Chapter

2

CHALLENGING DIAGNOSIS IN A PATIENT WITH CLEAR LYMPHOID IMMUNOHISTOCHEMICAL FEATURES AND MYELOID MORPHOLOGY: MIXED PHENOTYPE ACUTE LEUKEMIA WITH ERYTHROPHAGOCYTOSIS

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INTRODUCTION
Acute leukemia (AL) is classified according to commitment towards either the myeloid or lymphoid cell lineage. In about 4% of acute leukemias it is unclear whether the blasts are of myeloid or lymphoid origin. These AL with both myeloid and lymphoid characteristics represent a worse prognostic subgroup and it is still a matter of debate whether patients may benefit from an acute lymphoid leukemia (ALL) or acute myeloid leukemia (AML) treatment. In the WHO2008 criteria for defining mixed phenotype acute leukemia (MPAL), important changes have been made in defining cell line specific and characterizing markers in AL as compared to European Group for the Immunological Characterization of Leukemias (EGIL) criteria incorporated in the WHO2001. In the WHO2001 criteria a weighted points system is used for defining a Biphenotypic Acute Leukemia (BAL) to be Myeloid, B- or T-cell. In this scoring system at least one additional lineage defining marker for both lineages was necessary to diagnose a BAL. In the WHO2008 an AL expressing both myeloperoxidase (MPO) and CD19, can be considered as a MPAL (B/Myeloid) depending on expression of either cCD79a, CD10 or cCD22. Furthermore, the WHO2008 excludes AL with well-defined cytogenetic or clinical presentations from MPAL. For example, AL with t(8;21); t(15;17) or inv(16) are classified as AML, independent of their immunophenotypic marker expression. Here we describe a case in which cytochemistry showed Sudan Black (SB) negative, vacuole rich, leukemic blasts with a monocytoid appearance and erythrophagocytosis. Remarkably, flow cytometric analysis and immunohistochemistry showed clear expression of B cell markers and weak cytoplasmic (cyt)MPO. This unique case demonstrates the challenges in diagnosing an AL with ambiguous morphologic, immunohistochemical and flow cytometric characteristics.

CASE
A 65-year-old woman was admitted to the hospital with general discomfort and a fever of 2-week duration. She experienced a weight loss of 9 kg in the preceding month and complained of night sweats. Her medical history revealed diabetes mellitus type II, atrial fibrillation, a psychosis and a former drug addiction. Physical examination did not show relevant findings. Laboratory investigations revealed an elevated lactate dehydrogenase (948 U/l; normal values 0–250 U/l), leucopenia (2.0 × 10^9/l; normal values 4.0–10 × 10^9/l), thrombocytopenia (18 × 10^9/l; normal values 150-400 × 10^9/l) and a decreased hemoglobin level (4.8 mmol/l or 7.7 g/dl; normal values 7.5–10.0 mmol/l or 12.1–16.1 g/dl).

Morphological peripheral blood (PB) examination revealed anisocytosis, polychromasia, dyserythropoiesis, dysgranulopoiesis and the presence of monocytoid leukemic blasts (60%) with pronounced vacuolization (Fig. 1A). The leukemic blasts stained negative for SB (Fig. 1B). The bone marrow (BM) smear showed 64% blasts and a normal erythroid- and megakaryocytic lineage. Remarkably, erythrophagocytosis was observed in 4% of the leukemic blasts (Fig. 1C).
Figure 1. Morphology of leukemic cells at diagnosis (1000x). Bone marrow May-Grünwald Giemsa (MGG) staining of bone marrow smears at diagnosis: example of clear vacuole-rich blast cells (A), Sudan Black staining (B), arrow indicates erythrophagocytosis by blasts cells (C).

By flow cytometry two blast populations could be distinguished based on sideward scatter (SSC), CD45 and CD34 expression (Fig. 2). The largest population (97% of the total amount of blasts) was CD45 dim/neg, had a higher CD34 expression and an intermediate SSC. This population was positive for CD19, CD20, CD22, cytCD79a and cytTdT. Furthermore, these cells stained weakly for MPO and showed an over expression of CD90. The monocytic markers CD14 and CD36 were negative (data not shown). The smallest population (3% of the total amount of blasts) was CD45 positive, had a slightly lower CD34 expression and a low SSC. These blasts showed positivity for the following markers: CD13, CD117, CD33 and negativity for cytMPO, CD19, CD10, CD20, CD22, cytCD79a and cytTdT. This population most likely resembled the normal myeloid blast compartment.

