***Review***

# Mixed Phenotype Acute Leukemias

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**Mixed phenotype acute leukemia represents a small subset of acute leukemia that cannot be simply assigned as myeloid or lymphoid lineage, because of the ambiguous phenotype the leukemic cells exhibit. It encompasses leukemias containing separate populations of blasts of more than one lineage, or a single population of blasts co-expressing antigens of more than one lineage. The 2008 World Health Organization classification established strict criteria for diagnosis of mixed phenotype acute leukemia, emphasizing myeloperoxidase for myeloid lineage assignment, cytoplasmic CD3 for T lineage assignment, and CD19 and other B markers for B lineage assignment. A variety of cytogenetic lesions have been identified in this group of diseases, two of which, the t(9;22)(q34;q11) BCR-ABL1 translocation, and t(v;11q23) with MLL rearrangement are considered separate entities. Other categories include T/myeloid NOS, B/myeloid NOS and other rare types. Mixed phenotype acute leukemia is associated with poor outcome compared with other types of acute leukemias, particularly in those with Philadelphia chromosome, and clinically presents challenges in diagnosis and treatment.**

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**Key Words**: *acute leukemia, acute leukemia of ambiguous lineage, cytogenetics, diagnosis, mixed phenotype acute leukemia (MPAL)*

## INTRODUCTION

Most cases of acute leukemia can be classified based on the lineage of the leukemic cells as myeloid, B-lymphoblastic (B-ALL) or T-lymphoblastic leukemia (T-ALL). However, there are uncommon cases in which the blasts show differentiation towards more than one lineage. In the 2008 World Health Organization (WHO) classification these cases are identified as mixed phenotype acute leukemias (MPAL), under the category of acute leukemias of ambiguous lineage.[1] MPAL encompasses leukemias containing separate populations of blasts of more than one lineage (bilineal or bilineage), and a single population of blasts co-expressing antigens of more than one lineage (biphenotypic). Cases that can be classified in another category are excluded, including acute myeloid leukemia (AML) with recurrent translocations t(8;21), t(15;17) or inv(16), leukemias with *FGFR1* mutations, chronic myelogenous leukemia (CML) in blast crisis, myelodysplastic syndrome (MDS)-related AML and therapy-related AML, even if they have MPAL immunophenotype.[1] Of note, a diagnosis of MPAL should be reserved for patients who present with *de novo* acute leukemia.

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.[2,3] However, the true incidence is difficult to establish due to problems with definition, and perhaps variation between different laboratories. It affects both adults and children, more frequently adults and has slight male preference.[4] The prognosis for MPAL is poor comparing to other acute leukemias, with an overall survival of 18 months.[1,4]

## DIAGNOSIS

Before the publication of the 2008 WHO classification, the diagnosis and classification of MPAL were based on the scoring system proposed by the European Group for the Immunological Classification of Leukemias (EGIL).[5] The EGIL classification scheme assigns score points to major antigens to determine if certain lineage is present. According to the original EGIL scoring system MPAL is defined when scores are over two points for both myeloid and T- or B-lymphoid lineages (**Table 1**). CD117 was assigned for 0.5 point in the original EGIL scoring system and later considered as a reliable marker for myeloid commitment, and scored higher (1 point).[6] The EGIL criteria was very helpful in classification of MPAL. However, even the revised EGIL criteria can sometimes lead to an inaccurate classification. For instance, classical AML cases with t(8;21) frequently express multiple B-cell markers (CD19, CD79a and CD20), and cytoplasmic CD79a, considered to be specific in B-lineage determination, is positive in a significant percentage of T-ALL cases.

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**Table 1.** The European Group for the Immunological Classification of Leukemias (EGIL) scoring system.[5]

| Points | Myeloid | B-lineage | T-lineage |
|---|---|---|---|
| 2 | MPO | CD79a<br>Cyt IgM<br>Cyt CD22 | CD3, cyt and surface<br>TCR αβ<br>TCR γδ |
| 1 | CD13<br>CD33<br>CD65 | CD19<br>CD10<br>CD20 | CD2<br>CD5<br>CD8<br>CD10 |
| 0.5 | CD14<br>CD15<br>CD64<br>CD117 | TdT<br>CD24 | TdT<br>CD7<br>CD1a |

***Abbreviations:*** MPO, myeloperoxidase; TdT, terminal deoxynucleotidyl transferase; cyt, cytoplasmic; TCR, T-cell receptor. ≥ 2 points are required to assign a lineage.

