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CASE REPORT

Bone marrow-restricted aberrant myeloperoxidase expression in B-acute lymphoblastic leukemia: A diagnostic dilemma and mimicry of mixed phenotype acute leukemia

Wei J. Wang[1] | Brandon T. Gehris[1] | Daniel Rivera[1] | Sibel Ak[1] | David Feng[2] | Wei Wang[3] | Zhihong Hu[4]

[1]Department of Pathology and Laboratory Medicine, The University of Texas Health Science Center at Houston, Houston, Texas, USA

[2]Foster High School, Richmond, Texas, USA

[3]Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA

[4]Department of Laboratory Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA

Correspondence
Zhihong Hu, Department of Laboratory Medicine, The University of Texas MD Anderson Cancer Center at Houston, 1515 Holcombe Blvd, Houston, TX 77030, USA.
Email: Zhu6@mdanderson.org

Abstract

Myeloperoxidase (MPO) is the most specific marker of the myeloid lineage, essential for diagnosing acute myeloid leukemia and mixed phenotype acute leukemia with myeloid components. In this regard, we present a unique case of B-acute lymphoblastic leukemia (B-ALL) with isolated MPO expression in bone marrow blasts detected by flow cytometry and immunohistochemistry, while peripheral blood blasts were negative for MPO expression. In this report, our discussion encompasses diagnostic pitfalls from a laboratory testing perspective in similar cases and includes a literature review. Furthermore, we emphasize the necessity of conducting a comprehensive analysis for the accurate diagnosis of MPO-positive B-ALL cases.

KEYWORDS
acute myeloid leukemia, B-lymphoblastic leukemia, bone marrow, flow cytometry, myeloperoxidase, peripheral blood

1 | INTRODUCTION

Myeloperoxidase (MPO) is the most specific marker to define myeloid lineage and is commonly used in the diagnosis of acute myeloid leukemia (AML) and mixed phenotype acute leukemia (MPAL) with myeloid components [1, 2]. However, isolated MPO expression has also been reported in rare cases of B-acute lymphoblastic leukemia (B-ALL) [1]. The expression of MPO in B-ALL can cause diagnostic challenges. It is crucial to differentiate B-ALL with MPO expression from MPAL (B/myeloid) to adopt appropriate therapeutic strategies and ensure effective patient follow-up.

For MPO, there is a lack of consensus regarding its detection and interpretation using flow cytometry (FCM), leading to inconsistent and contradictory conclusions. According to the 4th edition of the World Health Organization (WHO) guidelines, weak MPO expression detected by FCM in otherwise typical B-ALL cases should not modify the diagnosis of MPAL unless other myeloid markers are present [1]. The 5th edition of the WHO guidelines and the International Clinical Cytometry Society (ICCS) recommend using MPO intensity in part exceeding 50% of mature neutrophil levels as one of the diagnostic criteria for MPAL in the myeloid lineage [2, 3]. We presented a case of B-ALL characterized by isolated MPO expression in blasts from bone marrow (BM) but its absence in blasts from peripheral blood (PB) and provided a comprehensive discussion on diagnostic pitfalls associated with such cases from a laboratory testing perspective.

