Reporting for B cell ALL with isolated dim MPO expression

Myeloid Lineage Assignment [Cancers 2025, 17, 871]
In both the WHO-HEM5 and the ICC classifications, the assignment of myeloid/ non-monocytic lineage relies on the expression of a single cell marker: myeloperoxidase (MPO). While this may seem straightforward, assessing adequate MPO expression for myeloid lineage commitment can be challenging due to the variability seen in MPO expression patterns across different leukemia cases. Consequently, the WHO-HEM5′s criteria for lineage assignment based on MPO expression remain somewhat ambiguous and subject to interpretation in certain cases. The ICC does not provide specific threshold criteria for MPO positivity. According to the WHO-HEM5, the hallmark of myeloid/non-monocytic lineage is the expression of MPO on aberrant blasts, particularly when the intensity of this expression exceeds 50% of what is observed in mature neutrophils, and/or when MPO expression is variable in a manner reminiscent of normal CD34-positive myeloid progenitors. The WHO considers dim MPO expression (i.e., not reaching the 50% intensity threshold of neutrophils) to be controversial, especially in cases where the immunophenotype of the aberrant blast population aligns with that of B-lymphoblastic leukemia/lymphoma (i.e., the blast population shows relatively homogenous expression of B-lineage markers).

Myeloid component of MPAL [Seminars in Diagnostic Pathology 42 (2025) 150893]: MPO, an enzyme localized into the primary granules of granulocytes, has been considered the most specific marker for myeloid lineage in prior classification systems. Cytoplasmic MPO can be detected by FCM, cytochemistry, or immunohistochemistry. Published studies have recommended various cut-offs for MPO expression. Currently, no specific threshold is set for MPO positivity.

Myeloid lineage of MPAL [WHO HEME5, IARC]: (no definitive criteria for dim expression of MPO)
The most specific feature of myeloid lineage is the expression of myeloperoxidase in the cytoplasm of the leukaemic blasts, particularly when its level exceeds 50% of that seen on mature neutrophils and/or it shows a variable pattern reminiscent of that seen on normal CD34-positive myeloid progenitors. The significance of dim expression of myeloperoxidase is controversial; it can be seen in otherwise typical B-lymphoblastic leukaemia/lymphoma, often relatively uniformly or on a discrete subset, so it is not likely to be lineage-specific in that context. However, the specificity of dim, variable myeloperoxidase is improved by the demonstration of a leukaemic subset with increased light-scatter properties or coordinated expression of other myeloid-associated antigens (e.g. CD117 or bright CD13/CD33) that differ from the remaining leukaemic population, which has more lymphoid characteristics. In cases where myeloperoxidase is dim or negative, demonstration of monocytic differentiation by the abnormal expression of more than one monocyte-associated antigen, including CD11c, CD14, CD36, CD64, or lysozyme, or diffuse positivity for nonspecific esterase by cytochemistry, is also considered sufficient. Various groups have proposed flow cytometry thresholds for

myeloperoxidase expression to be used for lineage assignment in acute leukaemia; these range from 3% to 28%. The 3% myeloperoxidase cut-off point, historically used for cytochemistry, was determined to have high sensitivity but poor specificity for general lineage assignment in acute leukaemia by flow cytometry. A threshold of > 10% for myeloperoxidase positivity seems to improve specificity, but no consensus cut-off point has been established.

The 1st method of setting the 50% intensity level for MPO by flow cytometry: using neutrophils (positive control)
[Seminars in Diagnostic Pathology 42 (2025) 150893]:

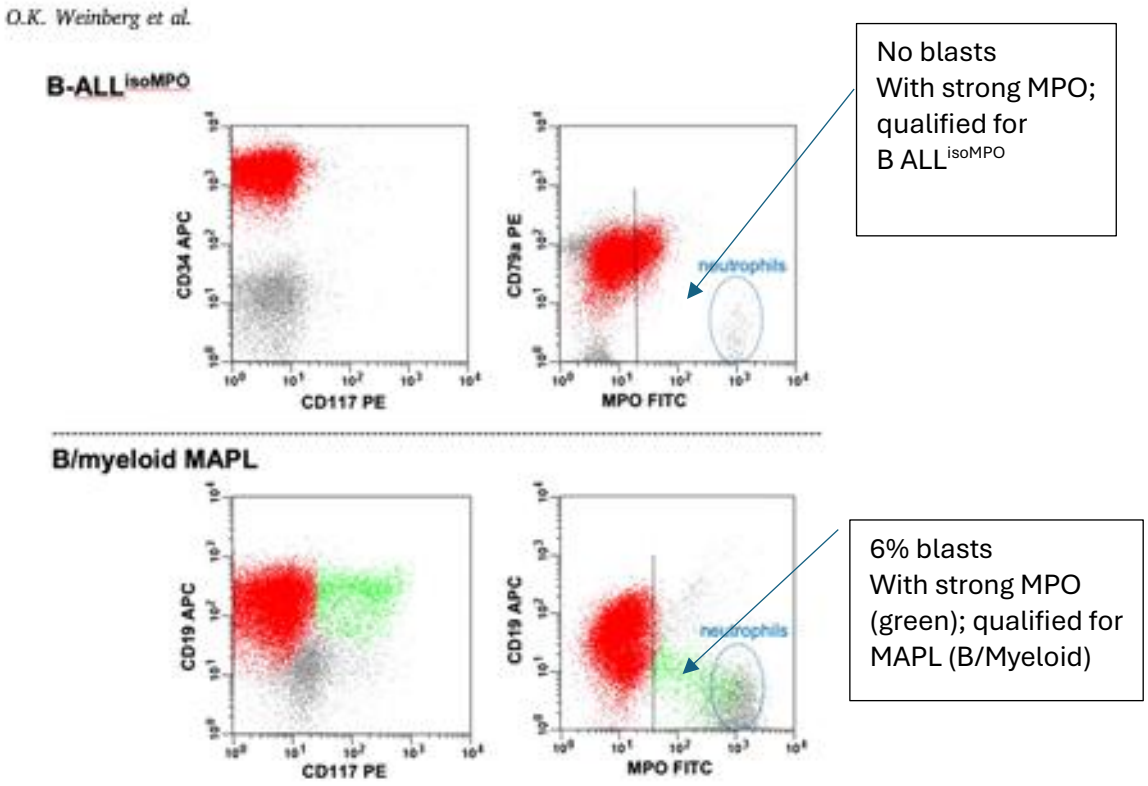
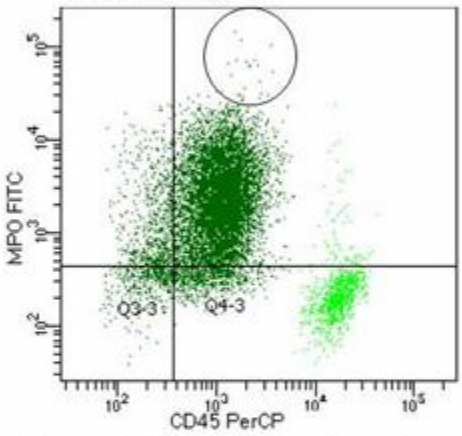


Fig. 1. Different (dim or strong) MPO expression patterns in B-ALL^{isoMPO} and B/myeloid MPAL. In a case of B-ALL^{isoMPO}, there is a homogenous, single B-lineage blast population (CD79a+/CD34+/CD117-); a subset of blasts expresses dim MPO (~20 %, with the brightest MPO at a level below the 50 % of background mature neutrophils). In a case of B/myeloid MPAL, there is a heterogenous antigen expression of CD117 and MPO, only present on a subset of blasts (~6 %, in green) but with strong MPO (the intensity at a level above 50 % of background mature neutrophils, reaching the intensity of mature neutrophils).

BLOOD, NOVEMBER 2021, VOLUME 138, Supplement1: 4475-4476

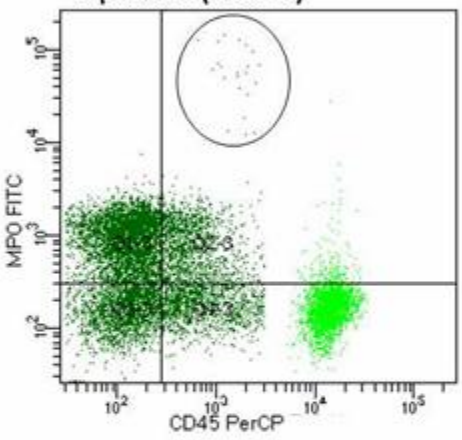
Figure 1. Representative flow cytometry data demonstrating use of study criteria for classification

A) Mixed phenotype acute leukemia (MPAL)



Maturational cytoplasmic myeloperoxidase (MPO) expression on the leukemic population with a maximal intensity >50% (1.5 logs) and approaching the maximal intensity of internal mature neutrophils (2.0 logs)

