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BONE MARROW WORKSHOP BOOK

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LEGAL ORGANISER & PCO

MCI Deutschland GmbH
Markgrafenstr. 56
10117 Berlin
Germany
www.wearemci.com
ea4hp-sh@wearemci.com

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EA4HP24-BMWS-412

Suspected hemophagocytic lymphohistiocytosis in young patient with systemic lupus erythematosis

Dr. Megan O. Nakashima, Dr. Sarah Ondrejka

Cleveland Clinic, Laboratory Medicine, Cleveland, USA

Case Description

08/2020 a 26 year-old man with recently diagnosed with systemic lupus erythematosus (SLE, ANA > 1:1280, anti-DNA+, anti-Sm+) presented with anemia, joint pain, abdominal pain, and a rash. He also had evidence of acute kidney injury and hepatic dysfunction. Imaging showed mildly enlarged cecal mesenteric lymph nodes and iliac lymph nodes bilaterally. He had a fever of 38.7 °C and was positive for hepatitis A. Skin biopsy showed a dermal hypersensitivity reaction. A left inguinal lymph node biopsy showed a depleted lymph node. Hemophagocytic lymphohistiocytosis (HLH) was suspected and a bone marrow biopsy performed. After infection was ruled out, he was started on hydroxychloroquine and prednisone. He met 5 HLH-94 criteria (fever, elevated IL-2R and ferritin, decreased NK cell activity, hemophagocytosis), however given that he had no splenomegaly and his fevers resolved, his "HLH" was attributed to SLE. He was discharged and followed-up in rheumatology with multiple later presentations to ED for joint and abdominal pain with diarrhea.

12/2023 he presented with a week of fevers and diarrhea. An infectious workup was positive for influenza B. Hematology suspected relapsed HLH; a second bone marrow biopsy was performed. He met 4/5 of his original HLH criteria at that time (see table in images). He was treated with steroids, but eventually developed hyponatremia, then hypovolemic shock. He began to have seizures and was moved into intensive care. Lymph node biopsies showed necrotic material and histiocytes. Blood cultures grew methicillin-sensitives *S. aureus*. His mental status continued to decline with generalized seizures. Eventually he was transitioned to comfort care and expired 17 days after admission.

Biopsy Fixation Details

Zinc-formalin. Trephine EDTA decalcified.

Frozen Tissue Available

None

Details of Microscopic Findings

2020: Aspirate smears: rare macrophages with engulphed erythrocytes and nucleated cells. Trephine: mildly hypocellular with an erythroid predominance.

2023: Aspirate smears: hemodiluted but abundant hemophagocytic cells present on touch imprints. Trephine: hypercellular with erythroid predominance.

Immunophenotype

2023: parvo B19-. Flow cytometry: no evidence of a lymphoproliferative disorder.

Cytogenetics

2020 and 2023: 46,XY [20]

Molecular Studies

2023: *TET2* p.Q622*, NM_001127208.2, c.1864C>T VAF: 17.4%. VUS in *ASXL1* p.D1265Y VAF: 50.4%. VUS in *BCOR* p.L808H VAF: 97%

TCR B and G: non-clonal

Proposed Diagnosis

Hemophagocytic lymphohistiocytosis

Interesting Feature(s)

This is a complicated case in which the patient had two episodes of severe systemic inflammation in which it was difficult to determine if there was HLH or just the underlying autoimmune disease and/or infection. HLH can be very difficult to diagnose because the symptoms and signs, including laboratory findings, overlap with other forms of inflammation. HLH can be triggered by autoimmune disease, however infection and malignancy should be excluded. In this case it is possible that the steroids the patient received masked the lymph node findings. The detection of the *TET2* variant also raises the possibility of an underlying undiagnosed hematolymphoid neoplasm, which would be difficult to detect given the reactive marrow findings. Databases indicate this truncating variant rarely is present in germline (0.0004%, gnomAD), however the VAF in this case is not suggestive of germline, while the two VUS detected had very high VAF suggesting they may be germline variants. *TET2* normally is involved with resolution of inflammation, including reducing IL-6, so a loss-of-function mutation would be pro-inflammatory.

Panel Diagnosis

MAS/HLH in the setting of autoimmune disease (SLE) with concurrent infections and TET2 mutation

EA4HP24-BMWS-11

Secondary hemophagocytic lymphohistiocytosis due to disseminated Histoplasmosis with underlying *STAT1* mutation

Dr. David D. Grier[1,2], Dr. Robert B. Lorsbach[1,2], Dr. Jason Meyer[3]

[1] Cincinnati Children's Hospital Medical Center, Pathology & Laboratory Medicine, Cincinnati, USA; [2] University of Cincinnati College of Medicine, Pathology & Laboratory Medicine, Cincinnati, USA; [3] Kettering Pathology Associates, Kettering, USA

Case Description

A 24-year-old woman with a presumed history of lupus and multiple infections (e.g., oral and esophageal candidiasis, MRSA) presented to an outside emergency room with arthralgias and shortness of breath. She was found to be hypotensive and pancytopenic. After discharge, her pancytopenia persisted and she suffered from generalized fatigue. A

bone marrow biopsy was performed and showed hemophagocytic histocytes and disseminated fungal organisms consistent with Histoplasmosis species. Her clinical condition worsened and she was transferred to a tertiary care hospital in acute respiratory failure and septic shock. She developed coagulopathic shock and required multiple blood products. The ferritin and soluble IL-2 receptor was 3381.4 ng/mL (normal 7.3 -270.7 ng/mL) and 1642 U/mL (normal 137 – 838 U/mL), respectfully. Whole exome sequencing was sent near the beginning of her hospital stay.

Biopsy Fixation Details

B-fix

Frozen Tissue Available

None

Details of Microscopic Findings

Examination of the bone marrow aspirate shows numerous monocytes and occasional neutrophils with intracellular organisms consistent with fungus. Scattered histocytes with ingested cells are identified. The bone marrow core biopsy is normocellular (80%) with multilineage hematopoiesis and a myeloid predominance. There was no increase in blasts or evidence of malignancy. GMS and PAS stains highlight numerous intracellular organisms consistent with Histoplasmosis species. AFB stain was negative.

Immunophenotype

Flow cytometry revealed no increase in blasts or monoclonal B-cell population. There was a subpopulation (7.7% of all T-cells) of CD4/CD8 negative CD3 positive cells with slightly downregulated CD5.

Cytogenetics

FISH studies for common AML abnormalities were negative. The karyotype was normal: 46,XX.

Molecular Studies

Whole exome sequencing revealed a heterozygous, pathogenic *STAT1* mutation (c.800C > T, p.Ala267Val). Of note, no *STAT1* mutation was detected in either of the patient's parents

Proposed Diagnosis

Secondary hemophagocytic lymphohistiocytosis due to disseminated histoplasmosis with underlying *STAT1* mutation.

Interesting Feature(s)

STAT1 (signal transducer and activator of transcription 1) is involved in multiple immune system functions and plays an important response to viral, mycobacterial, and fungal pathogens. There are four disease manifestations depending on the *STAT1* mutation type. The gain of function (GOF) mutation is the most common type. A267V is one of over 105 *STAT1* GOF mutations. Patients with *STAT1* GOF mutations are susceptible to bacterial, viral, fungal, and mycobacterial infections, including histoplasmosis as seen in this patient.

Histoplasmosis, which is endemic in the Ohio River Valley, leading to secondary hemophagocytic lymphohistiocytosis (HLH) is unusual. Disseminated histoplasmosis is typically seen in transplant and HIV positive patients.

Panel Diagnosis

Histoplasmosis with associated hemophagocytosis, in context of acquired STAT1 mutation

EA4HP24-BMWS-398

EBV-associated Hemophagocytic Lymphohistiocytosis with Monoclonal Expansion of T-cells in an Adolescent

Dr. Mats Ehinger[1], Dr. Despoina Violidaki[1]

[1] Lund University, Lund, Sweden; [2] Department of Pathology, Lund University, Lund, Sweden

Case Description

·Teenager presenting with fatigue and fever for 3 weeks with pancytopenia, icterus and splenomegaly.
·Initially kidney failure, liver failure and respiratory failure due to pleural effusion.
·Fulfils 6 criteria for HLH: fever, splenomegaly, bicytopenia, hypertriglyceridemia and/or hypofibrinogenemia, hyperferritinemia, elevated soluble IL-2 receptor.
·Bone marrow smear showed 11% atypical lymphocytes. Granulopoiesis and erythropoiesis were dysplastic. A few macrophages but no morphological hemophagocytosis.
·Bone marrow biopsy was hypercellular with phagocyting macrophages and a population of CD3+/CD5-/CD8+/EBER+ cytotoxic T-cells.
·Flow cytometry revealed 10% monoclonal T-cells CD3+/CD5-/CD2+/CD7dim/CD4-/CD8+/CD25+/TCR+/TRBC1-.
·Treated with etoposide 100 mg/m2 and dexamethasone 10 mg/m2.
·Two months after the initial presentation the patient was clinically recovered with normalized blood parameters except anemia (76 g/L) and slight hemolysis. Flow cytometry was normalized (no aberrant T-cell population).
·WBC 0.8 K/uL; HGB 91 g/L; PLT 42 K/uL
·High EBV-copy load in peripheral blood >1 million
·High CRP 154 mg/L
·High LD 106 µkat/L
·Very high ferritin 93304 µg/L
·Low fibrinogen 1 g/L
·Elevated triglycerides 5.4 mmol/L
·Elevated soluble IL-2 receptor
·Elevated conj bilirubin 84 µmol/L
·Elevated liver enzymes: ASAT 8.7 µkat/L; ALAT 1.9 µkat/L; ALP 6.2 µkat/L; GT 4 µkat/L
·Elevated kreatinin 100 µmol/L
·Low S-albumin 21 g/L

•Differential peripheral blood: 78% polys, 20% lymphs, 1% monos, 0% eos, 0% basos
•Differential bone marrow: 30% polys, 22% lymphs (including 11% atypical lymphs), 2% monos, 0% eos, 1% basos, 21% bands, 4% metamyelocytes, 3% myelocytes, 6% promyelocytes, 2% erythropoiesis, 1% blasts and occasional macrophages

Biopsy Fixation Details

10% formaldehyde

Frozen Tissue Available

No

Details of Microscopic Findings

Bone marrow aspirate: 11% atypical lymphocytes. A few macrophages but no morphological hemophagocytosis. Granulopoiesis and erythropoiesis were dysplastic.
Bone marrow biopsy: Hypercellular with phagocyting macrophages and reduced erythropoiesis.

