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Acute myeloid leukemia with RAM immunophenotype presenting with extensive mesenteric and retroperitoneal lymphadenopathy: A case report and review of the literature

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ABSTRACT

Acute myeloid leukemia (AML) with RAM immunophenotype is a rare recently described AML subtype. It is defined by blasts with strong expression of CD56 and weak to absent expression of CD45, HLA-DR....., and CD38 and characterized by significantly worse outcome [1]. Little is known about the clinical presentation and this immunophenotype is not widely recognized in clinical practice. We describe a case of AML with RAM immunophenotype in a 5-year-old male patient with a unique presentation, including extensive mesenteric and retroperitoneal lymphadenopathy. Diagnostic studies included bilateral bone marrow and lymph node biopsies, flow cytometry, cytogenetics, fluorescence in-situ hybridization (FISH), and next generation sequencing. Bone marrow biopsy revealed >90% blasts, positive for CD34, CD117, and CD56 by flow cytometry and immunohistochemistry. Next generation sequencing revealed *BCOR* loss and *CBFA2T3-GLIS2* fusion. Following induction chemotherapy, bone marrow biopsy showed residual disease and a stem cell transplant was performed. The patient relapsed three months after transplant and subsequently passed away eleven months after initial diagnosis. Limited literature is available describing this newly identified AML subset. The RAM immunophenotype has been identified as an independent prognostic factor for relapse rate and overall and disease-free survival [1]. Few case reports are available to characterize the genetic profile, typical presentation, and clinical course of patients with this unique immunophenotype.

1. Introduction

Acute myeloid leukemia (AML) with RAM* immunophenotype is a rare recently described subtype of acute myeloid leukemia. The RAM immunophenotype was initially observed in a non-COG protocol patient and named after the patient's initials (with documented informed consent), who was identified to have minimal residual disease (MRD) after day 100 post hematopoietic stem cell transplant. It is defined by blasts with strong expression of CD56 and weak to absent expression of CD45, HLA-DR....., and CD38 and characterized by significantly worse outcome [1]. Few case reports are available that encompass a wide range of initial presentations, necessitating further investigation to more clearly characterize the typical clinical presentation of these patients.

2. Case description

The patient is a 5-year-old male with recent history of COVID19 (SARS-CoV-2) infection four months prior to presentation with several weeks of fatigue, weight loss, and abdominal pain. Initial laboratory tests showed leukocytosis with eosinophilia and circulating blasts, normocytic anemia, and thrombocytopenia. Abdominal computed tomography (CT) scan revealed extensive mesenteric and retroperitoneal lymphadenopathy concerning for lymphoma. The abdominal lymphadenopathy displaced several major intra-abdominal vessels, with the external iliac vein partially non-enhancing and presumably occluded. A nodal mass in the region of the porta hepatis produced mass effect with secondary mild intrahepatic biliary ductal dilation (*Fig. 1*). In addition, the patient had splenomegaly, mild bilateral hydronephrosis, and bilateral calcified pulmonary lesions.

3. Methods

Initial diagnostic studies included bilateral bone marrow and lymph node biopsies, flow cytometry, cytogenetics, and next generation

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sequencing.

4. Results & discussion

Initial laboratory tests showed leukocytosis (25.1 thousand/mm^3) with eosinophilia (21.4%, absolute 5.37 thousand/mm^3) and circulating blasts (7.1%), normocytic anemia (RBC 4.09 million/mm^3, Hgb 11.4 g/dL, Hematocrit 33.1%, MCV 80.9 femtoliters), and thrombocytopenia (Platelets 69 thousand/mm^3).

The patient underwent an interventional radiology guided abdominal lymph node biopsy and bilateral bone marrow biopsies. The bone marrow biopsy findings are illustrated in *Fig. 1*. The bone marrow showed >90% blasts with smooth chromatin, variably prominent nucleoli and scant to moderate, agranular cytoplasm. Rare cytoplasmic blebs were seen. There was a mild background prominence of phenotypically normal eosinophils. Immunohistochemical stains demonstrated the blasts to be positive for CD34, CD117 (weak), and CD56 (diffuse and strong) (*Fig. 1*). An infiltrate of similar blasts was noted in the abdominal lymph node. Flow cytometry performed on the peripheral blood, lymph node biopsy, and bone marrow aspirate illustrated blasts with the following immunophenotype: CD13(partial dim +), CD33 (bright +), CD34(bright +), CD38(partial +), CD45(partial dim +), CD56(bright +), CD117(+), CD123(partially dim +), HLA-DR.....(-), MPO(-) (*Fig. 2*).