**Table 2.** 2008 WHO classification of acute leukemias of ambiguous lineage.[1]

| | |
|---|---|
| Myeloid lineage | Myeloperoxidase (flow cytometry, immunohistochemistry or cytochemistry)<br>*Or*<br>Monocytic differentiation (at least two of the following: NSE, CD11c, CD14, CD64, lysozyme) |
| T lineage | Cytoplasmic CD3 (flow cytometry with antibodies to CD3 epsilon chain)<br>*Or*<br>Surface CD3 |
| B lineage | Strong CD19 *AND* strong expression of at least one of the following: CD79a, cytoplasmic CD22, CD10<br>*Or*<br>Weak CD19 *AND* strong expression of at least two of the following: CD79a, cytoplasmic CD22, CD10 |

The most recent 2008 WHO classification has established new and strict criteria for the diagnosis of MPAL (**Table 2**). The T lineage is recognized by the presence of specific T-lymphoid antigens, cytoplasmic CD3 (cCD3) or surface CD3. Cytoplasmic CD3 expression is best demonstrated by flow cytometry with antibodies to CD3 epsilon chain. It should be noted that polyclonal CD3 antibodies used in immunohistochemistry also react with the T-cell receptor zeta chain present in NK cells, and therefore considered not specific for T lineage. Surface CD3 is rare but indicative of the T-lineage. The myeloid lineage is demonstrated with the presence of myeloperoxidase (MPO) by flow cytometry, immunohistochemistry or cytochemistry, or monocytic differentiation (requiring at least two of the following: non-specific esterase (NSE), CD11c, CD14, CD64, lysozyme. Since there is no single marker sufficiently specific for B-cell lineage, multiple antigens are required, including strong expression of CD19 with one of the other B-cell markers (CD79a, cytoplasmic CD22, CD10), or weak CD19 expression with at least two of the other B-cell markers.[1]

Compared with the EGIL scoring system, the current 2008 WHO criteria applied less but more specific markers to define the lineage of the blasts, and incorporated the intensity of markers expression into the diagnostic algorithm. The 2008 WHO criteria also emphasize MPO in the diagnosis of MPAL. MPO can be negative in an AML with minimal differentiation, but it has to be positive in MPAL with myeloid lineage, unless the myeloid lineage proven to be monocytic differentiation by expressing at least two of the monocytic markers. Practically, diagnosis of MPAL largely relies on flow cytometric immunophenotyping. Other diagnostic methods such as immunohistochemistry and cytochemistry can be helpful.

The B/myeloid MPAL is the most common among MPALs, followed by T/myeloid, and other rare types of MPALs. A cohort of 100 patients diagnosed as MPAL using the 2008 WHO criteria showed 59% of cases are B/myeloid immunophenotype, and 35% are T/myeloid immunophenotype. A small portion of the cases are B+T-lymphoid imminophenotype or trilineage (B+T+myeloid) immunophenotype.[4]

Morphologically most cases of MPAL display a single population of leukemic cell, with 43% showing ALL morphology, and 42% showing AML morphology in a large cohort study. [4] Majority of the cases with AML morphology are M1 (AML without maturation) or M5 (acute monoblastic and monocytic leukemia) according to the French-American-

British (FAB) classification system, and rarely M2 (AML with maturation) or M4 (acute myelomonocytic leukemia).[4]

The new WHO classification emphasizes MPO expression in myeloid lineage assignment. This was supported by the report by Bene, et al. showing 98% of the MPAL cases expressing MPO in at least 5% of blasts, 76% of the cases with more than 20% of blasts expressing MPO. Majority of the cases have variable populations of blasts coexpressing MPO and lymphoid markers. Other myeloid markers are variably expressed, including CD13 (74%), CD33 (66%), and CD117 (52%), and frequently coexpressed with MPO. Expression of monocyte-associated markers include lysozyme (31%), CD15 (12%), and CD14 (8%).[4]
There are four major categories listed under MPAL in the 2008 WHO classification: B/myeloid, NOS; T/myeloid, NOS; MPAL with t(9;22)(q34;q11.2); *BCR-ABL1*; and MPAL with t(v;11q23); *MLL* rearranged.

**MPAL WITH t(9;22)(q34;q11.2); *BCR-ABL1***
This is the most frequent recurrent genetic abnormality occurring in MPAL and considered a distinctive entity. It accounts for 20% of all MPAL.[1,4] It is a leukemia meeting the diagnostic criteria for MPAL with the blasts bearing the t(9;22)(q34;q11.2) translocation or *BCR-ABL1* rearrangement (Ph+) in patients with no history of CML. It occurs more often in adults than in children.[4] Clinically, the patients present similarly as other patients with acute leukemias, with white blood cell counts likely to be high, resembling $Ph^+$ ALL.