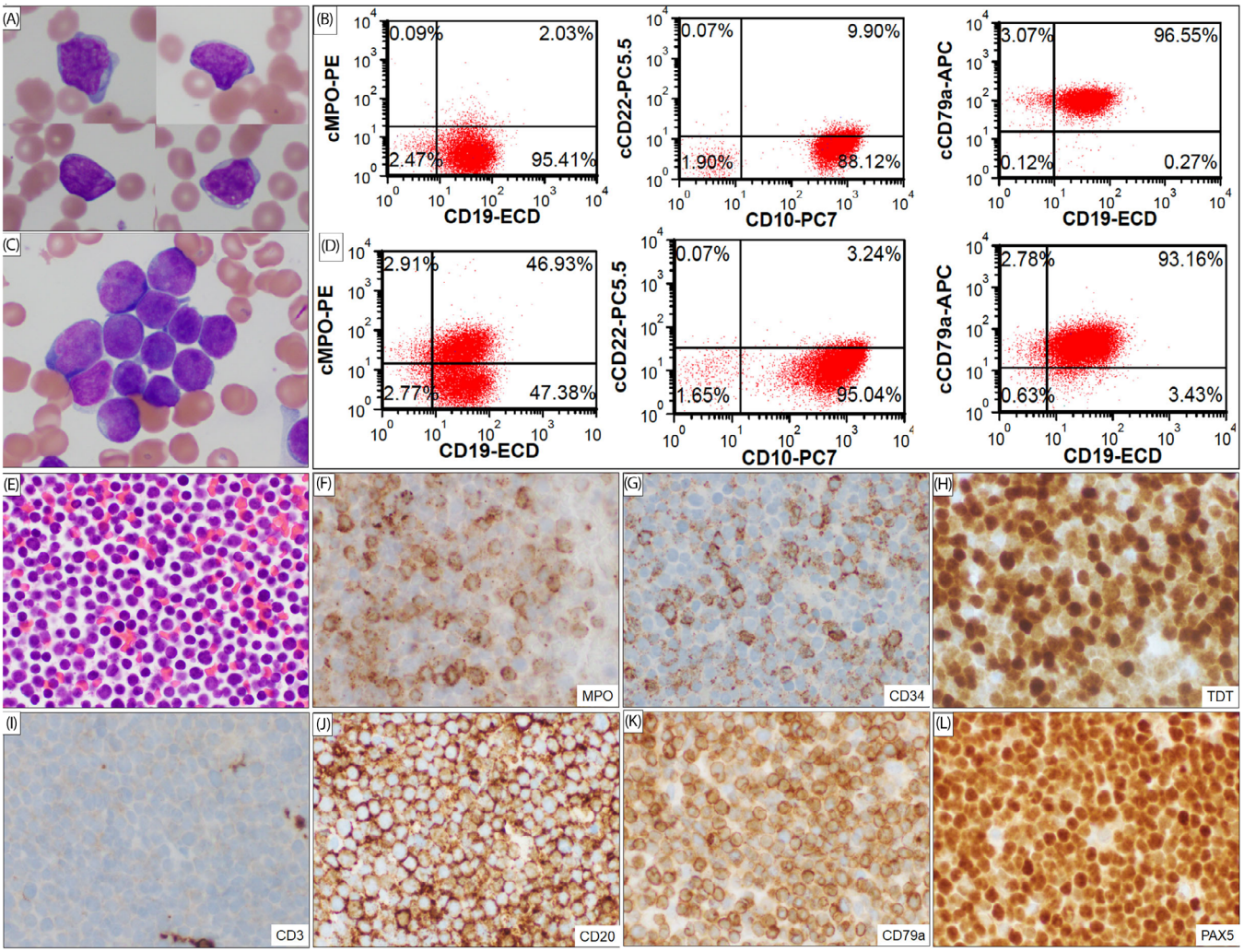

FIGURE 1 Differential expression of myeloperoxidase (MPO) in the blasts of B-cell acute lymphoblastic leukemia (B-ALL) in the peripheral blood (PB) and bone marrow (BM). (A) Wright–Giemsa stain of the PB smear (1,000×) showed blasts with intermediate to large sizes, irregular nuclear contours, fine chromatin, distinct nuclei, scant agranular cytoplasm, and occasional cytoplasmic vacuoles; no Auer rods were seen. (B) Flow cytometry immunophenotyping of the PB specimen, blasts positive for CD10, CD19, and CD79 and negative for cCD22 and cMPO. (C) Wright–Giemsa stain of the BM aspirate (1,000×) showed blasts with similar morphologic characteristics. (D) Flow cytometry immunophenotyping of the BM aspirate specimen: blasts positive for CD10, CD19, CD79, and cMPO, and negative for cCD22. Hematoxylin and eosin (H&E) staining of the BM clot showed predominant blasts within the marrow particles (500×, E). Immunohistochemical (IHC) staining (500×) showed that these blasts were positive for MPO (F), CD34 (G), terminal deoxynucleotidyl transferase (TdT) (H), CD20 (J), CD79a (K), and PAX5 (L) and negative for CD3 (I).

2 | CASE PRESENTATION

A 33-year-old man with no significant medical history presented with fever, lethargy, and dizziness. Initial blood work revealed pancytopenia, including leukopenia (white blood cells: 1.4×10^9/L; reference range 3.7–10.4 $\times$ 10^9/L) with severe neutropenia (neutrophils: 0.1×10^9/L; reference range 2.0–7.1 $\times$ 10^9/L), anemia (hemoglobin: 5.1 g/dL; reference range 14–18 g/dL), and thrombocytopenia (4.0×10^9/L; reference range 133–450 $\times$ 10^9/L). A PB smear showed 46% of blasts that were intermediate to large-sized with irregular nuclear contours, fine chromatin, distinct nucleoli, scant agranular cytoplasm, and occasional cytoplasmic vacuoles. No Auer rods were noted (Figure 1A). FCM analysis of the PB specimens revealed an aberrant immature B-cell population, accounting for 95% of total cells analyzed, with the following immunophenotype: positive for CD7 (partial, dim), CD10, CD19, CD20, CD34 (partial), CD38 (dim), CD45 (dim), and cytoplasmic CD79a (cCD79a) and negative for CD2, CD3, CD4, CD5, CD8, CD11b, CD11c, CD13, CD14, CD15, CD16, CD33, CD64, CD117, Kappa, Lambda, cCD3, cCD22, and cMPO (Figure 1B).