Immunophenotype

Immunohistochemistry of bone marrow: Atypical T-cells CD3+/CD5-/CD2+/CD7+/CD4-/CD8+/granzymeB+/TIA-1+/CD56-/EBER+
Flow cytometry:
10% monoclonal T-cells CD3+/CD5-/CD2+/CD7dim/CD4-/CD8+/CD25+/TCR+/TRBC1-

Cytogenetics

Not done

Molecular Studies

Not done

Proposed Diagnosis

EBV-associated hemophagocytic lymphohistiocytosis (with monoclonal expansion of T-cells)

Interesting Feature(s)

This case illustrates the gray zone between EBV-associated HLH and Systemic EBV-positive T-cell lymphoma (SEBVTCL) of childhood.
The distinction between EBV-HLH and SEBVTCL of childhood is often difficult, as aberrant T cell clones can be seen in both entities. Karyotyping (not done in this case) can be used to make the distinction, as an abnormal karyotype would argue in favour of lymphoma. Clonality of T-cells can be determined by flow cytometry (as in this case) or PCR (not done in this case).
This case also clearly shows that monoclonal expansion of T-cells in a patient with EBV-associated hemophagocytic lymphohistiocytosis does not immediately imply lymphoma.

Panel Diagnosis

EBV associated HLH with monoclonal CD8+ T-cell expansion

EA4HP24-BMWS-433

Hemophagocytic Lymphohistiocytosis associated with B-cell maturation antigen (BCMA)-directed chimeric antigen receptor (CAR) T cell therapy

Faryal Fatima[1], Siraj El Jamal[1,2], Shafinaz Hussein[1], Bruce Petersen[1], Christian Salib[1], Alina Dulau-Florea[1], **Amy Duffield**[1]

[1] Icahn School of Medicine at Mount Sinai Hospital, Pathology, Molecular and Cell-Based Medicine, New York, USA; [2] Rutgers - Robert Wood Johnson Medical School, Pathology, New Brunswick, USA

Case Description

A 51 year-old man with plasma cell myeloma (diagnosed 2018) who had previously failed multiple lines of therapy began treatment with B-cell maturation antigen (BCMA)-directed chimeric antigen receptor (CAR) T cell therapy on March 7, 2023. Prior to CAR T infusion, laboratory studies showed leukopenia (2,200/uL), anemia (Hct 32.1%), and thrombocytopenia (123,000/uL), as well as mildly elevated ferritin (467 ng/mL; range 22-275 mg/mL), and normal triglycerides and fibrinogen.

On March 8 the patient was diagnosed with grade I cytokine release syndrome, which was managed with tocilizumab. IL-2 receptor alpha levels were found to be elevated 2 weeks after the CAR T infusion (1365 U/mL; range 223-710 U/mL). 4 weeks after the infusion, ferritin began to increase relatively rapidly, rising from 735 ng/mL to 1,134 ng/mL in 3 days. 5 weeks after the CAR T infusion he developed a neutropenic fever, and was treated with antibiotics. The next week the patient complained of dizziness, fatigue and loss of appetite, and was noted to have leukopenia (900/uL), anemia (Hct 22.9%), and thrombocytopenia (19,000/uL), along with elevated ferritin (1,377 ng/ml).Triglycerides and fibrinogen were normal. He was treated with anakinra, steroids, intravenous immunoglobulin, and growth factors. The cytopenias persisted, and a bone marrow biopsy was performed 8 weeks after CAR T infusion.

Rising free kappa light chains were noted approximately 4 months after the CAR T infusion, compatible with relapse. Currently, the patient is responding well to treatment with a bispecific antibody that targets both CD3 and BCMA.

Biopsy Fixation Details

The biopsy was fixed in B-Plus fixative solution.

Frozen Tissue Available

No

Details of Microscopic Findings

The bone marrow biopsy shows patchy cellularity, and is overall hypocellular (~20%). There is progressive maturation of the myeloid and erythroid lineages, with patchy areas that exhibit left-shifted maturation. Megakaryocytes are decreased in number with normal morphology. Plasma cells are absent. Lymphocytes are not increased, and no atypical lymphoid aggregates are seen.

The aspirate smears demonstrate patchy cellularity, with hypocellular and hypercellular areas. The myeloid and erythroid lineages show no overt dysplasia. Plasma cells are essentially absent. Lymphocytes are not increased, and show no morphologic atypia. Occasional hemophagocytic histiocytes are seen. Storage iron is markedly increased.

Immunophenotype

CD68 and CD163 highlight increased histiocytes. CD3 marks singly-scattered T-cells that are a mixture of CD4+ and CD8+ cells. CD61+ megakaryocytes are relatively decreased, and CD34+ blasts are not increased. CD138 does not highlight any plasma cells.

Cytogenetics

Not applicable

Molecular Studies

T-Cell Receptor (TCR) gene rearrangement studies (peripheral blood; April 29): Positive for clonal TCR gamma and beta gene rearrangements

Proposed Diagnosis

Hemophagocytic lymphohistiocytosis (HLH) associated with chimeric antigen receptor (CAR) T cell therapy

Interesting Feature(s)

- The patient was diagnosed with antecedent cytokine release syndrome, which has been reported in HLH associated with immune effector cell therapy.
- The laboratory anomalies meet criteria for HLH; however, the alterations are more subtle than often seen in HLH associated with other etiologies, and the patient was successfully managed without etoposide.
- The marrow shows patchy cellularity with a marked increase in storage iron.
- Evaluation of T-cell subsets is complicated by prior CAR T therapy.

Panel Diagnosis

HLH associated with CAR-T therapy

EA4HP24-BMWS-91

Lenalidomide-associated B-ALL: a rare case with transient, spontaneous regression after discontinuing lenalidomide

Dr. Wenbin Xiao[1], Dr. Mark Geyer[2], Dr. Brian Shaffer[2], Dr. Hani Hassoun[2], Dr. Yanming Zhang[1], Dr. Maria Arcila[1], Dr. Mikhail Roshal[1], Dr. Ahmet Dogan[1]

[1] Wenbin Xiao, Pathology/Memorial Sloan Kettering Cancer Center, New York, USA; [2] Mark Geyer, Medicine/Memorial Sloan Kettering Cancer Center, New York, USA

Case Description

40-year-old male presented with anemia, renal failure, and lytic bone lesions in 4/2012 and was diagnosed as IgG lambda restricted multiple myeloma. He was treated with CyBorDx4 and achieved partial remission with <5% plasma cells in the marrow. Autologous stem cell transplant with melphalan-200 conditioning was performed in 8/2014 and VGPR (very good partial response) was achieved for which he was put on lenalidomide maintenance (dose 10-15mg/day). His myeloma disease status had improved to stringent CR. A routine bone marrow examination on the lenalidomide maintenance trial in 12/2016 showed no evidence of plasma cell neoplasm but noted 8% blasts in the marrow, with an abnormal immature B-cell phenotype. He was initially observed with normalization of cytopenia and blasts (<5%) upon stopping lenalidomide but subsequently progressed to morphologic B-ALL in 9/2017. He was treated with R-HyperCVAD and then Blinatumomab and achieved MRD negative remission. Haploidentical allogeneic HCT was performed in 3/2018 and he remains disease free since.

Biopsy Fixation Details

Decalcified FFPE

Frozen Tissue Available

none

Details of Microscopic Findings

2012 (Multiple myeloma): Bone marrow biopsy is hypocellular for the age of the patient (30% cellularity) with increased plasma cells. CD138 shows increased plasma cells, 30% of total cells.

2017 (BALL): Bone marrow biopsy is normocellular (50% cellularity) with increased blasts. CD34 and TdT show increase blasts, accounting for 50% of total cells. The aspirate smears show an expanded population of small to medium size blasts (57%), with round nuclei, fine chromatin,

occasional prominent nucleoli, and scant basophilic cytoplasm.

Immunophenotype

2012 (Multiple myeloma) IHC: the plasma cells are positive for CD138 and lambda, negative for kappa.

2017 (BALL): flow—the blasts abnormally express CD10 (uniform bright), CD33 (partial dim), CD38 (dim), CD45 (dim to absent), with normal expression of CD19, CD58 and CD34; and without CD20.

Cytogenetics

2012 (Multiple myeloma): Karyotype was normal. FISH showed additional copies of 1q25, MLL (11q23) gene in 16% and ETV6 gene and p53 gene in 17% of the cells.

2017 (BALL): normal

Molecular Studies

2012 (Multiple myeloma): not done

2017 BALL: Clonal rearrangement involving the IGH is detected. Lymphotrack assay shows IGHV Family: V4-31 and IGHJ Family: J6

2017 NGS BALL: 1. TET2 (NM_001127208) exon3 p.Q888* (c.2662C>T) VAF28%, 2. GRIN2A (NM_001134407) exon13 p.T1164M (c.3491C>T) VAF 31%

Proposed Diagnosis

2012: Multiple myeloma

2017: B-lymphoblastic leukemia/lymphoma, lenalidomide associated

Interesting Feature(s)

- Lenalidomide associated B-ALL can occur in patients receiving lenalidomide maintenance therapy for multiple myeloma.
- This type of B-ALL is enriched for poor-risk genetics including TP53 mutations and hypodiploidy, however the treatment response and outcome is favorable.
- The B-ALL appears clonally unrelated to prior plasma cell neoplasm.
- Our case showed spontaneous regression of abnormal B-blasts after discontinuation of lenalidomide, supporting an association. Two more similar cases with spontaneous regression were included in our MSK series (Geyer, et al. Blood Adv, PMID: 36827680). Recently, a patient on pomalidomide maintenance also developed B-ALL suggesting a class effect.