B) MPAL with isolated MPO expression (isoMPO)



Low-level MPO expression, in this case on a dichotomous subset of the leukemic population, with a maximal antigen intensity <50% (0.5 logs) of the maximal intensity of internal mature neutrophils (2.0 logs)

Populations: dark green = leukemia, circled: mature neutrophils, light green = mature lymphocytes
Logs: estimated from archival pdf dot plots to the nearest 0.5

<u>The 2nd method of setting the 50% intensity level for MPO by flow cytometry</u>: dynamic range
using B lymphocytes (negative control) and neutrophils (positive control)
[Cancers 2025, 17, 871]

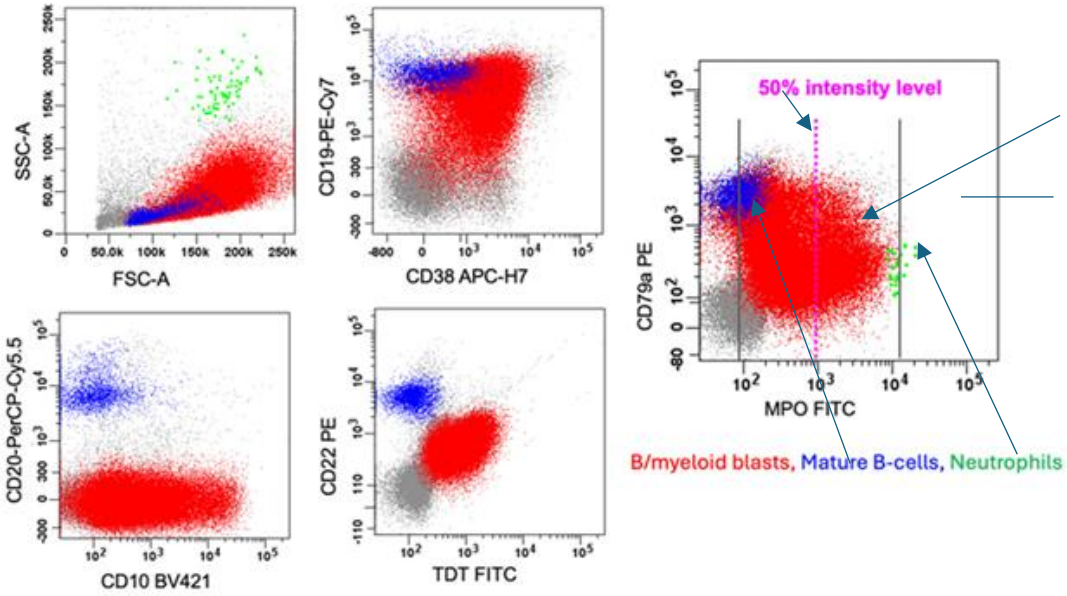


Figure 3. MPAL with *P2RY8::CRLF2* fusion, B/myeloid (biphenotypic, pattern 1a). This figure illustrates an example of a biphenotypic leukemia (reported recently in [8]) with a single blast population showing differentiation along more than one lineage (biphenotypic, pattern 1a). Blue represents normal, mature B-lymphocytes. Red represents the biphenotypic blast population. This biphenotypic population meets both the WHO-HEM5 and ICC criteria for B-lineage assignment with strong expression of CD19, along with strong expression of CD10, CD22, and cCD79a, and for myeloid lineage assignment with strong expression of MPO (intensity in a part of blasts exceeding 50% of the mature neutrophil level). The enlarged plot shows the midpoint (i.e., 50% intensity level in a log-axis) of the dynamic range for MPO expression established using the midpoint of the neutrophils (green) as the positive control and the mature B-lymphocytes as the negative control.

Clinical Significance of Isolated Myeloperoxidase Expression in Pediatric B-Lymphoblastic Leukemia

[Am J Clin Pathol 2017;147:374-381]

Three groups of patients with acute leukemia: patients with B-ALL who express MPO by flow cytometry in the absence of other myeloid lineage markers (B-ALL-isoMPO), patients with B ALL, and patients with MPAL(B/myeloid).

Compared with B-ALL with no myeloid antigen expression, patients with B-ALL with isolated MPO expression experience a significantly increased risk of relapse despite similar therapy.