Conventional cytogenetics showed an add(11)(p15) and der(18)t (18;21)q23;q22) abnormalities. FISH studies detected an extra copy of *RUNX1* and deletion of 3′- end of *NUP98* (11p15.4), consistent with the cytogenetics results. Next generation sequencing identified *CBFA2T3-GLIS2* fusion, resulting from a cryptic inversion of chromosome 16, and BCOR loss. Both have been previously associated with a worse prognosis and shown to be independent marker of adverse outcomes in intensively treated patients with acute myeloid leukemia [2–4]. It is important to note that the fusion resulting from a cryptic inversion of chromosome 16 involves the genes CBFA2T2 and GLIS2, in contrast to the classic inversion involving chromosome 16 (CBFB-MYH11) which is associated with a favorable prognosis.

Following induction chemotherapy per COG trial AAML1831, abdominal lymph nodes showed significant decrease in size. However, a post-induction bone marrow biopsy showed residual disease by flow cytometry. Due to the high-risk status associated with the diagnosis and the presence of residual disease, a matched unrelated donor stem cell transplant was performed after completing the third course of chemotherapy. The patient relapsed three months after transplant and subsequently passed away eleven months after initial diagnosis.

Limited literature is available describing this newly identified subset of acute myeloid leukemia.

Few case reports are available to fully characterize the typical presentation, genetic profile, and complete clinical course of patients with this unique immunophenotype.

Abu-Arja et al. described a unique case of 2-year-old monozygotic twins diagnosed simultaneously. The twins were born prematurely at 27 weeks gestation but had no other significant medical history. Clinical presentation for both twins was similar and characterized by intermittent fevers and pancytopenia. After intensive chemotherapy, both patients underwent unrelated umbilical cord blood transplants. Twin A remained in remission three years post-transplant, whereas Twin B relapsed and died 13 months after initial diagnosis. Twin A's leukemia cells were near triploid, whereas Twin B's leukemia cells had a normal karyotype (46, XX). Neither harbored *FLT3-ITD, CEPBa*, or *NPM* mutations. [5] A similar presentation was described by Conces et al. of a 5-year-old patient with persistent fever, pancytopenia, epistaxis, and petechial rash. In the bone marrow aspirate, the leukemic blasts showed unique cohesive clusters, which is an unusual pattern for a hematopoietic neoplasm. Chromosome analysis showed an abnormal male karyotype with an unbalanced translocation resulting in a gain of 1q and loss of 20p. FISH were normal with probes for 5q33-q34, 5p14.2, 7q31, 7p11.1-q11.1, and chromosome 8 centromere. The patient underwent a matched unrelated hematopoietic stem cell transplant with recurrence 9 months post-transplant. The recurrence was characterized by left face swelling, and imaging revealed a left temporal soft-tissue mass with bony destruction. A biopsy of the mass showed myeloid sarcoma. Bone marrow biopsy at the time of recurrence confirmed 40% blasts morphologically and immunophenotypically identical to the blasts seen at initial diagnosis. The patient ultimately passed away 18 months following his initial diagnosis [6].

An extensive study on the subject by Eidenschink Brodersen et al.