Panel Diagnosis

Lenalidomide-associated B-ALL

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EA4HP24-BMWS-15

Differentiation syndrome in a case of *FLT3*-ITD-positive AML treated with gilteritinib

Prof. Alexandar Tzankov

University Hospital Basel, Pathology, Basel, Switzerland

Case Description

M, 58 with a history of AML (FAB: M4, ICC 2022: NOS, WHO-5: myelomonocytic) that has been diagnosed in February 2021 and treated with standard double-induction chemotherapy + midostaurin. The patient progressed, was salvaged and underwent allogenic HCT in April 2022, upon which he remained MRD+ (0.02% on FCM/FACS of the peripheral blood) and has therefore been put on gilteritinib.

A bone marrow biopsy for estimation of treatment's efficacy was obtained in May 2022.

At that time his CBC were:

- WBC 0.57 x 10^9/L
- RBC 3.78 x 10^{12}/L
- Hb 116 g/L
- PLT 23 x 10^9/L
- No peripheral blasts

Biopsy Fixation Details

FFPE, EDTA decalcified

Frozen Tissue Available

No

Details of Microscopic Findings

Hypercellular atypical bone marrow with myeloid hyperplasia and maturational arrest at the promyelocyte level.

Immunophenotype Initial AML phenotype: CD34, CD117, HLA-DR, CD36, CD13, CD33, CD11b and cMPO+

Negativity for CD34 and CD117 in the post-treatment biopsy, in spite of initial blast positivity. Expression of MPO and CD33.

Cytogenetics

49,XY,+8,+13,+15

Molecular Studies

At initial presentation:

- *WT1* R385Sfs*7 (VAF 42%)
- *WT1* K393Sfs*3 (VAF 32%)
- *FLT3*-ITD ins69bp+

Remaining *FLT3*-ITD still postive at the time of biopsy.

Proposed Diagnosis
Differentiation syndrome in *FLT3*-ITD-positive AML treated with gilteritinib
Interesting Feature(s)
Interesting (and already documented) pattern of morphologic (promyelocytic) and phenotypic (loss of CD34 and CD117) differentiation syndrome in a case of a *FLT3*-ITD-positive AML treated with gilteritinib (i.e. ASP2215 - a small-molecule FLT3/AXL inhibitor) that hematopathologists should be aware of.
Reference:
Kondo et al. Myelomonocytic differentiation of leukemic blasts accompanied by differentiation syndrome in a case of FLT3-ITD-positive AML treated with gilteritinib. Hematology 2021;26:256-260. doi: 10.1080/16078454.2021.1889111. PMID: 33631087.

Panel Diagnosis
Differentiation syndrome in FLT3-ITD-positive AML treated with gilteritinib

EA4HP24-BMWS-39

38 Year-old man with severe anemia, marrow fibrosis, and lymphoplasmacytic infiltrate

Dr. Mariko Yabe

Memorial Sloan Kettering Cancer Center, Hematopathology, New York, USA

Case Description
The patient developed severe anemia in 2015 (Hgb 5g/dl) and was diagnosed with aplastic anemia. He received steroid, ATG, cyclosporin A and Rituximab, however, hemoglobin level did not improve. He was referred to our institution in November 2016. At the time of initial visit at our institution, he was RBC transfusion dependent and was not on specific therapy. CBC showed severe anemia and mild thrombocytopenia (WBC 4900/ul, Hgb 5.8 g/dL, MCV 90 fL, Plt 85000/ul, neutro 58.9%, lymph 37.0%, mono 3.5%, eosino 0.4%, baso 0.2%). LDH was normal (128 U/L; Ref 120-246). Total bilirubin, vitamin B12, folate, iron, ferritin, haptoglobin were all normal. He had no significant past medical history or history of toxin or chemical exposure. Radiology exam did not show lymphadenopathy or hepatosplenomegaly.
Biopsy Fixation Details
10% formalin
Frozen Tissue Available
Not available
Details of Microscopic Findings
Bone marrow biopsy: Erythroid predominant trilineage hematopoiesis with marked myelofibrosis and lymphoplasmacytic infiltrate. Dysmegakaryopoiesis and osteosclerosis are not evident.

Peripheral blood smear: No circulating blasts. No dysgranulopoiesis. Leukoerythroblastic features are not evident.

Immunophenotype

CD138, kappa, and lambda light chain stains highlight increased polytypic plasma cells. CD3, CD4, and CD8 highlight increased interstitial lymphocytes. CD71 highlights increased erythroid precursors. CD34 shows no increase in blasts. CD117 highlights scattered mast cells. Myeloperoxidase highlights myeloid population. CD61 highlights megakaryocytes. CD20 highlights scattered B-cells. HHV8, IgG4, and pan-cytokeratin are negative.

Flow cytometry showed no PNH clone on red cells, granulocytes, or monocytes.

Atypical NK cell with CD5 expression was detected (2.5% of WBC).

Cytogenetics

46, XY[18]. FISH for chromosome 5, 7, 8 show no abnormality. DEB assay is negative

Molecular Studies

No evidence of mutation in tested genes by NGS.

T-cell receptor monoclonal rearrangement: not detected.

Proposed Diagnosis

Primary autoimmune myelofibrosis

Interesting Feature(s)

Findings commonly seen in primary myelofibrosis were not observed. Cytogenetic and molecular studies did not detect clonal hematopoiesis. Based on the ineffectiveness of steroid, ATG, cyclosporin A and Rituximab therapy, as well as the presence of numerous polytypic plasma cells in the bone marrow, treatment with anti-CD38 (daratumumab) was attempted. After anti-CD38 therapy, his hemoglobin level improved up to 10g/dL, and he became transfusion independent for 10 months. However, he again gradually became anemic, and this time, he presented with pancytopenia. Bone marrow biopsy obtained in November 2017 showed virtually acellular marrow, and myelofibrosis was not observed. The etiology of aplastic marrow is unclear. This could represent progression of underlying marrow failure syndrome, or, although bone marrow failure related to daratumumab has not been reported so far, anti-CD38 could have targeted CD38 positive stem cell niche in this case. He received allogeneic stem cell transplant in December 2017. He has been showing excellent CBC recovery since then.

Panel Diagnosis

Hypoplastic bone marrow with fibrosis in a patient with refractory vitamin B12 deficiency and ANA positivity

EA4HP24-BMWS-45

Recurrent idiopathic hemophagocytic lymphohistiocytosis (HLH) with cryptogenic hemolytic anemia, pancytopenia and massive splenomegaly

Dr. Yong Zhang[1], Dr. Ioana Capa[2], Dr. Tiffany Bohr[1], Dr. Inga-Marie Schaefer[2], Dr. Melissa S. Gildenberg[2], Dr. Erik Washburn[1], Dr. Evelyn M. Potochny[1], Dr. Julie Fanburg-Smith[1], Dr. Jozef Malysz[1], Dr. Michael Bayerl[1], Dr. Sam Sadigh[2], Dr. Melissa R. George[1], Drs. Yong Zhang and Ioana Capa contributed equally to this case submission.

[1] PennState Health Hershey Medical Center, Department of Pathology, Hershey, USA; [2] Brigham and Women's Hospital, Department of Pathology, Boston, USA

Case Description

46 y.o. M patient presented with life-threatening, recurrent, idiopathic HLH. He had a history of Coombs (-) hemolytic anemia with hepatosplenomegaly since 1990. On his first hospital admission, he had worsening pancytopenia and marked splenomegaly of unknown etiology. He had a second hospitalization due to pancytopenia, recurrent fevers and back pain. He had low fibrinogen, liver dysfunction and severely elevated inflammatory markers. The diagnosis of idiopathic HLH was made. He was then treated with dexamethasone, etoposide, IVIG, Ruxolitinib and Tacrolimus. Splenectomy was performed to assess for occult malignancy and treat coagulopathy. He had repeated PET CT scan, liver and skin biopsy without evidence of lymphoma. Additional infectious disease and rheumatologic work-ups were negative. Given the life-threatening nature of his recurrent idiopathic HLH, he was scheduled for an allogenic stem cell transplant. Repeat bone marrow biopsy showed extensive necrosis. He developed multi-drug resistant bacteremia and fungemia, and did not survive to transplant. Autopsy demonstrated multiorgan failure in the setting of disseminated fungal and bacterial infections. No evidence of lymphoma or other malignancy. Bone marrow showed extensive histiocytosis with active erythrophagocytosis.

Biopsy Fixation Details

Acetic Zinc Formalin for bone marrow biopsy. The bone marrow specimen in 10/2020 to be submitted for additional molecular: 2024-EA4HP-45-#1.

Frozen Tissue Available

N/A

Details of Microscopic Findings

A bone marrow biopsy in 10/2020 demonstrated hypercellular marrow with extensive histiocytic proliferation with erythrophagocytosis; repeat biopsy in 6/2021 showed mildly hypercellular marrow with persistent erythrophagocytosis. The autopsy bone marrow exhibited hypocellular marrow with areas of degeneration, increased histiocytes with erythrophagocytosis.

Immunophenotype

Two pre-mortem bone marrow biopsies, splenectomy, and post-mortem bone marrow demonstrated numerous histiocytes strongly positive for CD163 or CD68. Moreover, bone marrow biopsy in 10/2020 was negative for S100, BRAF and CD1a. CD56 was positive in scattered rare large atypical cells, with a subset of large atypical cells exhibiting variable expression of CD30. Immunostains for CD21, CD23, GMS and AFB stains, and in situ hybridization for EBER were all negative. Repeat bone marrow biopsy in 06/2021 was negative for S100, CD1a and ALK1.