Greater than 3% MPO-positive cells by enzyme cytochemistry is the minimum threshold to establish myeloid lineage. Such a threshold has not been formally established for MPO positivity by flow cytometry. Reliance on cytochemical and/or immunohistochemistry MPO evaluation may give a false negative due to sensitivity issues

Our study provides the first published data on outcomes in pediatric patients with isolated MPO expression in otherwise typical B-ALL and suggests that these cases represent a high-risk leukemia, which are best classified as MPAL. If MPO is not measured in every case of leukemia by flow cy tometry, patients may be at risk of undertreatment if diag nosed as having B-ALL.

Flow Cytometric Patterns of MPO Expression in the B-ALL-isoMPO Cases: Three general patterns are noted:

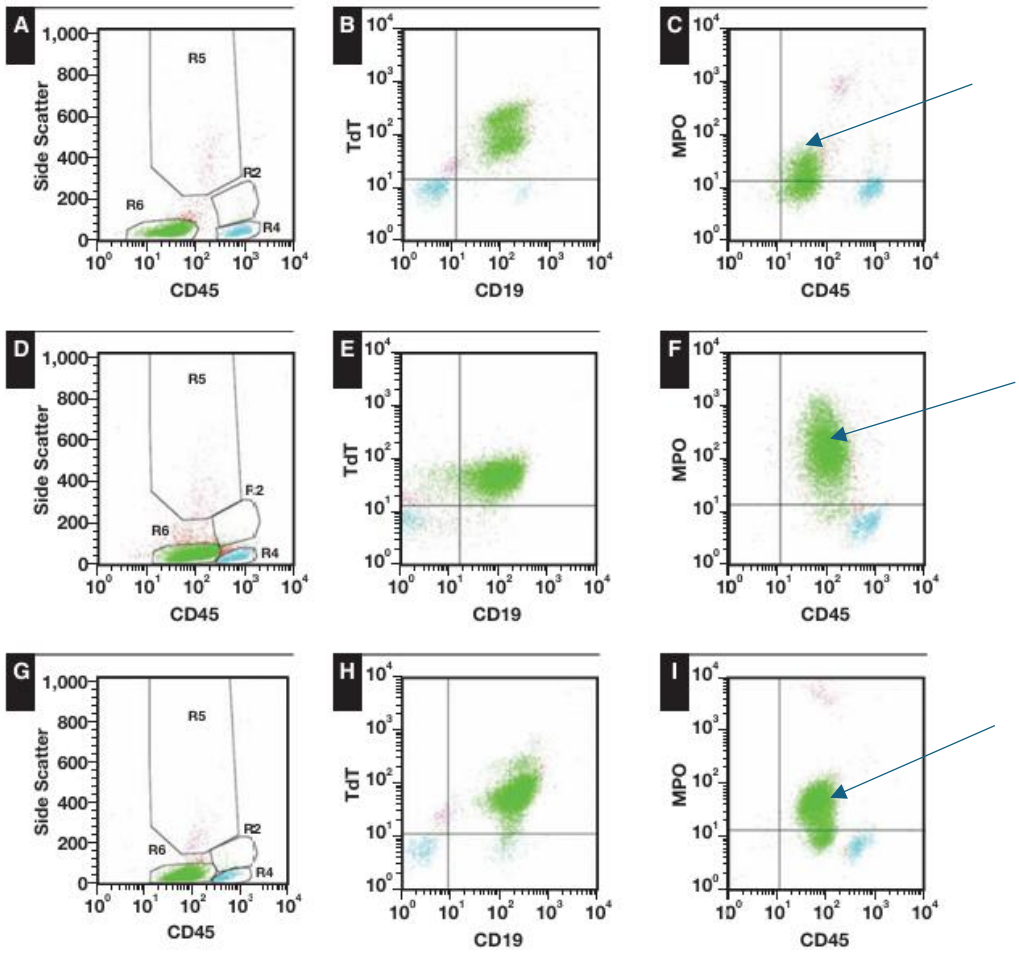


Figure 2 Three patterns of myeloperoxidase expression are identified: (**A-C**) tailed partial expression pattern, (**D-F**) uniformly positive expression pattern, and (**G-I**) discrete partial expression pattern. Leukemic blasts in all cases were gated based on CD45 and side-scatter characteristics (green). Lymphocytes were gated based on high CD45 expression and low side-scatter characteristics (aqua). Granulocyte and monocyte populations were gated based on intermediate CD45 expression and high side-scatter characteristics (purple). All live-cell events are shown in all of the dot plots after gating out debris based on forward scatter characteristics. TdT, terminal deoxynucleotidyl transferase.

