

Acute myeloid leukemia with RAM immunophenotype: a pediatric case with unusual morphologic features

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Abstract

The RAM immunophenotype has been recently described as a subtype of acute myelogenous leukemia (AML) that is characterized clinically by extremely poor prognosis. We present a case of AML with RAM immunophenotype in a 5-year-old patient that resulted in poor outcome despite early hematopoietic cell transplant. We describe the unusual morphologic features that, along with the distinct immunophenotype, may provide initial diagnostic clues and further justify the classification of this AML variant as a rather distinct subtype.

Introduction

A newly described subtype of Acute Myelogenous Leukemia (AML), with a distinct immunophenotype (referred to as RAM immunophenotype), was reported recently by Eidenschink Brodersen *et al.*¹ It is characterized by high intensity CD56 expression, dim-to-negative expression of CD45 and CD38, and a lack of HLA-DR expression. Clinically, RAM cases showed a distinctly high induction failure rate and extremely poor outcome. In this report, we describe a case of RAM subtype, highlighting its unusual morphologic features and challenging clinical course, despite early recognition and treatment with hematopoietic cell transplant (HCT).

Case Report

Clinical presentation

The patient, a 5-year-old boy, presented to our hospital with a history of persistent fever, epistaxis, petechial rash, and pancytopenia. His laboratory workup revealed hemoglobin 6.7 g/dL, platelet count $4 \times 10^9/L$, WBC $9.7 \times 10^9/L$, uric acid 5.7 mg/dL, and LDH 14,398 U/L.

Pathologic findings

Peripheral blood smear

An initial peripheral blood smear showed a blast population characterized by large cells with increased nuclear to cytoplasmic ratios and a small amount of blue-gray cytoplasm. The nuclear borders were irregular. A prominent nucleolus was present. The chromatin was fine and open in appearance. The blast population represented 12% of the white blood cell differential count. The non-neoplastic white blood cells, as well as the red blood cells and platelets, showed no morphologic abnormality.

Bone marrow aspirate smears

The blast population comprised 70% of the white blood cell differential count. The blasts were morphologically similar to those seen in the peripheral blood, and, in addition, showed unusual cohesiveness and clumping with nuclear molding, mimicking non-hematopoietic small round cell tumors (Figure 1). Large binucleate cells were present. Cytoplasmic blebs were present at the periphery of some cells (Figure 2).

No cytoplasmic granules or Auer rods were identified. Occasional blasts showed cytoplasmic vacuoles. No morphologically recognizable megakaryocytes were seen. The myeloid and erythroid cells were decreased in number but showed normoblastic maturation. Flow cytometry identified a population of cells within the progenitor cell gate (CD45/SSC) with low side scatter and intermediate expression of CD45. These cells expressed CD56, CD22, CD33, CD41, CD117, and cytoplasmic CD61.

Bone marrow core biopsy

The core biopsy showed replacement of the marrow space by cohesive sheets of

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blasts. Islands of residual hematopoiesis and rare megakaryocytes were present (Figure 3). By immunohistochemistry, the blasts were immunoreactive for CD33, CD117, CD56 (strong), and CD43. They showed weak and partial positivity for CD45. They were negative for CD57, CD68, CD3, CD15, lysozyme, TCL-1, myeloperoxidase, CD61 (note discrepancy with Flow Cytometric finding), CD42b, and von Willebrand factor (vWF). EBV-encoded RNA in situ hybridization was negative. A cytokeratin immunostain showed weak, dot-like positivity. Immunohistochemistry for additional non-hematopoietic antigens was negative and included CD99, vimentin, myogenin, and neuron specific enolase (NSE). Chromosome analysis showed an abnormal karyotype of 46, XY, der(20)t(1;20)(q12;p12.2)[10]. The abnormal clone of cells demonstrated an unbalanced translocation between chromosomes 1 and 20 that resulted in a gain of 1q and a loss of 20p. Fluorescent in situ hybridization analysis with probes localizing to 5q33-q34, 5p14.2, 7q31, 7p11.1-q11.1, and

the chromosome 8 centromere showed normal signal numbers.