Cytogenetics

46, XY

Molecular Studies

- RBC membrane and enzyme evaluation: No abnormalities.
- Hereditary Hemolytic Anemia Gene Panel: Negative for variants in 37 genes.
- PNH testing: Negative for CD55 and CD59 deficiency.
- NeoTYPE Myeloid Disorders Profile: Negative.
- B-Cell Gene Rearrangement: Negative
- T-Cell Receptor Beta Gene Rearrangement: Negative
- Gaucher and other inborn errors of metabolism: Negative

Proposed Diagnosis

Recurrent, idiopathic hemophagocytic lymphohistiocytosis

Interesting Feature(s)

- Life-threatening, recurrent, idiopathic HLH with:
  - Cryptogenic hemolytic anemia
  - No evidence of:
    - Hematolymphoid malignancy.
    - Predisposing genetic abnormality.
    - RBC disorders.
    - Gaucher's disease or other storage diseases.
    - Infectious disease or rheumatologic etiology.

Panel Diagnosis

Recurrent, idiopathic HLH

EA4HP24-BMWS-46

Secondary HLH induced by human granulocytic anaplasmosis

Dr. Yong Zhang[1], Dr. Tiane Chen[1], Dr. Hyma Polimera[2], Dr. Matthew Evans[2], Dr. Daniela Mihova[3], Dr. Michael Bayerl[1], Dr. Melissa R. George[1]

[1] Penn State Health Hershey Medical Center, Department of Pathology, Hershey, USA; [2] Penn State Health Hershey Medical Center, Division of Hematology/Oncology, Hershey, USA; [3] Frederick Memorial Hospital, Department of Pathology, Frederick, USA

Case Description

67 y.o. M presented with a 5-day history of fever, severe fatigue, shortness of breath, poor appetite and diarrhea. He developed shock requiring pressor support and acute hypoxic respiratory failure requiring intubation. He had a history of Lyme disease. Workup demonstrated pancytopenia with WBC of 700/µL, absolute neutrophil count 80/µL, platelets 12,000/µL. His laboratory workup was also significant for a ferritin level greater than 50,000 ng/ml, elevated triglyceride level and AST/ALT, increased soluble interleukin 2 receptor (sCD25) and low fibrinogen. CT abdomen showed hepatosplenomegaly. By using an on-line calculator (http://saintantoine.aphp.fr/score/), his H-Score was obtained as 293, >99.9% probability of having hemophagocytic syndrome. He was started on empiric therapy with vancomycin, cefepime and flagyl for presumed sepsis from a GI source due to diarrhea. He was also given dexamethasone and etoposide treatment following the HLH-94 protocol. A peripheral blood smear was flagged for pathologist review for "Howell-Jolly body like inclusions" in neutrophils. Blood smear also showed possible morulae of *Anaplasmaphagocytophilum,* clinical team was doubtful due to serologic tests being negative. Later, *Anaplasma* PCR was positive confirming *Anaplasma phagocytophilum* infection. Other infectious workup testing was uniformly negative. IV doxycycline treatment was initiated. Further dose of etoposide was discontinued and dexamethasone was tapered. The patient had dramatically rapid improvement for his HLH symptoms within only 2 days after doxycycline treatment, with successful extubation on day-3 of admission. He was weaned to room air and became clinically stable. At his one week follow up visit after the discharge, the patient continued to improve and his WBC increased to 2000 /µL, ANC 1180/µL, Hb 10.3 g/dL, platelets 100,000/µL.

Biopsy Fixation Details

Acetic Zinc Formalin for bone marrow core biopsy (2024-EA4HP-46)

Frozen Tissue Available

N/A

Details of Microscopic Findings

A bone marrow biopsy was performed and revealed hypercellular bone marrow (60–70%) due to extensive histiocytic proliferation with hemophagocytosis. The peripheral blood smear demonstrated multiple neutrophils with toxic changes, and the presence of light blue-purple intracytoplasmic inclusions consistent with human granulocytic anaplasmosis.

Immunophenotype

Bone marrow biopsy demonstrated numerous histiocytes strongly positive for CD68.

Cytogenetics

Not performed

Molecular Studies

Anaplasma PCR was positive confirming *Anaplasma phagocytophilum* infection.

Proposed Diagnosis

Secondary HLH induced by human granulocytic anaplasmosis

Interesting Feature(s)

We report a case with marked hyperinflammatory syndrome accompanied by shock-like symptoms and ARDS with laboratory findings consistent with hemophagocytic lymphohistiocytosis (HLH) in the setting of *Anaplasma* infection. Bone marrow demonstrated extensive histiocytic proliferation with hemophagocytosis, confirmed by CD68 immunostain. A peripheral blood smear still has great value to detect Anaplasmosis during the first week of infection, and simultaneously evaluate for other tick-borne co-infections. Upon detection of *Anaplasma*, our patient stopped unnecessary chemotherapy to spare the side effects from etoposide, and showed dramatic rapid improvement for his HLH symptoms within only 2 days of starting doxycycline treatment. Our case further indicates that *Anaplasma* should be considered as a possible triggering pathogen for unexplained HLH, especially in endemic areas with high tick activity.

Panel Diagnosis

Human granulocytic anaplasmosis with associated HLH

EA4HP24-BMWS-52

Fatal Hemophagocytic Lymphohistiocytosis (HLH) at presentation of Chronic Myelomonocytic Leukemia (CMML)

Dr. Katelynn M. Wilton[1,2], Dr. Daniel DeAngelo[3], Dr. Geraldine Pinkus[1], Dr. Sam Sadigh[1]

[1] Brigham and Women's Hospital, Pathology, Boston, USA; [2] Massachusetts General Hospital, Pathology, Boston, USA; [3] Dana Farber Cancer Institute, Hematology / Oncology, Boston, USA

Case Description

A 62-year-old man with limited past medical care presented with altered mentation, 20kg weight loss and fatigue. On physical exam, he had chronic lower limb venous stasis ulcers. Clinically his presentation was consistent with cellulitis and distributive shock. Laboratory testing showed normocytic anemia (Hgb 9.3g/dL, MCV 81.3), thrombocytopenia (37K/uL) and leukocytosis (103.81 K/uL) with mostly neutrophils (65%, ANC 53.27K/uL) and monocytes

(28%, 29.07K/uL). No previous CBCs were available for review. Cultures grew Serratia marcescens and Stenotrophomonas maltophilia. Imaging showed marked splenomegaly. A bone marrow biopsy was performed, but despite intensive care, he died rapidly from multiorgan failure.

Biopsy Fixation Details

Bouin's fixative; 15 minute decalcification in RapidCal-Immuno.

Frozen Tissue Available

No

Details of Microscopic Findings

Aspirate: myeloid predominant with increased monocytes, trilineage dysplasia, and 6% blasts. Frequent histiocytes showed phagocytosis of lymphocytes, erythroid and myeloid precursors, including mature, immature and dysplastic forms. Iron stain highlighted histiocytes with internalized erythroid elements.

Core biopsy: markedly hypercellular and myeloid predominant with dysplastic megakaryocytes, and evident histiocyte phagocytosis .

Immunophenotype

No immunophenotypic evaluation.

Cytogenetics

Karyotype: 46,XY,del(12)(?p12p11.2)[cp8]/46,XY[cp12]

Molecular Studies

NGS panel detected pathogenic variants in *ASXL1* (37.5%), *CBL* (95.7%), *CUX1* (49.3%), *NF1* (13.2%), *SETBP1* (46.4%), and *U2AF1* (50.3%).

BCR-ABL1 (p210) testing was negative.

Proposed Diagnosis

Hemophagocytic lymphohistiocytosis (HLH), in setting of chronic myelomonocytic leukemia (CMML), and sepsis.

Interesting Feature(s)

This is a highly unusual case of a patient presenting with florid HLH, in the setting of a new CMML diagnosis, and concurrent sepsis, and illustrates the potential interplay between three complex diseases. It is unclear what the relative contribution of sepsis and CMML might have been to development of HLH in this patient: although HLH is traditionally associated with lymphoid malignancies and acute leukemias, rare cases have been reported in the setting of chronic myeloid neoplasms. Furthermore, immune system dysfunction from CMML itself may be predisposing to infection, and contributing to HLH, thus this may represent a rare fatal complication of untreated CMML progression. From a diagnostic point, this case also highlights the challenges of utilizing a single time point, and potentially confounded criteria within a complex medical presentation. Although persistence of monocytosis could not be documented in this patient, the overall morphologic features combined with the mutational profile best fit with CMML, though furthermore, there are overlapping criteria and findings between sepsis, CMML, and HLH. Finally, it is of interest that dysplastic hematopoietic elements appear engulfed by histiocytes and are similarly 'vulnerable' to phagocytosis in HLH.

Panel Diagnosis

CMML with sepsis and HLH

EA4HP24-BMWS-55

Gelatinous marrow transformation (GMT) in a patient with myeloid chronic leukemia treated with Ponatinib and with a concomitant Borrelia infection

PhD/MD Margherita Vannucchi[1], Prof. Maria Paola Martelli[2], Dr. Stelvio Ballanti[3], Dr. Elisabetta Agliani[3], Dr. Lucia Mariucci[3], Dr. Nicola Leone[1], Dr. Giovanni Martino[2], Prof. Brunangelo Falini[2], Prof. Stefano Lazzi[1]

[1] University of Siena, Medical Biotechnology, Siena, Italy; [2] University Hospital of Perugia, Center of Research in Hemato-Oncology (CREO), Perugia, Italy; [3] University Hospital of Perugia, Hematology and Clinical immunology Section, Perugia, Italy

Case Description

We present a case of a50-year-old man with a medical history of chronic myeloid leukemia (CML) diagnosed in 2020 and treated in the first line with Dasatinib. The disease was characterized by a complete hematologic response (CHR) albeit a minor molecular response (MR1). For this reason, the patient was treated with Nilotinib as second-line therapy, without any improvement. In February 2023 he underwent therapy with Ponatinib that demonstrated a better efficacy in terms of molecular response (MR2). After 7 months of therapy with Ponatinib, the patient came to our attention for evening fever, fatigue, lack of appetite, migrating joint, muscle and lumbar spine pain. Blood tests revealed pancytopenia (WB:2740/mCL, (N44%, L28%, M 22%, E 4%, B 2%) HgB: 5.9 gm/dL; PLT: 11.000/mCL).The serological test for infection revealed IgM anti-Borrelia burgdorferi antibodies indicating a recent infection. Moreover, magnetic resonance imaging (MRI) showed a lumbar thickening suspicious of neoplasia. Due to the pancytopenia and the MRI results, a bone marrow (BM) aspirate and a BM biopsy (BMB) were performed. Currently, the patient has interrupted the treatment with Ponatinib and started antibiotic therapy for Borrelia infection and the patient's blood count is slowly improving.