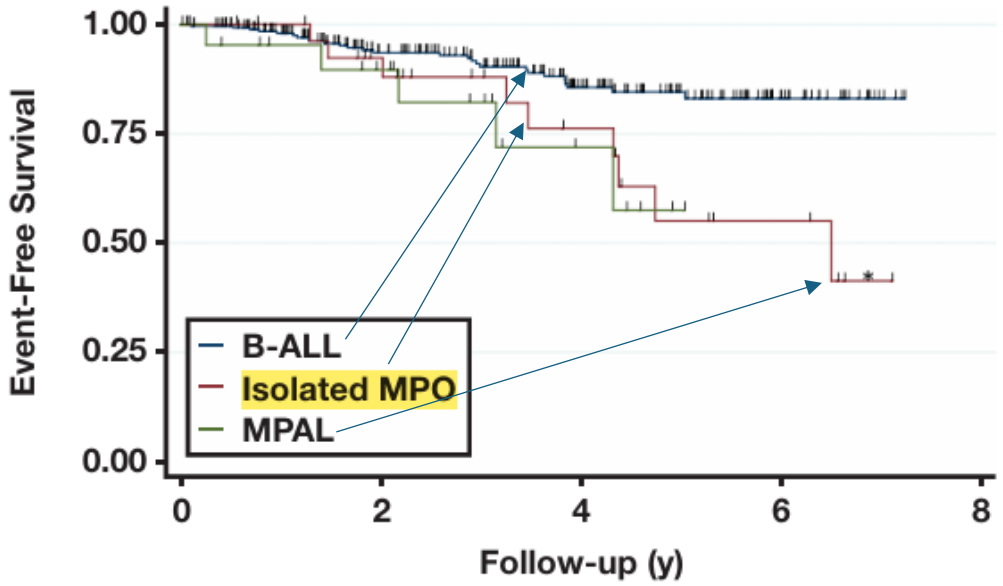


Figure 4 Kaplan-Meier plot of the event-free survival (EFS). Univariate analysis of EFS shows a significantly worse outcome in patients with isolated myeloperoxidase expression in otherwise immunophenotypically standard B-cell acute lymphoblastic leukemia. *Log-rank $P = .0066$. MPAL, mixed-phenotype acute leukemia.

Two other studies looking at Pediatric patients with B ALL and MPO Expression:

1.[Cytometry. 2021;100:446–453]: this study categorized cases as MPO positive (MPO+) or negative (MPO−) using a threshold of ≥20% blasts expressing MPO at intensity greater than the upper limit of normal lymphocytes on diagnostic bone marrow flow cytometry. The study also includes all cases with additional myeloid antigen expression. The conclusion from this publication cannot be used here for comparison due to different study design (the study includes cases that are not considered isolated MPO expression).

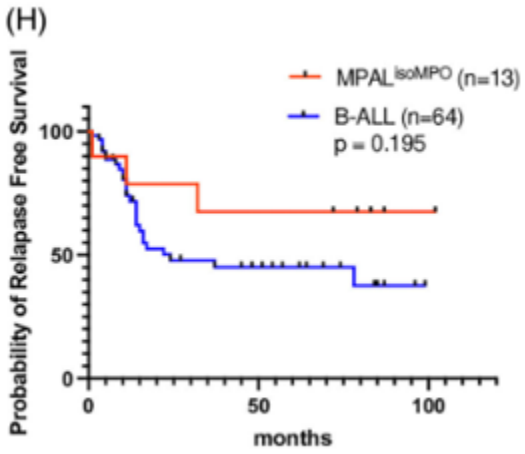
2.[Blood, 1 FEBRUARY 2018 | VOLUME 131, NUMBER 5, pp 573-577]:
This study includes patients with MPAL B/myeloid isoMPO that also express myeloid-associated markers, such as CD13 (52%), CD33 (10%), and CD15 (14%). The conclusion from this publication cannot be used here for comparison due to different study design (the study includes cases that are not considered isolated MPO expression).