Cerebrospinal fluid

There was no evidence of leukemia.

Pathologic diagnosis

A diagnosis of AML with predominantly megakaryoblastic differentiation was made based on the morphologic and immunohistochemical features. The subtype known as RAM was suspected based on the distinct immunophenotypic and morphologic findings, though quantitative flow cytometry was not available at the time of diagnosis, and the predicted aggressive course was discussed.

Clinical course

The decision was made to proceed with AML treatment off protocol (AAML1031) followed by HCT at remission. Treatment included induction chemotherapy following the standard arm of Children's Oncology Group (COG) study AAML1031. Bone marrow examination (BME) at the end of induction showed persistent disease with 6% residual blasts. A second induction was given using a Mitoxantrone/Ara-C regimen with intrathecal cytarabine. Repeat BMA/Bx demonstrated complete remission.

A 10/10 HLA allelic-matched, unrelated donor was identified. The patient received myeloablative conditioning with fractionated total body irradiation (1,200 cGy) and cyclophosphamide (60 mg/kg/d for two days). Graft-versus-host disease (GvHD) prophylaxis included methotrexate and tacrolimus. On D100, BME demonstrated complete remission. Peripheral blood chimerism on D30, D60, D100 and D180 post-HCT showed >95% donor DNA at each post-transplant time point. Transplant-related complications included WHO grade 4 mucositis, respiratory syncytial virus, and rhinovirus upper respiratory tract infection.

Follow-up and relapse post-bone marrow transplant

At 9 months post-HCT, the patient developed swelling of the left side of his face associated with left ear drainage and headaches. CT scan of the head and sinuses showed a left temporal soft tissue mass with bony destruction and intracranial extension. He underwent a facial mass biopsy that demonstrated myeloid sarcoma with diffuse infiltration of the soft tissue by leukemic cells similar to those at initial diagnosis.

A BME showed 48% blasts that were morphologically and immunophenotypically identical to the initial bone marrow spec-

imen seen at diagnosis. A similar pattern of patchy involvement of bone marrow was noted on sections from aspirate clot (Figure 4). The cerebrospinal fluid had no evidence of leukemia.

Multiple reinduction attempts failed to

induce remission after relapse (Figure 5). Given the refractory nature of the patient's AML, the patient proceeded to a second allogeneic HCT using a different allogeneic donor. The patient received myeloablative conditioning with targeted daily Busulfan