Biopsy Fixation Details

The biopsy was fixed in 10% buffered formalin and 10% EDTA

Frozen Tissue Available

No

Details of Microscopic Findings

A fine needle aspiration of the bone marrow was carried out and yielded a dry tap. Bone marrow biopsy was markedly hypocellular (less than 5% cellularity) with a reduction in the number of hematopoietic cells, an increase of hemosiderin pigment-laden macrophages without hemophagocytosis and a reduction in the number and size of adipocytes. Massive deposition of an amorphous and gelatinous substance, staining

positive with Alcian blue at pH2.5 was detected in the extracellular matrix. A slight increase of reticulin fibers was present (MF1). Infiltration of reactive B and T lymphocytes arranged in a few nodular aggregates along with scattered interstitial infiltrate of CD8+ T lymphocytes were also found.

Immunophenotype

Glycophorin, CD71, CD15, MPO, CD31, CD34, CD117, CD68KP, CD68PGM1, CD20, CD3, CD4, CD8, TIA-1, GRANZYME-B

Cytogenetics

46 XY, t(9;22) (q34,q21)

Molecular Studies

In progress

Proposed Diagnosis

Gelatinous marrow transformation

Interesting Feature(s)

Gelatinous marrow transformation is a rare and poorly understood condition. It can be due mostly to cachexia but also to neoplasia, anorexia and acute infection. Furthermore, few cases of correlation between GMT and the use of Tyrosine Kinase inhibitors (TKI), especially with Imatinib, have been reported. Ponatinib is a third-generation ABL-inhibitor and it binds ABL in its inactive form as Imatinib, with the additional capability to be effective also in cases carrying resistance mutations to the TKIs, especially the T315I mutation. Interestingly, to the best of our knowledge, cases of GMT that occurred during treatment with Ponatinib have not been described in the literature. Moreover, even if probably, in our case, the most reasonable cause of GMT is the therapy with Ponatinib, the concomitant Borrelia burgdorferi infection or the CML itself, broadens the spectrum of the possible causes of the BM starvation. These patients represent a therapeutic challenge, especially with CML. Clinicians should be aware of this type of side effect and carefully monitor the patients. Further studies in CML patients treated with Ponatinib are needed to confirm these findings.

Panel Diagnosis

Gelatinous transformation, in patient treated with Ponatinib for CML and with Borrelia Burgdorferi infection

EA4HP24-BMWS-69

HIV-associated HHV8-related multicentric Castleman disease involving bone marrow with concurrent hemophagocytic lymphohistiocytosis

Dr. Tapan M. Bhavsar

George Washington University Hospital, Pathology, Washington DC, USA

Case Description

A 41-year-old male with HIV/AIDS presented with pancytopenia, very low CD4 count, worsening constitutional symptoms, significant lymphadenopathy and clinical/laboratory findings consistent with hemophagocytic lymphohistiocytosis (HLH). A bone marrow biopsy was performed.

Biopsy Fixation Details

Bone marrow biopsy; fixed in 10% neutral buffer formalin

Frozen Tissue Available

Not applicable

Details of Microscopic Findings

Sections of the bone marrow shows overall 95-100% cellularity, with an increase in megakaryocytes in all stages of maturation including immature/regenerating forms. The M:E ratio was 1:1-1.5 (mildly erythroid-dominant) with overall reduced myeloid and erythroid elements. There was a marked increase in variably-sized plasmacytoid/plasma cells, mostly in clusters, mature – approximately 70-80% of total cellularity. Many hemosiderin-laden macrophages were noted. No lymphoid/lymphohistiocytic aggregates, granulomas, viral inclusions or atypical cell population were identified. Bone marrow aspirate showed many hemophagocytic histiocytes, a marked increase in plasmacytoid/plasma cells and an overall markedly reduced myeloid and erythroid elements.

Immunophenotype

CD138 stains many clusters/few scattered polyclonal (kappa and lambda) plasma cells ~ 70-80% of total cellularity. HHV8 highlights few scattered interstitial positive cells (some with nuclear dot-like positivity); the clonality (if any) cannot be assessed due to background polyclonal plasma cells. EBER in-situ hybridization stains few scattered positive cells (focally up to 17/HPF) – the co-expression of HHV8 and EBER in-situ hybridization (if any) cannot be assessed. CD61 highlights an increase in number of megakaryocytes (with morphology as described); CD31 highlights megakaryocytes and small subset of plasma cells. MPO and spectrin/E-cadherin stain rare myeloid and few scattered/clusters of erythroid elements respectively, confirming the slightly inverted M: E ratio. CD20/CD79a and CD3 stain occasional scattered small B-lineage cells including plasma cells and few scattered/small collections of reactive T-cells respectively, within normal limits. CD68 stains many histiocytes; no definitive hemophagocytic forms identified. CD34 stains 1-2% scattered positive cells. CD117 stains few scattered immature cells and rare scattered mast cells. CD30

shows non-specific staining, and is largely negative. Special stains for AFB, Fite, GMS and PAS are negative for infectious elements.

Cytogenetics

Not performed

Molecular Studies

Not performed

Proposed Diagnosis

HIV-associated changes with marked polyclonal plasmacytosis and few EBV-positive cells; scattered interstitial HHV8-positive cells, consistent with marrow involvement by HHV8-related multicentric Castleman disease; many hemophagocytic histiocytes; negative for lymphoma

Interesting Feature(s)

Rare case of HIV-associated HHV8-related multicentric Castleman disease involving bone marrow with concurrent hemophagocytic lymphohistiocytosis

Panel Diagnosis

Reactive bone marrow with HLH in the setting of immune dysregulation (HIV associated myelopathy), multicentric Castleman disease and EBV reactivation

EA4HP24-BMWS-77

Fibrin-ring granulomas occurring in association with myelodysplastic syndrome/neoplasm

Dr. Derek Loneman, Prof. Robert Hasserjian

Massachusetts General Hospital, Department of Pathology, Boston, USA

Case Description

A 70-year-old man with a history of autoimmune disease and recent COVID-19 infection presented to an outside hospital with persistent fatigue, fever, and dyspnea on exertion, as well as acute abdominal pain. Review of systems revealed 15-lb weight loss and night sweats in the preceding 3 months. CT showed acute diverticulitis, and he was admitted and started on antibiotics with course complicated by spontaneous splenic hematoma. Bone marrow biopsy showed fibrin-ring granulomas with negative special stains for EBV, CMV, and parvovirus, as well as bacterial, fungal, and mycobacterial organisms. Infectious disease workup was negative, including for *Coxiella burnetii*. Workup for HLH was also negative. The patient was transferred to our institution where additional history revealed no significant travel/exposure history, and infectious disease testing was negative, including for EBV, CMV, HIV, and HBV/HCV. Blood and pleural fluid cultures were negative. CT chest/abd/pelvis confirmed splenic hematoma but showed no splenomegaly/lymphadenopathy. CBC/diff was remarkable for macrocytic anemia (Hgb

8.1 g/dL, MCV 100.8 fL), thrombocytopenia (PLT 43 K/uL), and normal WBC/ANC (4.88 K/uL, 81.2% neutrophils); there were no peripheral blasts. SPEP was negative for monoclonal gammopathy.

Biopsy Fixation Details

B-plus followed by decalcification in RapidCal Immuno.

Frozen Tissue Available

No

Details of Microscopic Findings

Bone marrow biopsy performed at our institution revealed a left-shifted, hypercellular (70%) marrow with an increased M:E ratio. Megakaryocytes were decreased, with focal clustering, as well as small and hypolobated forms. Several fibrin-ring granulomas were noted.

Aspirate smear showed marked granulocytic dysplasia, including hypogranulation and Pelgeroid cells. Erythroid maturation was also dysplastic, with occasional lobated nuclei and nuclear irregularities. Megakaryocytes were infrequent and were mostly small and hypolobated forms. There was no increase in blasts (<1%). Plasma cells were increased at 12%.

Immunophenotype

Immunostains revealed no increase in CD34+ blasts (<5%). CD138 showed singly scattered and clustered plasma cells constituting approximately 10% of marrow cellularity and which were polytypic by kappa/lambda in-situ hybridization. Scattered T cell (CD3+) and fewer B cells (CD20+) were seen. A p53 immunostain showed strong staining in 1-2% of cells, indeterminate for mutation. AFB, GMS, spirochete, and Warthin-Starry stains were negative for organisms.

Flow cytometry showed no increase in blasts.

Cytogenetics

Karyotype revealed 44~46,XY,inv(6)(p22q21)[2],del(7)(q22q36),+8[2],-12[9],add(14)(p11.2)[8],idic(14)(p11.2)[9],psu dic(16;17)(q11.2;p11.2),del(18)(q21)[9],+1~2mar[cp18]/46,XY[2].nuc ish(KMT2Ax3)[24/100].

Molecular Studies

NGS detected a *TP53* variant (c.1018delA) at 80% VAF and a 1-copy deletion of *TP53*.

Proposed Diagnosis

Myelodysplastic neoplasm (MDS) with bi-allelic TP53 inactivation (WHO 5th edition)/Myelodysplastic syndrome with mutated TP53 (ICC) and fibrin-ring granulomas.

Interesting Feature(s)

Fibrin-ring granulomas are commonly associated with infection, especially with EBV or *Coxiella burnetii*. However, the fibrin-ring granulomas lack a definitive etiology in this patient with multiple comorbidities. The indeterminate p53 immunostaining pattern despite multi-hit *TP53* mutation also illustrates that p53 IHC does not always correlate with *TP53* mutation status. In this case, in which the clinical presentation and fibrin ring granulomas suggested an infectious process, the dysplastic morphology and abnormal molecular results helped avoid potential misdiagnosis.