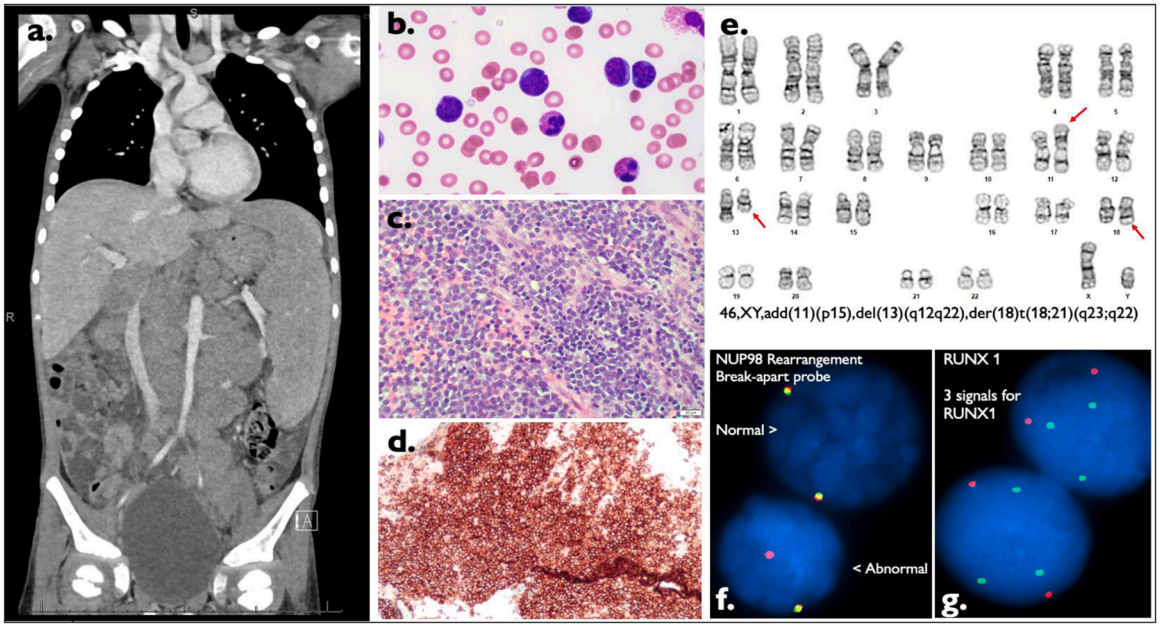

Fig. 1. (a.) Computed tomography (CT) scan coronal view illustrating significant abdominal and retroperitoneal lymphadenopathy, including displacement of intrabdominal major vessels and organs. (b.) Peripheral smear illustrating blasts and eosinophilia. (c-d.) Bone marrow core biopsy (H&E) demonstrating strong and diffuse CD56 + by immunohistochemical stain. (e.) Conventional cytogenetics. (f.) FISH for NUP98 (break-apart probe). (g.) FISH for RUNX1.