Clinical Significance of Isolated Myeloperoxidase Expression in Adult B-Lymphoblastic Leukemia

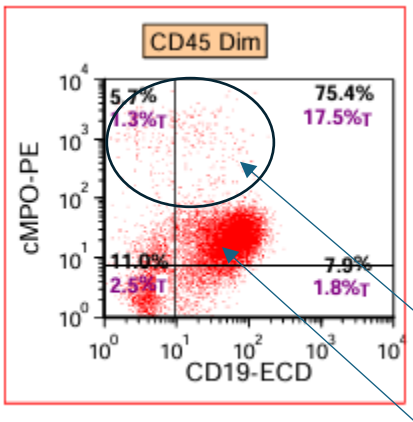
[IJLH Volume45, Issue2, April 2023, Pages 170-178]

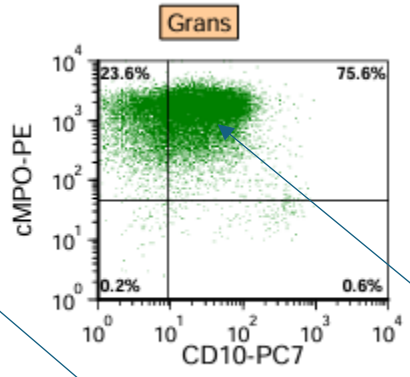
Cases of B/myeloid MPAL^{isoMPO} showed a fairly homogenous single B-lineage blast population with dim MPO expression whereas cases of other MPAL subtypes displayed heterogeneous antigen expression and moderate/strong MPO expression. The percent of MPO positive blasts in these two groups was similar.

Expressions of CD15, CD117, and monocytic markers were more common in other MPAL than in B/myeloid MPAL^{isoMPO}. B/myeloid MPAL^{isoMPO} patients had similar overall and leukemia free survivals as B-ALL patients and better than other MPAL patients.



Recent 12 y/o patient at MHH-TMC (patient T, Flow study HF-25-xxx)





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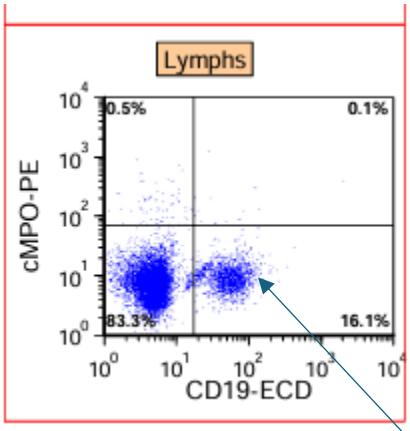
MPO Pos control for granulocytes including neutrophils

Subset of MPO-strong positive blasts using 50% cut-off by 2 methods, no established threshold criterion for % of this subset

MPO-dim blasts (MPAL by ICC and "B-ALL with aberrant dim expression of MPO" by WHO)



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MPO Neg control with B lymphocytes

SUMMARY:

Myeloperoxidase (MPO) is considered a specific marker of myeloid/non-monocytic lineage in the diagnosis of mixed phenotype acute leukemia (MPAL). Isolated dim MPO expression in otherwise typical B lymphoblastic leukemia (without myeloid markers) is referred to as:
(1)B/myeloid MPAL$^{iso\ MPO}$ in some literature sources [IJLH Volume45, Issue2, April 2023, Pages 170-178].
In adult patients, B/myeloid MPAL$^{iso\ MPO}$ patients had similar overall and leukemia free survivals as B-ALL patients and better than other MPAL patients.
(2) B-ALL-isoMPO in some literature sources [Am J Clin Pathol 2017;147:374-381].
Pediatric patients with isolated MPO expression in otherwise typical B-ALL represent a high-risk leukemia, which are best classified as MPAL. If MPO is not measured in every case of leukemia by flow cytometry, patients may be at risk of undertreatment if diagnosed as having B-ALL.

HIGHLIGHTS:

1.Highlights inconsistent reporting nomenclature (for cases with isolated dim MPO expression in otherwise typical B lymphoblastic leukemia) and the need of pathology report to clearly show the details of marker profiles and the associated clinical ramification, mostly correlated with patient's age group, optimally with literature references for critical patient management

2.Recommended process for signing out flow cytometry cases with isolated dim MPO expression in otherwise typical B lymphoblastic leukemia (without myeloid markers). Note that all blasts must be below the 50% threshold of MPO for PMNs, negative control by B lymphocytes can be used for dynamic range if vailable:

Diagnosis: B cell ALL with isolated dim MPO expression in otherwise typical B lymphoblastic leukemia

-Comment for adult patients: these patients have similar overall and leukemia free survivals as B-ALL patients and better than other MPAL patients (see Reference article). Clinical correlation is suggested. Ref: International Journal of Laboratory Hematology, Volume 45, Issue 2, April 2023, pp 170-178

-Comment for pediatric patients: this may represent a high-risk leukemia; patients may be at risk of undertreatment with chemotherapy regiment designed for B-ALL (see Reference article). Clinical correlation is suggested. Ref: Am J Clin Pathol 2017;147: pp 374-381