Panel Diagnosis

Myelodysplastic neoplasm (MDS) with bi-allelic TP53 inactivation (WHO-2022) / MDS with mutated TP53 (ICC) and fibrin-ring granulomas

EA4HP24-BMWS-81

The hidden cause of massive erythrophagocytosis.

PhD/MD Konnie Hebeda[1], PhD/MD Suzanne van Dorp[2]

[1] Radboudumc, pathology, Nijmegen, Netherlands; [2] Radboudumc, hematology, Nijmegen, Netherlands

Case Description

Previously healthy male 70 yrs, August 2023 analysis of pancytopenia: Hb 4 mmol/L (8.4-10.8), Leucocytes 0.4*10^9/L (4-10), Trombocytes 7*10^9/L (150-400)

Clinically HLH: pancytopenia, high ferritin, elevated triglyceride, AST, fibrinogen, fever and splenomegaly (HScore 253 >99% probability HLH).

Infection with Leishmania after visit to Spain and Portugal confirmed by PCR on blood and bone marrow.

After therapy with etoposide, dexamethasone, anakinra and ambisome clinical improvement. December 2023 new BMB because of persisting pancytopenia: Hb 7 mmol/L, L 2.8*10^9/L, T 87*10^9/L.

Biopsy Fixation Details

Burckhardt fixative, overnight microwave enhanced fixation and EDTA decalcification

Frozen Tissue Available

not available

Details of Microscopic Findings

August 2023

BM smears: poor quality with sparse hematopoiesis with relatively abundant erythropoiesis with maturation defects, 22% sideroblasts, <15% ring sideroblasts. Myelopoiesis is decreased, no megakaryocytes. Many macrophages, but no hemophagocytosis. No parasites.

Trephine biopsy: hypercellular with massive erythrophagocytosis, reduced myelopoiesis and dysmegakaryopoiesis. Strong inflammatory component. No EBV (ISH EBER) or Leishmania (H&E, Giemsa) detected.

December 2023

BM smears*:* cell rich with increased erythropoiesis with > 15% ring sideroblasts. Trilinear dysplasia, 2% blasts. No hemophagocytosis or parasites**.**

Trephine biopsy: hypercellular with erythroid hyperplasia, dyshematopoiesis and slight increase of blasts (<5%), suggestive of myelodysplastic syndrome. No hemophagocytosis.

Immunophenotype

December 2023: Flowcytometry on BM: no indication of lymphoma. Ogatascore = 2. Percentage CD34+ myeloid precursor cells normal (0.6%). % B-precursor cells decreased. SSC-ratio is decreased. Eryscore = 4 with decreased CD71 median, increased CD71 CV and CD36 CV and increased % CD71dim cells. Flowscore = 2 with aberrant CD11b/CD13/CD16 maturation patterns on myeloid cells. These patterns can indicate MDS.

Cytogenetics

46,XY[10]

Molecular Studies

PCR for Leishmania on blood and bone marrow: positive

NGS:

1. *IDH2* NM_002168 c.419G>A p.(Arg140Gln) VAF 44.0%
2. *SRSF2* NM_003016 c.284_307del p.(Pro95_Arg102del) VAF 46.5%No *SF3B1* mutation

Proposed Diagnosis

WHO-2022: MDS-LB, ICC2022: MDS, NOS with multilineage dysplasia (and RS) with Leishmania infection related hemophagocytic syndrome (HS)

Interesting Feature(s)

- Leishmania infection with massive erythrophagocytosis without parasites detectable in the bone marrow by microscopy.
- An underlying MDS became apparent after treatment of the infection-related HS with etoposide, dexamethasone, anakinra and ambisome.
- The MDS belongs to a newly recognized subgroup of MDS-RS with *SRSF2* instead of *SF3B1* mutation, as described by Todisco et al.

Panel Diagnosis

MDS-LB (WHO-2022) / MDS, NOS with multilineage dysplasia (and ring sideroblasts) (ICC) with Leishmania infection and associated HLH

EA4HP24-BMWS-83

A case of disseminated histoplasmosis in a patient with sarcoidosis

Dr. Ion Prisneac[1], Dr. Jeffrey A. Vos[2], Dr. Rocco P. LaSala[3], Dr. Cara Randall[1]

[1] West Virginia University, Pathology, Anatomy and Laboratory Medicine, Morgantown, USA; [2] Essentia Health-St. Mary's Medical Center, Pathology, Duluth, USA; [3] UConn Health, Pathology and Laboratory Medicine, Farmington, USA

Case Description

A 69-year-old male with a history of long-standing sarcoidosis was admitted to the hospital due to intermittent fevers for several months and pancytopenia. His past medical history is significant for sarcoidosis which was diagnosed 21 years prior, initially involving the lungs and eventually showing cardiac involvement, requiring a pacemaker; he was also treated with methotrexate and prednisone. His recent medical history was also significant for COVID-19 infection, diagnosed 3 months before admission. His fevers were initially attributed to sarcoidosis and his pancytopenia to methotrexate. However, his symptoms continued despite discontinuation of his medications, and further workup was initiated. Computed tomography showed hepatomegaly, splenomegaly, and lymphadenopathy, concerning for a lymphoproliferative disorder. The patient underwent a bone marrow biopsy and a right supraclavicular lymph node biopsy as well.

Biopsy Fixation Details

Bone marrow:

- Bone marrow biopsy was inaspirable. No bone marrow aspirate and no flow cytometry were performed.
- The bone marrow core was submitted in Formical for decalcification and histologic evaluation.

Lymph node:

- Lymph node biopsy tissue was submitted in formalin for histologic evaluation.
- Additional fresh lymph node tissue was submitted for fungal culture.

Frozen Tissue Available

Not available

Details of Microscopic Findings

Bone marrow core biopsy showed hypercellular marrow for age (70%) with non-caseating granulomas and adequate trilineage hematopoiesis on hematoxylin and eosin (H&E) stain. A Grocott methenamine silver (GMS) stain was performed, showing round-ovoid yeast forms (3-5µm) with occasional narrow-based budding.

Lymph node touch preparations (TP) showed the microorganisms predominantly within the macrophages on Diff-Quick and H&E stains.

Immunophenotype

Flow cytometry was not performed due to inaspirable bone marrow.

Bone marrow immunohistochemistry (IHC) showed:

- No increase in blasts (< 5% by CD34 IHC).
- Adequate trilineage hematopoiesis highlighted by Factor VIII, myeloperoxidase, and CD71, respectively.
- CD20 and CD3 highlight scattered B and T lymphocytes respectively.
- CD68 highlights the granulomas and mildly increased histiocytes.
- Pancytokeratin is negative.
- No mast cell aggregates (by tryptase IHC)

Cytogenetics

Chromosome analysis revealed a normal male karyotype.

AML/MDS FISH panel was negative.

Molecular Studies

Not performed.

Proposed Diagnosis

BONE MARROW, ASPIRATE AND CORE BIOPSY:

- Hypercellular marrow (70%) with non-caseating granulomatous inflammation.
- Positive for fungal organisms, morphologically consistent with *Histoplasma*.

LYMPH NODE, RIGHT SUPRACLAVICULAR, BIOPSY:

- Granulomatous lymphadenitis.
- Positive for fungal organisms, morphologically consistent with *Histoplasma*.

Interesting Feature(s)

- Sarcoidosis reduces T-cell activity, and treatment with steroids causes further immunosuppression and vulnerability to the development of disseminated infection.
- COVID-19 also presumably increases the predisposition to acquire bacterial or fungal co-infections.
- Clinicians and pathologists should be aware of the overlap in clinical, radiologic, and pathological presentations of sarcoidosis and histoplasmosis to make the correct diagnosis and administer the appropriate treatment.

Panel Diagnosis

Histoplasmosis, in setting of sarcoidosis, methotrexate and prednisone

EA4HP24-BMWS-89

BM findings in a pediatric patient with a clinical diagnosis of new-onset Systemic Lupus Erythematosus (SLE) - LE cells, Tart cells and beyond

PhD/MD Yen-Chun Liu

St. Jude Children's Research Hospital, Department of Pathology, Memphis, USA

Case Description

A 9 y/o girl who started ethosuximide ~1 month ago for absence seizure presented with weight loss and acute renal failure. CBC showed anemia. Imaging showed mild splenomegaly and generalized lymphadenopathy. BM showed no diagnostic evidence of malignancy but flow cytometry performed on PB showed an expanded alpha beta double negative T cell population (6% of the CD3+ lymphocytes). Further workup revealed positive direct antiglobulin test, positive ANA (>1:2560) and positive anti-ds DNA antibody (1:1280). Her C3 and C4 levels were low; plasma soluble FAS ligand level was normal. With a clinical diagnosis of SLE, she received steroid and mycophenolate. Her kidney failure and lymphadenopathy have improved.

Biopsy Fixation Details

Bouin and nitric acid (10%)

Frozen Tissue Available

NA

Details of Microscopic Findings

There was maturing trilineage hematopoiesis with no increased blasts. The M:E ratio was normal. Cytologic atypia was identified but found in <10% of the cells in all 3 lineages. Amorphous homogeneous globular materials were readily identified in the aspirate smears and felt likely representing dying/degenerate cells. Rare neutrophils engulfing these amorphous homogeneous globular materials (the so-called “LE cells”) were found. The marrow was 80-90% cellular in the biopsy. Maturing erythropoiesis and granulopoiesis were identified. Megakaryocytes were abundant in number. There were several lymphohistiocytic aggregates reminiscent of granulomas showing a focal paratrabecular distribution pattern. The lymphohistiocytic aggregates composed of a mixture of B cells and T cells did not appear associated with follicular dendritic cellular meshworks. Polytypic plasmacytosis was also identified.