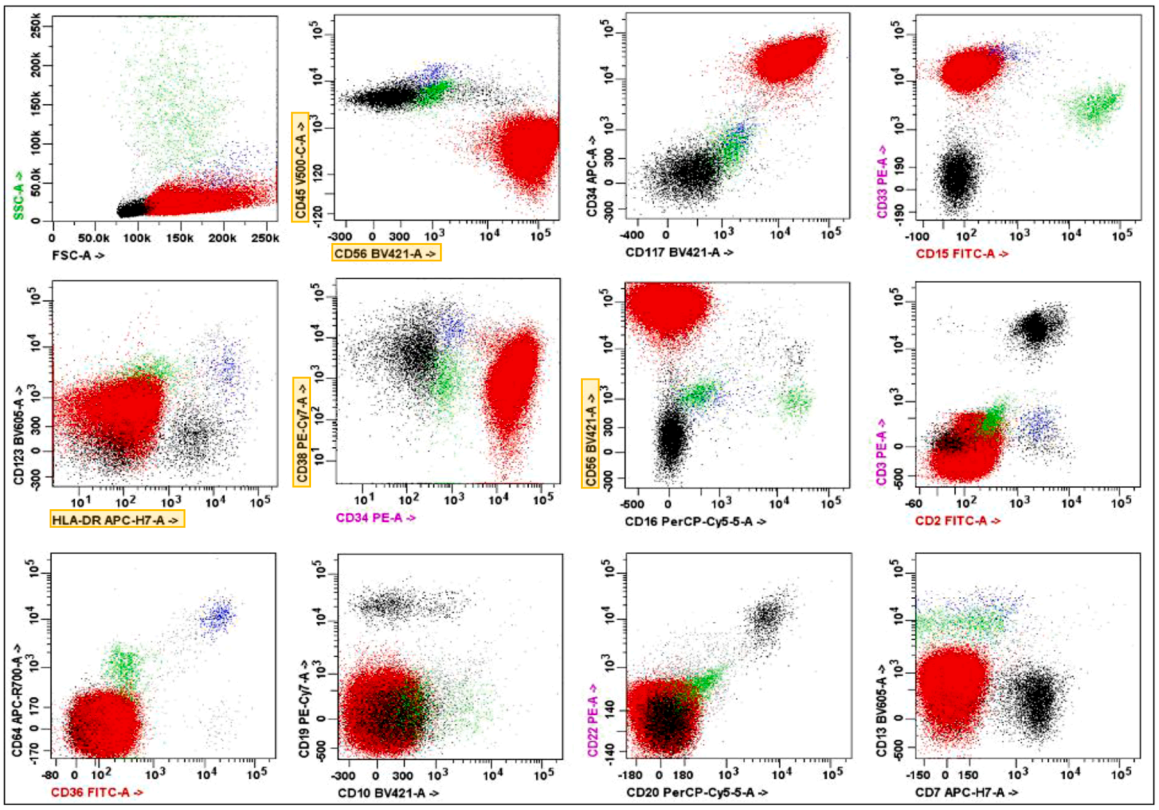

Fig. 2. Flow cytometry of lymph node core biopsy, illustrating medium-large blasts (CD34+ and CD117+) with strong expression of CD56 and dim-absent CD45 and HLA-DR..... expression. Blasts (red), granulocytes including eosinophils and neutrophils (green), lymphocytes (black), monocytes (blue).

compared RAM phenotype patients ($N = 19$) to a subdivided non-RAM cohort including CD56+ non-RAM AML patients ($N = 166$) and CD56– AML patients ($N = 636$). In this study, RAM phenotype patients presented at significantly younger age, with intermediate-risk cytogenetics (seven patients with normal cytogenetics, one patient with trisomy 8, and eleven patients with other cytogenetic abnormalities not currently known to correlate with prognosis) and lacked currently known high-risk molecular features such as *FLT3*-ITD or other mutations such as *CEBPA* or *NPM1*. Despite the favorable presentation, complete response rate was observed to be lower in the RAM cohort ($p < 0.001$). Event-free survival and overall survival were also significantly lower in the RAM cohort ($p < 0.001$). Although an AML immunophenotype has not been previously used to risk-stratify patients, this study illustrated a robust statistically significant difference in patient outcomes with the RAM phenotype acting as an independent prognostic factor for overall survival, relapse rate, and disease-free survival. [1] A subsequent study examining the differences among CD56+ AML patients ($N = 769$) in the Children's Oncology Group Trial AAML0531 and identified three distinct phenotypic clusters of patients. Cohort 1 showed a prevalence of t(8;21) and had a 69% 5-year event-free survival. Cohort 2 showed a prevalence of 11q23 and had a 39% 5-year event-free survival. Cohort 3 (RAM immunophenotype) showed a prevalence of the *CBFA2T3-GLIS2* fusion and a 19% 5-year event-free survival. The high prevalence of the CBFA2T2-GLIS2 fusion in this study (10 of 16 RAM cohort patients; 63%) and the presence of this fusion in this patient's case may implicate this fusion as a pathogenic driver of the disease in this cohort of patients [4] In addition, recent research has described potential treatment implications of targeting the cellular pathways affected by this fusion with GLI inhibitors such as GANT61 with promising initial results in vitro studies [7, 8].

Detailed characterization of patients with the RAM phenotype is needed to fully delineate the clinical presentation of these rare cases and to identify the genetic drivers and clinically actionable targets to guide future treatment of this high-risk patient group.

5. Authorship contributions

The following authors contributed to composition of the manuscript: NW, OW, PK, DR....., and FF. All authors reviewed and approved the final manuscript. Images were provided by NW, OW, PK, and FF.

Declaration of Competing Interest

No conflict of interest

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N/A

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