new definition. Leukemia. 2010 Nov;24(11):1844-51. doi: 10.1038/leu.2010.202. Epub 2010 Sep 16.

17. LingzhiYan, NanaPing, MinggingZhu, AiningSun, YongquanXue, Changgeng Ruanetal. Clinicalimmunophenotypic, Cytogenetic, And Molecular Genetic Features In 117 Adult Patients With Mixed-Phenotype Acute Leukemia Defined By WHO-2008 Classification.Haematologica November 2012;97: 1708-1712. DOI. 10 .3324/ HAEMATOL. 2012.064485.

18. Maciejewski JP, Haferlach T. Introduction: molecular pathogenesis of hematologic malignancies. Semin Oncol. 2012 Feb;39(1):9-12. doi: 10.1053/j.seminoncol.2011.12.002.

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How to cite this article?

Namrata N.R, Raghavendra H.V, Appaji, Jayadev naik, Madhumathi D S' ' B+Myeloid MPAL: boys have it all? : *Int J Med Res Rev* 2016;4(4):550-556. doi: 10.17511/ijmrr.2016.i04.13.

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