Immunohistochemical analysis of the trephine biopsy revealed a hypercellular marrow with approximately 50% blast cells. The blasts were variable in size and showed irregular nuclear morphology with fine chromatin, small nucleoli and occasionally multi-lobulation.
The cells stained positive for CD34, cytTdT, CD20 and cytCD79a, and also showed a weak staining for MPO.

A normal female karyotype (46,XX) in 20/20 cells was present while no molecular aberrancies could be detected (PCR or FISH for t(15;17), t(8;21), inv(16), t(9;22), t(10;17), MLL abnormalities, and FLT3-ITD were all negative).

To summarize, this patient presented with an AL with monocytoid morphology, erythrophagocytosis and a clear B-lymphocytic marker expression combined with positivity for cytMPO in both flow cytometry and immunohistochemistry. Based on the combined marker expression of cytCD79a, CD19, CD20 and cytMPO, this patient was diagnosed as a MPAL (B/Myeloid) according to the WHO2008 criteria. She was treated with AML like chemotherapy combined with intrathecal methotrexate prophylaxis resulting in complete

Figure 2. Immunophenotype of leukemic cells. Immunophenotype of leukemic cells analyzed with Infinicyt software (Cytognos, Salamanca, Spain). Plot A and B represent gating strategy for the two different blast populations. Plots C–I represent marker expression on the two populations.
remission (CR). Unfortunately she relapsed after 11 months of follow up. Because she refused further therapy only maximal supportive care was provided.

**DISCUSSION**

Overall, this case has a paradoxical appearance: morphology revealed a clear monocytoid picture with prominent vacuolization and erythrophagocytosis, both mostly seen in myeloid leukemia. In contrast, immunohistochemistry and immunophenotypical analysis showed clear expression of B cell lineage defining and specific markers combined with MPO. Unlike other myeloid markers such as CD13 and CD33, MPO expression is very rarely found in AL with a clear B-ALL phenotype. Although immunophenotypical and immunohistochemical MPO expression was found to be of low intensity, it was clearly positive and considered specific. Strikingly, cytological examination did not stain positive for SB in this case. Following the WHO2001 criteria, this AL would have been classified as an ALL. However, based on additional expression of solely MPO, this case was classified as MPAL according to the WHO2008 guidelines.

The most paradoxal and unique feature in this case is the erythrophagocytosis. This rare finding is reported in less than 1% of AL cases and is most commonly associated with acute myeloid leukemia, especially those from a monoblastic or monocytic origin. Erythrophagocytosis has also been associated with other subtypes such as acute undifferentiated AL, AML without maturation, AML with maturation and acute megakaryoblastic leukemia. Erythrophagocytosis as seen in AL is often associated with cytogenetic abnormalities on chromosome 8(p11) involving the C-MOZ gene. Most frequently, the t(8;16)(p11;p13) translocation is found that gives rise to the CMOZ/CBP chimeric transcript. Recently, AML with t(8;16)(p11;p13) has been described as a distinct entity with unique features like erythrophagocytosis, positive MPO with strong positive non-specific esterase staining and poor prognosis. Furthermore, other translocations have been described such as t(16;21)(p11;q22), t(10;17)(p13;p12), inv(8)(p11q13), as well as 20q- deletion. However, in the present case none of these cytogenetic aberrancies could be detected. In biphenotypic leukemia erythrophagocytosis is only described twice, both associated with a cytogenetic aberrancy; t(9;22) and inv(8)(p11q13). Considering WHO2001 guidelines no cases of lymphoid AL with erythrophagocytosis have previously been described; the case presented here shows clear lymphoid marker expression and erythrophagocytosis. Moreover, based on the WHO2008 criteria, this is the first MPAL to show erythrophagocytosis without cytogenetic aberrancies.

Although the introduction of the WHO2008 classification makes the diagnosis of a MPAL more clear and less frequent compared to the older EGIL criteria, diagnosing a MPAL remains difficult but important. Prospective trails are still needed to clearly understand its clinical impact. This case highlights the challenges in diagnosing an AL with ambiguous morphologic, immunohistochemical and flow cytometric characteristics.
REFERENCES