Majority of the cases occurring in adults have B/myeloid phenotype, while some show T/myeloid, B and T lineage, or trilineage leukemias. Morphologically many cases show a dimorphic blast population, one resembling myeloblasts and the other lymphoblasts. Some cases do not have distinguishing phenotypes.[4,7,8]

Cytogenetic abnormalities are identified by conventional karyotyping for t(9;22), or FISH or PCR for *BCR-ABL1* translocation. Additional cytogenetic abnormalities are shown in many cases, including complex karyotypes. Ph+ is a poor prognostic factor for MPAL, with a reported median survival of 8 months in 12 patients, significantly worse than patients of all other types of MPAL.[4]

**MPAL WITH t(v;11q23); *MLL* REARRANGED**
This is a leukemia meeting the diagnostic criteria for MPAL with blasts bearing a translocation involving the 11q23 breakpoint (*MLL* gene). *MLL* rearrangement is more often seen in patients with a precursor B-ALL with aberrant expression of myeloid markers, especially CD15 and CD65 but not MPO. These cases should not be considered MPAL.[1,9]

MPAL with *MLL* rearranged is rare and accounts for 8% of all patients with MPAL. It is more often seen in children and relatively common in infancy.[1,4] The clinical presentation is similar to other patients with acute leukemias. High white blood cell counts are common as with other leukemia patients with *MLL* translocations. Commonly these leukemias display a biphenotypic blast population, with one resembling monoblasts and the other resembling lymphoblasts. The lymphoblast population often shows a CD19-positive, CD10-negative B precursor immunophenotype, frequently positive for CD15. Expression of other B markers is usually weak.[1]

The translocations involving *MLL* gene include t(4;11)(q21;q23), t(11;19)(q23;p13), and t(9;11)(p22;q23), with confirmed partner genes being *AF4* on chromosome 4q21 and *AF9* on 9p22.[4] However, cases with chromosome 11q23 deletion should not be classified in this category. The prognosis for this type of leukemia is poor.[1,4]

**MPAL B/MYELOID, NOS**
This type of leukemia meets the diagnostic criteria for assignment to both B and myeloid lineages and lacks the above mentioned recurrent cytogenetic abnormalities. B/myeloid acute leukemia accounts for 59% of all MPAL[4] cases and about 1% of all leukemias.[1] It more commonly occurs in adults, but can be seen in children as well.

Morphologically, the blasts have no distinguishing features in most cases, with dimorphic populations, resembling lymphoblasts and myeloblasts, or a single population resembling ALL. CD19 is strongly expressed in greater than 90% of the cases, with majority positive in greater than 50% of the blasts. The blasts are also positive for CD10, cytCD22, and/or cytCD79a.[4] Multiple different cytogenetic changes have been demonstrated, however none is proven to be specific in this subtype (will be discussed below).

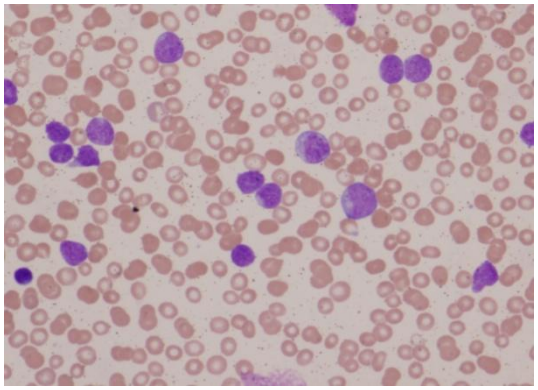

**Figure 1**. Wright-Giemsa-stained bone marrow smear from a patient with T/myeloid leukemia. Dimorphic populations of blasts are observed, one is small lymphoid appearing, the other has dispersed chromatin, prominent nucleoli and a moderate amount of pale cytoplasm, resembling myeloblasts, and positive for MPO.

**MPAL T/MYELOID, NOS**
This category meets the requirements for assignment to both T-lymphoid and myeloid lineages without recurrent cytogenetic abnormalities. It accounts for one third of MPAL and less than 1% of overall leukemias.[1,4] It can occurs in both children and adults, but more commonly in children.