Immunophenotype

Flow cytometry performed on BM did not show increased blasts. There were hematogones, heterogeneous T cells and B cells with no definitive evidence of light chain restriction. Flow cytometry performed on PB showed an expanded alpha beta double negative T cell population (6% of the CD3+ lymphocytes).

Cytogenetics

46,XX[20]

Molecular Studies

Pathogenic/likely pathogenic variants not detected by targeted sequencing (362 cancer-related genes)

Proposed Diagnosis

Normocellular marrow with maturing trilineage hematopoiesis and multiple lymphohistiocytic aggregates; LE cells identified.

Interesting Feature(s)

This unusual case demonstrated findings infrequently encountered in daily practice. The LE cells described in the 1948 landmark paper (PMID: 18119558) regarding SLE BM were used in the SLE classification criteria until 1997, although largely replaced by ANA testing nowadays. The presence of the LE cells and Tart cells, cells that phagocytosed naked nuclei of dying cells induced by autoantibodies, is a sign of an autoimmune process but not specific for SLE. Nonphagocytized extracellular nuclear bodies (seen in our case), histologically identical to the LE cell inclusions, can also be identified (PMID: 13984037). These cytologic findings have been reported to facilitate lupus diagnoses in undiagnosed patients even in the modern era. This case also showed lymphohistiocytic aggregates reminiscent of granulomas and polytypic plasmacytosis. The expanded alpha beta double negative T cell population in the current case highlights the overlapping clinical features of autoimmune diseases and the importance of detailed diagnostic workup. On the other note, it is unclear if the patient's disease may potentially represent drug-induced lupus in light of the patient's recent start of ethosuximide treatment; ethosuximide-associated lupus and cytologic atypia in BM associated with antiseizure treatment have been described.

Panel Diagnosis

Reactive changes in setting of SLE

EA4HP24-BMWS-92

EBV negative, aggressive NK cell leukemia/lymphoma associated with FAS mutation and hemophagocytic lymphohistiocytosis

Dr. Wenbin Xiao, Dr. Ying Liu, Rohan Sardana, Kimon Argyropoulos, Filiz Sen, Maria Arcila, Mikhail Roshal, Ahmet Dogan

Wenbin Xiao, Pathology/Memorial Sloan Kettering Cancer Center, New York, USA

Case Description

65-year-old male presented with an incidentally detected mediastinal mass on X-ray during routine workup. Chest CT scan showed multiple lung nodules (largest 17mm, SUV max 15.9). Past history was significant for a germ cell tumor of testis for which the patient had

undergone orchidectomy 40 years ago, however no chemotherapy was given then. Wedge resection of the right lower lobe was performed and a diagnosis of EBV positive DLBCL (compatible with lymphomatoid granulomatosis, grade 3) was made. Peripheral blood EBV DNA titer was 2758. After a month of completion of therapy, the PET/CT revealed complete resolution and the EBV titers were negative. Patient developed breathlessness and dyspnea 4 months after treatment. PET-CT revealed a recurrence. The lung nodule biopsy and flow cytometry performed on FNA revealed an atypical NK cell population. The patient was put on steroids however his condition worsened with high fever and splenomegaly. CBC showed pancytopenia. The bone marrow biopsy was performed.

Biopsy Fixation Details

FFPE and decalcified

Frozen Tissue Available

none

Details of Microscopic Findings

Lung Wedge resection: Polymorphous lymphohistiocytic infiltrate composed of aggregates of large atypical lymphoid cells. There is an extensive area of necrosis/infarction where the tumor cells show a predominantly an angiocentric distribution.

Lung biopsy: Polymorphus lymphohistiocytic infiltrate. Scattered atypical, large cells with round to oval nuclei and distinct nucleoli are noted. No sheets or clusters of large cells are seen.

Bone marrow biopsy: Hypercellular for the age of the patient with interstitial clusters of atypical medium sized lymphoid cells with clumped chromatin, irregular nuclear membrane, and moderate amounts of eosinophilic cytoplasm. Small clusters of histiocytes were also noted. Hemophagocytosis is noted.

Immunophenotype

Bone marrow biopsy:

Immunohistochemistry:

Express: CD2, CD3 (cytoplasmic), CD56, CD30, Granzyme-B, TIA-1

Negative: CD20, CD19, OCT-2, PAX5, CD8, CD5, PD-1, ICOS, ALK1, CD138, Kappa, Lambda, BCL6, TCRdelta, EBER ISH and LMP1 (EBV)

Flow cytometry: Positive: CD2, CD45, CD56, CD94, Granzyme B, TIA-1 Negative: CD7, CD8, CD335, CD3, CD4, CD5, CD10, CD14, CD25, CD26, CD38, CD279 (PD1), TCR gamma/delta, TCRCbeta1, cytoplasmic CD3

Cytogenetics

Bone marrow Aspirate:

48,XY,add(1)(p36.3),+8,add(9)(q34),+12,add(17)(q25),del(22)(q11.2q13)[3]/96~104<4n>,idemx2,+add(1)(p22),+6,del(6)(q21q25)x2,+12,+12,+14,+20,inc[cp3]/46,XY[14]

Molecular Studies

Lung biopsy:

TCR beta and gamma: No clonal rearrangement

NGS: FAS (NM_000043) exon9 p.S230Lfs*4 (c.688_709delAGTAAATATATCACCACTATTG)

Proposed Diagnosis

Lung Wedge resection: EBV positive diffuse large B-cell lymphoma, compatible with lymphomatoid granulomatosis, grade 3

Lung biopsy: atypical NK-cell proliferation, consistent with NK-cell lymphoproliferative disorder
Bone marrow biopsy: EBV negative, aggressive NK cell leukemia / lymphoma with secondary hemophagocytic lymphohistiocytosis

Interesting Feature(s)

- Patient with EBV+ DLBCL developed an aggressive EBV negative NK cell leukemia / lymphoma subsequent to treatment.
- FAS mutation, which is uncommon in T/NK-cell lymphoma, is detected in neoplastic NK cells.
- The correlation between FAS mutation and HLH is raised.
- We retrospectively reviewed a large cohort of T- and NK-cell lymphoma patients (n=473) at MSK and confirmed the association between somatic FAS mutations and secondary HLH, providing evidence of genetic susceptibility for secondary HLH.
- Data will be submitted as a companion abstract

Panel Diagnosis

Aggressive NK cell leukemia with associated HLH, in setting of FAS mutation

EA4HP24-BMWS-94

Young man with common variable immunodeficiency and pancytopenia.

PhD/MD Ivo N. SahBandar[1], PhD/MD Chandler B. Sy[1], Dr. Georgi Lukose[1], Dr. Julia T. Geyer[2]

[1] NewYork Presbyterian/Weill Cornell Medical Center, Pathology and Laboratory Medicine, New York, USA; [2] Weill Cornell Medicine, Pathology and Laboratory Medicine, New York, USA

Case Description

The patient is a 20-year-old man with history of common variable immunodeficiency (CVID) and bipolar disorder treated with lithium. CVID was diagnosed after he presented with Coombs positive hemolytic anemia, leukopenia and thrombocytopenia, and he remained cytopenic and unresponsive to pegfilgastrim (GCSF) injections. The patient's laboratory results at admission showed: Hgb 8.7 (13.3-17.7 g/dL), platelets 51 (150-450x10e3/uL), WBC 1.1 (3.4-11.2x10e3/uL), with the differential count of: neutrophils 0.6%, bands 2%, lymphocytes 97%, monocytes 0.5%, eosinophils 1.8%. IgG subclass 4 was 4 (7-89 mg/dL), IgA 20 (70-312 mg/dL), IgE <0.25 (1.31-165.3 IU/mL), IgM 84 (56-352 mg/dL). The patient was treated and responded well to IVIg.

Biopsy Fixation Details

Bouin

Frozen Tissue Available

No

Details of Microscopic Findings

Bone marrow (BM) cellularity was close to 100%. Myeloid to erythroid ratio was markedly decreased due to erythroid hyperplasia and virtual absence of granulopoiesis. Erythroid maturation was left-shifted with many megaloblastoid forms seen. Megakaryocytes were increased in number and showed a normal spectrum of morphology. Scattered interstitial lymphoid aggregates composed of small mature-appearing lymphocytes were seen. Reticulin stain showed a moderate increase in reticulin fibers (2+). BM aspirate demonstrated 3% blasts, 4% promyelocytes, 1% myelocytes, 0% bands and neutrophils, 5% pronormoblasts, 24% normoblasts and 62% lymphocytes. Maturation beyond myelocyte stage was virtually absent. Occasional megaloblastoid erythroid cells were noted.

Immunophenotype

Immunostains revealed a borderline increase in CD34 positive blasts. Very few MPO positive myeloid cells were present. No micromegakaryocytes were seen. There was evidence of marked T-cell lymphocytosis. The majority of the T-lymphocytes corresponded to CD8(+) cells with an interstitial and intrasinusoidal distribution. The CD4(+) T-cells were predominantly confined to the lymphoid aggregates. Approximately half of the interstitial CD8(+) lymphocytes expressed cytotoxic antigens TIA1 and Granzyme B. The number of plasma cells was decreased. E-cadherin highlighted numerous left-shifted erythroid precursors.

Cytogenetics

Conventional cytogenetic analysis showed a normal male karyotype.

Molecular Studies

The patient's DNA was used for T-cell receptor gamma chain PCR analysis. Several distinct bands were detected, suggestive of the presence of oligoclonal T cell populations.

Proposed Diagnosis

Hypercellular BM with erythroid and megakaryocytic hyperplasia, prominent T-large granular lymphocyte proliferation and markedly decreased granulopoiesis, consistent with autoimmune cytopenias in a patient with CVID.

Interesting Feature(s)

- Autoimmune hemolytic anemia and thrombocytopenia are the most common autoimmune complications in patients with CVID, and often time are the first manifestation of the immune defect in the patients.
- The bone marrow findings are highly characteristic of CVID patients with presence of erythroid and megakaryocytic hyperplasia that correlates with patient's history of hemolytic anemia and thrombocytopenia. Additionally, large nodular and diffuse CD3+ T-cell infiltrates are a characteristic finding that may be related to CVID-associated autoimmune cytopenia.
- Neutropenia is a rare finding in patients with CVID, is generally associated with presence of other cytopenias and is of autoimmune origin.