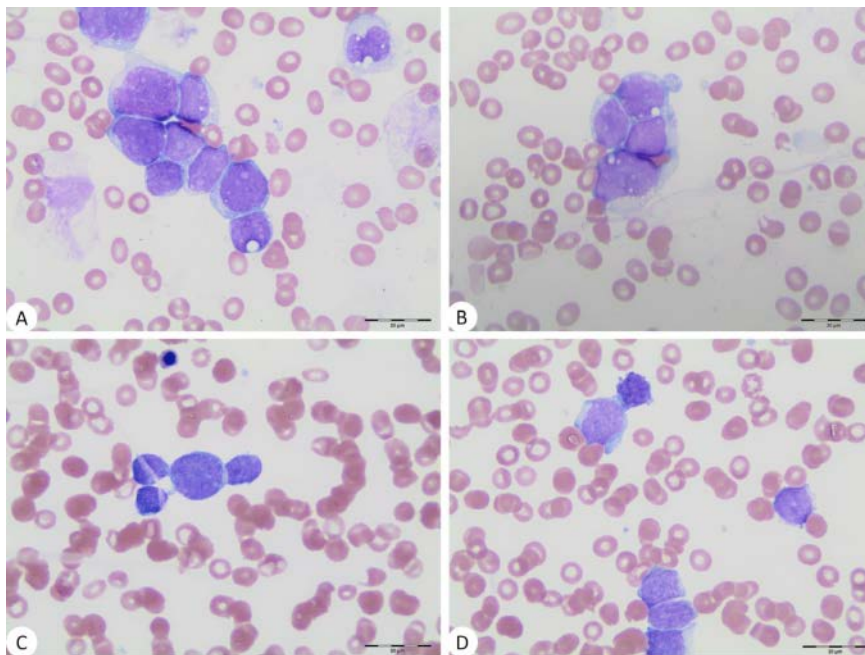


Figure 1. The blasts were present as small clusters of cohesive cells (bone marrow aspirate, 100× oil).

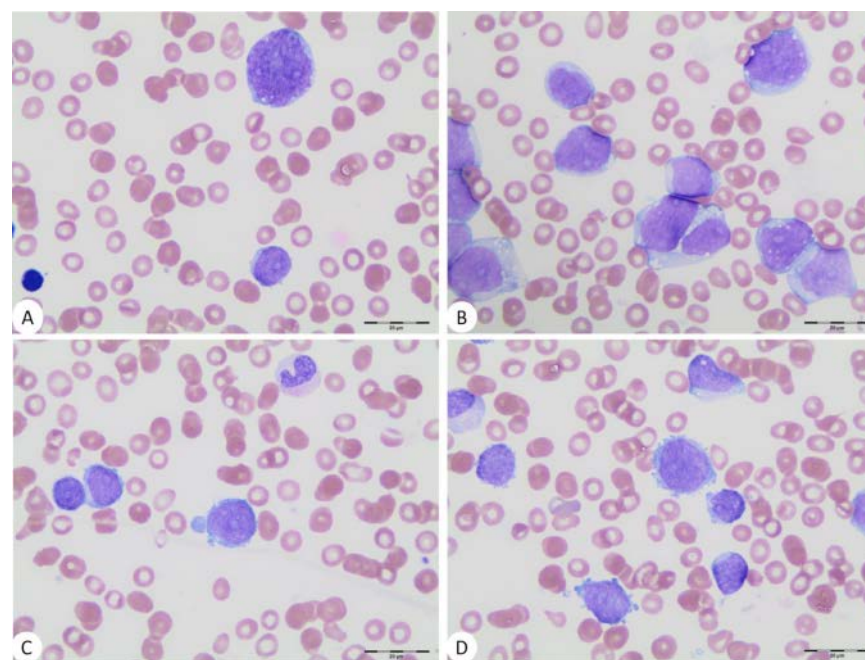


Figure 2. A subset of the blast population had large, binucleate nuclei, and cytoplasmic blebs (bone marrow aspirate, 100× oil).

and Cyclophosphamide. His GvHD prophylaxis included methotrexate and tacrolimus. The patient received unprocessed, G-CSF mobilized, peripheral blood stem cells (D0) and was discharged on D18 post-HCT with a relatively uncomplicated course. D30 BMA/Bx demonstrated persistent disease. He was placed on hospice care until he died, approximately 18 months following initial diagnosis.

Discussion

Pediatric leukemias are risk stratified by molecular and cytogenetic markers.² Not all pediatric leukemias demonstrate markers with a known prognostic significance. Previous studies have proposed risk stratification systems that include the immunophenotype of AML.^{3,4} Acute myeloid leukemia (AML) with RAM immunophenotype is a newly described subtype characterized by a unique immunophenotype and a poor prognosis. The RAM immunophenotype is characterized by bright CD56, dim to negative CD45, dim to negative CD38 and negative HLA-DR.¹ While the original description noted a high incidence of acute megakaryoblastic leukemia (AMKL) cases among RAM patients, no detailed morphologic features were described. The morphologic features in the case presented here include those which can be seen in AMKL; *i.e.* cytoplasmic blebs, multinucleation, and absence of cytoplasmic granules and Auer rods. However, there are also features that are unusual for hematopoietic neoplasms and leukemia, in particular. Cohesive clumps of blasts, nuclear molding, and patchy islets of blasts are unusual features in acute leukemia. Although blast clusters may be seen in myeloid malignancies with extremely high blast counts, clustering is striking in this case and is particularly important to recognize as it is usually encountered in non-hematopoietic round cell tumors of childhood.⁵ These unusual morphologic features may precipitate an extensive workup for non-hematopoietic tumors and perhaps mislead even the experienced diagnostician along the wrong path, particularly since the RAM immunophenotype is characterized by the expression of bright CD56 and weak, or even negative, CD45.^{6,7} It is possible that the unusual cohesiveness and clumping of blasts in this subtype is somehow related to the high expression of CD56; a cell surface glycoprotein known to be involved in cell adhesion.^{8,9} The unique immunophenotypic and morphologic characteristics, coupled with