The BM aspirate smears revealed blasts comprising 95% of total events, exhibiting morphological features similar to those observed in the PB (Figure 1C). FCM analysis of the blasts in the BM aspirate exhibited an immunophenotype resembling that observed in the PB, with the exception of MPO positivity in approximately 50% of the blasts

(Figure 1D). Mature neutrophils constituted less than 0.5% of total events in the BM, precluding a comparison of MPO intensity between blasts and mature neutrophils. To further confirm MPO expression in blasts, MPO immunohistochemistry (IHC) was conducted on a BM core biopsy specimen, revealing MPO positivity in approximately 50% of the blasts. Additional immunohistochemical staining of the BM clot demonstrated positive expression for CD20, CD34, CD79a, PAX5, and terminal deoxynucleotidyl transferase in blasts (Figure 1E–I). The final diagnosis was B-ALL with MPO expression in the BM.

Cytogenetic analysis of the BM aspirate specimen yielded no metaphase cells available for analysis. Fluorescent in situ hybridization (FISH) analysis performed on the PB specimen using a specific panel of FISH probes (Supplementary Materials) was negative. Furthermore, the Neotype ALL profile showed no genetic alterations (Supplementary Materials).

Treatment wise, the patient underwent induction chemotherapy using hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (hyper-CVAD). After completing one cycle, the patient achieved complete remission with no residual leukemia cells detected by FCM. However, the patient was subsequently lost to follow-up.

3 | DISCUSSION

Cases of B-ALL with MPO expression are rare [1, 4–10]. When making such a diagnosis of B-ALL with aberrant MPO expression, it is crucial to be aware of potential pitfalls associated with FCM measuring MPO expression and take necessary precautions to avoid false positivity. According to the 5th edition of the WHO guidelines and the ICCS, the intensity of MPO, as detected by FCM, in part should exceed 50% of mature neutrophil levels, constituting one of the diagnostic criteria for MPAL in the myeloid lineage [2, 3]. In FCM, Fc receptors on the cell surface can contribute to false positivity. Particularly large cells with more Fc receptors that can nonspecifically bind to heavy chains of fluorescent monoclonal antibodies (mAbs) generate nonspecific positive signals. In our current case, the detection of cytoplasmic MPO after membrane permeabilization helped exclude the nonspecific binding caused by Fc receptors. Furthermore, cells with abundant cytoplasmic granules may exhibit autofluorescence, potentially leading to false positivity for certain markers. However, in our patient's case, blasts lacked granules, eliminating autofluorescence as a confounding factor. Lastly, factors such as suboptimal compensation and insufficient washing may induce false positivity. These issues can be addressed by comparing the MPO expression on residual normal neutrophils and lymphocytes in the same specimen, serving as internal positive and negative controls, respectively.

When interpreting and comparing results across different laboratories, the choice of fluorescent mAb is a critical factor in FCM testing. Table 1 illustrates the usage of at least four different types of fluorescent mAbs in previous studies. There are contradictory results regarding the 8E6 clone of fluorescent mAbs. In one study, the authors suggested that the 8E6 clone led to false positivity of MPO in B-ALL cases, since positive MPO could not be confirmed by other clones of MPO antibodies and IHC did not detect MPO expression in these cases [9]. Similarly, Oberley et al. reported 29 cases of typical B-ALL with MPO expression detected using the 8E6 clone [7]. However, MPO positivity was confirmed by IHC staining in only one (4.8%) of 21 cases. On the contrary, McGinnis et al. detected MPO expression in 32 typical B-ALL cases using the same 8E6 clone, and in four cases with MPO IHC available, all showed positive MPO by IHC [5]. Of note, different clones of MPO antibodies were used in IHC staining across these three studies, making it challenging to compare the MPO results directly. The discrepancies among FCM, IHC, and cytochemistry have been documented in AML. Studies suggest that FCM, using a cutoff of 10%, is less sensitive than cytochemistry and IHC for MPO detection [11]. Therefore, further research is necessary to determine the optimal mAb clone for MPO detection via FCM.