There are no distinctive clinical features in patients with T/myeloid acute leukemia. The blasts are composed of either a single population or dimorphic populations (**Figure 1**). Cytoplasmic CD3 is expressed in virtually all cases, with

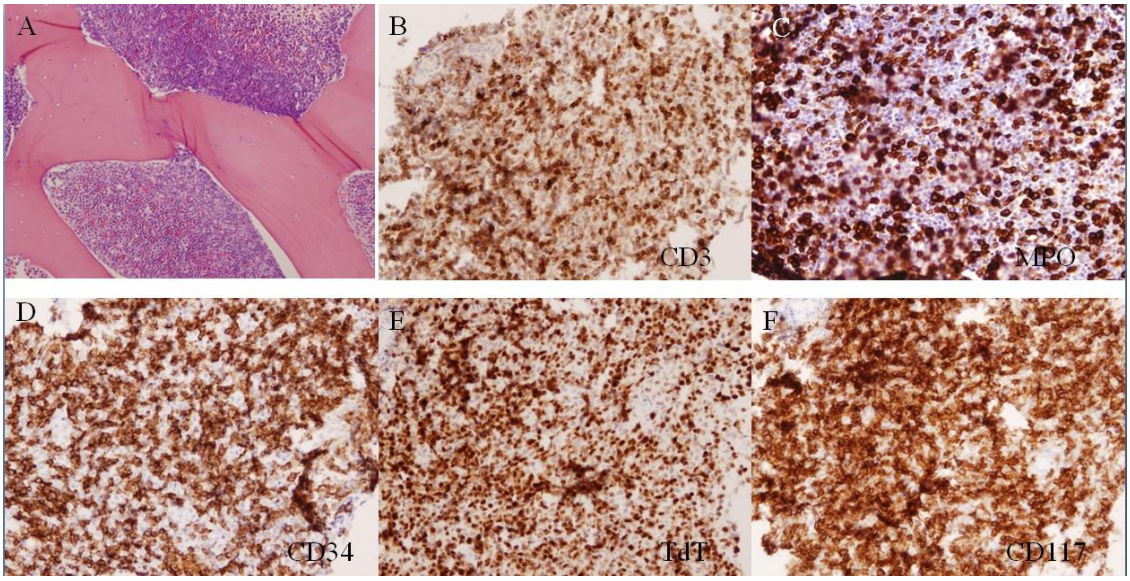


**Figure 2.** T/myeloid leukemia with *FLT3* mutation. Bone marrow section shows 100% cellularity with extensive replacement of marrow by sheets of blasts (**A**). Immunohistochemistry stains show a subset of blasts positive for CD3 (**B**), MPO (**C**), CD34 (**D**), TdT (**E**) and CD117 (**F**).

more than 20% of the blasts positive in majority of the cases. Other commonly expressed T-lineage markers include CD2, positive in 27-98% of the blasts in 67% of the cases, and CD7, positive in 24-99% of the blasts in 91% of the cases.[4] Interestingly, the FMS-like tyrosine kinase 3 gene (*FLT3*) mutations is demonstrated to be specifically associated with T/myeloid lineage. The immunophenotypic profile of CD117(bright), terminal deoxynucleotidyl transferase (TdT), CD7, CD13 and CD34 is reported to be highly sensitive (100%) and specific (94%) for predicting *FLT3* mutation in T-ALL and T/myeloid acute leukemia (Figure 2).[10] Targeted therapy with *FLT3* inhibitors has been developed and undergone clinical trials.[11]

## CYTOGENETICS

The incidence of cytogenetic abnormalities is high in MPAL, with only 13% of the cases showing a normal karyotype. About one third of the cases have a complex karyotype with three or more structural chromosome abnormalities, and 27% have other abnormalities. The most commonly involved chromosomal abnormalities include del(6)(q11-21), 7q-, -7, t(2;7), del(5q) or -5, trisomy 4, and hyperdiploid karyotype. *ETV-6-RUNX1* rearrangement has been reported.[12] Except for t(9;22), *MLL* rearrangement, and *FLT3* mutation, there is no significant correlation between the other cytogenetic abnormalities with age, sex, morphology, FAB subtype or immunophenotype.[4]

## PROGNOSIS

Young age, normal karyotype and ALL induction therapy are associated with favorable survival, and Ph+ is a predictor for poor prognosis. Study shows median survival is 139 months for children versus 11 months for adults, 139 months for patients with normal karyotype, versus 8 months for Ph+, and 139 months for patients receiving ALL regimens, versus 11 months for those receiving AML schedules.[4]

In summary, MPAL is a rare disease with poor prognosis. In order to establish the diagnosis, a panel of markers are suggested including: MPO, CD3 (cytoplasmic and surface), CD19 plus three other B-lineage markers (CD22, CD79a, CD10), and two or three monocytic markers. Adult and Ph+ patients have worse prognosis, and bone marrow transplantation should be considered in first remission in these patients.[1,4]

**CONFLICT OF INTEREST**
None.

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