the resistance to treatment, high relapse risk, and extremely poor prognosis of RAM subtype have been well documented in the original article. This case further support these observations hold true, even when

early transplant is offered. Furthermore, the unusual clinical, immunophenotypic and morphologic findings raise questions about the true nature of the underlying neoplastic cell.

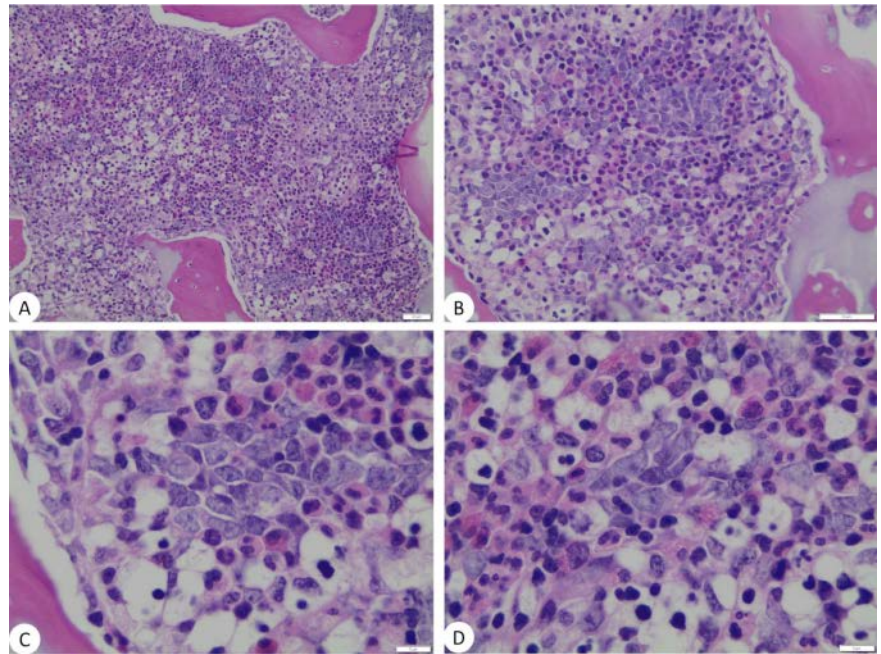


Figure 3. Sheets of large blasts were present within the bone marrow biopsy. Residual hematopoiesis was present in the background [(A) 20×, (B) 40×, (C,D) 100× oil].