Recognizing that weak MPO expression in otherwise typical B-ALL cases should not be exclusively relied upon as a lineage marker for classification as MPAL due to the potential for false positivity caused by nonspecific binding has been acknowledged [1]. However, a related question emerges: Does strong MPO expression in B-ALL cases suffice to define myeloid lineage? It has been reported that isolated expression of MPO without detectable expression of other myeloid or monocytic markers in a typical case of B-ALL results in similar clinical behaviors to B-ALL [12]. Therefore, isolated MPO expression in B-lymphoblasts may be insufficient to categorize as MPAL, B/myeloid. Further complicating the matter, previous studies seem to provide support for the presence of MPO expression through the detection of MPO mRNA in certain B-ALL cases [13]. However, it is crucial to note that none of these studies employed cell sorting to purify lymphoblastic leukemia cells before RNA extraction. The high sensitivity of reverse transcription-polymerase chain reaction makes it susceptible to false positivity, even with a small number of myeloid cells present.

In our current case, a notable difference was observed in the expression of MPO between the PB and BM specimens, highlighting immunophenotypic discrepancies between these anatomical sites. The mechanisms of this discrepancy are uncertain but may be attributed to multiple factors. First, the BM microenvironment provides a favorable environment for the growth and survival of blasts [14]. While the PB serves as a conduit for circulating cells throughout the body, and certain cell populations may be restricted or diminished in the circulation. Second, the homing and trafficking of blasts can be influenced by adhesion molecules and chemokine receptors [15]. Differential expression of these molecules between the BM and PB can result in distinct migration patterns and cellular distribution. It is plausible that blasts expressing MPO in our current case has limited capacity to migrate into the peripheral circulation. Moreover, the localization of blasts in the BM and limited dissemination into the PB may be influenced by specific signaling pathways or genetic alterations within the blasts themselves. Further investigations are warranted to comprehensively understand the underlying mechanisms governing cell trafficking and localization in B-ALL.

TABLE 1 Fluorescence-conjugated monoclonal antibodies in flow cytometry (FC) for the identification of myeloperoxidase (MPO)-positive B-cell acute lymphoblastic leukemia cases in the literature.

Reference #	Case number	Fluorescence	Company	Clone	Specimen	FC result of MPO	FC result of other myeloid markers	Other test results
[4]	1	PE	BD Biosciences	5B8	BM	Positive	None	Cytochemistry positive
[5]	32	eFV450	eBioscience	8E6	BM	Positive	CD13 (18/32 cases) CD33 (20/32 cases) CD117 (1/32 cases)	IHC positive (4/4 cases) (clone number is NA)
[6]	1	PE	BD Biosciences	5B8	BM	Positive	None	NA
[7]	29	NA	Invitrogen	8E6	BM	Positive	None	IHC positive (1/29 case) (clone 59A5, Leica Biosystem) Cytochemistry positive (1/29 case)
[9]	3	FITC FITC FITC PE	Invitrogen Dako Beckman-Coulter BD Biosciences	8E6 MPO-7 CLIB-MPO-1 5B8	BM BM BM BM	Positive Negative Negative Negative	CD33 (2/3 cases)	IHC negative (3/3 cases) (clone A0398, Dako)
Current case	1	PE	Beckman-Coulter Beckman-Coulter	CLIB-MPO-1 CLIB-MPO-1	PB BM	Negative Positive	None None	NA IHC positive (clone A0398, Dako)

Abbreviations: BM, bone marrow; FITC, fluorescein isothiocyanate; IHC, immunohistochemistry; MPO, myeloperoxidase; NA, not available; PB, peripheral blood.

4 | CONCLUSION

We present a unique case of blasts expressing cytoplasmic MPO in the BM while lacking MPO expression in the PB. Given the absence of expression of other myeloid and monocytic markers, our final diagnosis is B-ALL with aberrant MPO expression in the BM. The discordant MPO expression between the PB and the BM underscores the critical importance of conducting a comprehensive analysis to ensure an accurate diagnosis when dealing with acute leukemia. Specifically, this case highlights the necessity to distinguish MPO-positive B-ALL cases from MPAL, B/myeloid.

AUTHOR CONTRIBUTIONS

Wei J. Wang conceived the case report and wrote the manuscript. Brandon T. Gehris revised the manuscript. Daniel Rivera wrote parts of the manuscript. Sibel Ak assisted with the data interpretation. David Feng edited the figures. Wei Wang assisted with the data interpretation and revised the manuscript. Zhihong Hu conceived the case report, made the data interpretation, provided the figures, and revised and approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

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DATA AVAILABILITY STATEMENT

The authors of the article agree to share the research data.

ETHICS STATEMENT

The submission of this paper was approved by the Institutional Ethics Committee.

PATIENT CONSENT STATEMENT

The authors have confirmed patient consent statement is not needed for this submission.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

No material from external sources has been used in this paper.

CLINICAL TRIAL REGISTRATION

Not applicable as it is the report of only one case.

ORCID

Wei J. Wang https://orcid.org/0000-0002-1957-3226
Wei Wang https://orcid.org/0000-0001-6821-4556
Zhihong Hu https://orcid.org/0000-0001-8809-6729

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