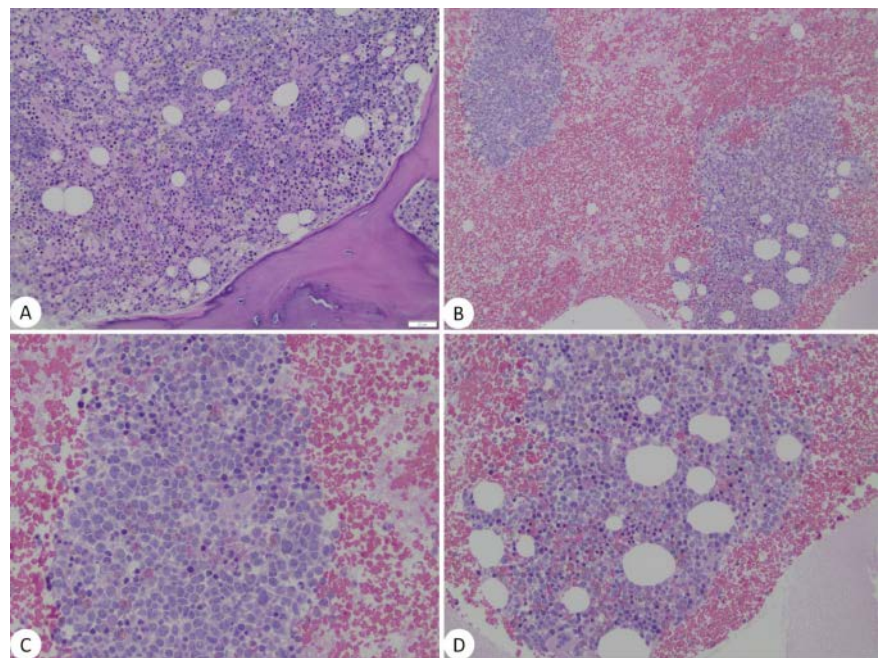


Figure 4. Sheets of blasts were identified in the post-transplant bone marrow biopsy.

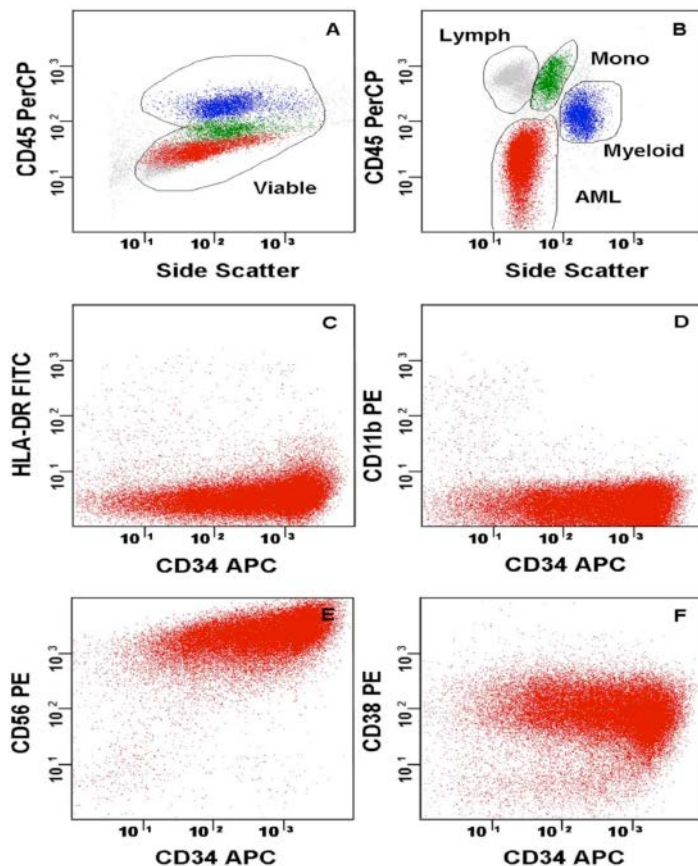


Figure 5. Quantitative multidimensional flow cytometry of the bone marrow aspirate at end of reinduction. The abnormal myeloid progenitor cells are characterized by low expression of CD45 and log side scatter (red events on A and B). The abnormal cells express CD34, high levels of CD56 (E) and reduced expression of CD38 (F) without expression of HLA-DR (C) or CD11b (D).

Conclusions

The current WHO classification of hematopoietic tumors incorporates morphologic, immunophenotypic, and genetics findings into the diagnostic process.¹⁰ As future classifications consider including the RAM immunophenotype, it will be important to further describe cases with this distinct subtype and consider new treatment strategies.

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