Panel Diagnosis
Reactive changes in setting of CVID with T-cell proliferation

EA4HP24-BMWS-110

A fatal outcome of chronic active EBV infection (CAEBV), NK subtype in an immune competent patient, diagnostic and management dilemma!

Dr. Merit Hanna

North Shore Hospital, Haematology Department, Auckland, New Zealand

Case DescriptionA 48 year old Samoan gentleman had multiple admissions to North Shore Hospital with undifferentiated illness. He had fever, constitutional symptoms, systemic lymphadenopathy and splenomegaly.
Submental lymph node biopsy revealed reactive changes with a necrotic component. He subsequently had two non diagnostic lymph node biopsies.
Septic screen and extensive infectious serology screen was negative. He had evidence of past Epstein-Barr virus infection (EBNA IgG +).
Infectious panel on the lymph nodes by immunohistochemistry was also negative.
A bone marrow biopsy was non-contributory.
He was discharged home with a diagnosis of pyrexia of unknown origin.
Three weeks later, he represented to hospital critically unwell with fever, jaundice and shock. He was noted to have multi-organ failure and pancytopenia with haemoglobin 110 g/L, MCV 85 fL, thrombocytopenia (68 E+9/L), and neutropenia (1.3 E+9/L). He was coagulopathic with a fibrinogen of 0.9 g/L.
Biochemistry showed AST > 6000, LDH >4500, Ferritin > 16500, and creatinine peaked at 250.
He needed admission to intensive care unit for circulatory support.
Bone marrow biopsy was repeated. It showed haemophagocytosis of nucleated elements in keeping with haemophagocytic Lymphohistiocytosis. Additionally, a population of large abnormal, sometimes granular lymphoid cells were noted.
Lymph node biopsy result triggered EBV viral load. He had a high DNA viral load of 4.99 log IU/ml.
He was initially treated as per the HLH-2004 protocol with etoposide and high dose dexamethasone and subsequently commenced on Bortezomib and Valganciclovir for CAEBV.
Unfortunately, he suffered a fulminant course with persistent EBV viremia.
He progressed very rapidly and died of recurrent infective complications before his planned allogeneic stem cell transplant.

Biopsy Fixation Details

Standard technique

Frozen Tissue Available

No

Details of Microscopic Findings

Expansion of the interfollicular space by oedema and necrosis. Surrounding the necrotic areas is a population of polymorphic lymphoid cells, with small to intermediate sized cells predominating. The cells are arranged in sheets, but also in a noticeable perivascular distribution. Infiltration into the vessel walls is seen. No significant large cell population is seen. At the periphery of the node, residual follicular and perifollicular B-cell areas were present.

Immunophenotype

A population of 15% lymphocytes, which consist predominantly of NK cells (75%) with an expected immunophenotype CD2+, CD3-, CD7+, CD56+. Smaller numbers of CD4 and CD8 positive T cells within normal limits. No significant B cell population present. The presence of a relatively expanded population of NK cells is non-specific and may be a reactive phenomenon particularly in the setting of infection.

Cytogenetics

NA

Molecular Studies

A polyclonal pattern was observed for both sets of TCRg PCR primers. This result was demonstrable on both lymph node and bone marrow biopsy.

Proposed Diagnosis

Chronic active EBV infection- NK subtype. ?Polyclonal versus monoclonal type

Interesting Feature(s)

Epstein Barr virus can cause B cell lymphoproliferative disorders. Our case represents a rare disorder in which Epstein Barr virus infected the NK/T cells with an accompanying HLH in an individual who have no known immune deficiency. The polyclonal T cells and lack of T cell clonality on molecular tests made it quite challenging to establish the diagnosis. Whilst NK-cell CAEBV has a somewhat better prognosis than T-cell disease, our patient did not respond to antiviral therapy nor to intensive immune suppression and he died soon prior to his planned transplant.

Panel Diagnosis

EBV-positive LPD, favor systemic CAEBV disease, NK cell type, probably progressing to aggressive NK cell leukemia, with associated HLH

EA4HP24-BMWS-113

A Rare Case of an EBV-positive B-cell Lymphoproliferative Disorder Presenting in the Bone Marrow of an Immunocompetent Patient

Dr. Swati Gite, Dr. Sucheta Malik, Dr. Nourhan Ibrahim, Dr. Hanadi El Achi, Dr. Jacob Armstrong, Dr. Brenda Mai

University of Texas Health Science Center at Houston, Department of Pathology and Laboratory Medicine, Houston, Germany

Case Description

62-year-old female with a history of liver cirrhosis, hypothyroidism, hypertension, paroxysmal atrial fibrillation, and nephrolithiasis who presented to our facility with fever, night sweats, neck/bone pain, and weight loss.

Biopsy Fixation Details

Formalin-fixed formic acid-decalcified paraffin-embedded tissue

Frozen Tissue Available

None

Details of Microscopic Findings

The bone marrow biopsy displayed dense lymphohistiocytic infiltrate comprising heterogeneous lymphocytes, histiocytes, and scattered large atypical lymphocytes. The atypical cells had enlarged nuclei, irregular nuclear contours, and occasional prominent nucleoli.

Immunophenotype

- Flow cytometry performed on bone marrow aspirate showed no aberrancies.
- Immunohistochemical stains show many small T cells (CD3-positive), many histiocytes (CD68-positive), scattered large B-cells (positive for CD45, CD30, and PAX5 and negative for CD79a and CD15). EBER shows a few dimly positive large cells.

Cytogenetics

Cytogenetics analysis showed a normal female karyotype 46,XX [20] and myelodysplastic syndrome (MDS) fluorescence in situ hybridization (FISH) panel was negative.

Molecular Studies

Molecular studies were not performed.

Proposed Diagnosis

Polymorphous EBV-positive B-cell lymphoproliferative disorder.

Interesting Feature(s)

- EBV positive B-cell LPDs represent a wide and expanding clinicopathological spectrum ranging from indolent, self-limiting and localized conditions to highly aggressive lymphomas.

- Particularly in the recent years there has been an increased understanding of indolent EBV positive B-cell LPDs, which have historically been regarded as aggressive neoplasms.
- Although EBV is the main factor in etiopathogenesis, there are other factors like genetic rearrangement and state of host immune response which play an important role in these diseases.
- Lymphoproliferative disorders associated with immunodeficiency have been well described in the setting of transplantation.
- This rare case of primary bone marrow EBV-positive B-LPD occurred in the absence of obvious immunodeficiency or transplant.
- Therefore, polymorphic B-LPDs should still be considered as a possible diagnosis in an immunocompetent patient.
- Another interesting feature in this case was although numerous cases of polymorphic B-LPDs have been described in lymphoid tissue, primary involvement of bone marrow in B-LPD is uncommon.
- The management of these lesions in immunocompromised patients is usually removal of immunosuppressants with the lesion regressing after withdrawal of immunosuppression. In this case, interestingly, this patient needed treatment with chemotherapeutic agents. The patient was treated with two cycles of COP-based regimen and was disease free for a small interval before the lesion recurred again.
- Additional studies are needed for treatment optimization of this entity in the immunocompetent population.

Panel Diagnosis

Classic Hodgkin lymphoma

EA4HP24-BMWS-116

Medication – induced HLH: amox-clave/doxycilline related DRESS syndrome inducing HLH (forme fruste?).

Dr. Gorana Gasljevic[1], Dr. Biljana Grcar Kuzmanov[1], Prof. Veronika Kloboves-Prevodnik[2], Dr. Lucka Boltezar[3]

[1] Institute of Oncology Ljubljana, Department of Pathology, Ljubljana, Slovenia; [2] Institute of Oncology Ljubljana, Department of Cytopathology, Ljubljana, Slovenia; [3] Institute of Oncology Ljubljana, Division of Medical Oncology, Ljubljana, Slovenia

Case Description

25-yrs-old healthy male presented with high fever up to 40° and night sweating after returning from south Dalmatia. Initial lab: CRP 145, Leu 5, Ly 0.67, Hb 144, Plt 85. Antipyretics were prescribed. Since fever and sweating persisted, hemocultures and serologies were

tested for numerous viral, tick and mosquito-borne illnesses, STDs all being negative. He was treated with Doxycycline; liver enzymes became slightly elevated. After the partial improvement of the symptoms (in 2-3 w), he got worse again TT> 40° C, Leu 11.1, weight loss (10kgs); Amox-clav administration. Generalized rash appeared. Urgent rheumatologic investigations excluded rheumatic causes. He was re-directed to haematologists (Leu 8, Eos 6.1, Hb 93, PLts 136, CRP 153). Abd. US showed hepatosplenomegaly. PET CT: increased uptake in BM and abdominal LNs (SUV 5). First BMT (August): suspicious for 30-40% infiltration by unclassified LPN; further diagnostics from enlarged LN/spleen is needed. Liver functional tests started to rise, ferritin to 800, triglycerides to 1.89 and sIL2 receptor to 806 U/ml. Fibrinogen 6.5. LDH 5.7. 5/8 HLH criteria, HScore 173.

In 2w diagnostic splenectomy was performed and BMT repeated. Perioperatively, he was administered Cephazolin developing generalized skin rash again. Spleen weighted 700 g.

Additional work-up showed decreased population of B ly in peripheral blood, with increased activated T-cells.

After the splenectomy, general condition and laboratory parameters were improving in the course of a few w/mths, with no additional th. At the last control (Dec23), he was doing well. CRP 83, SR 39, Leu 8, E 4.86, Hb 124, Plts 590, Eos 3.9.

Biopsy Fixation Details

Spleen and LN node 10% BF.

BM Schaffer.

Frozen Tissue Available

Spleen and LN.

Details of Microscopic Findings

Band like infiltrates and poorly formed granuloma-like structures in BM occupying 30-40% of volume, consisting of histiocytes, small ly, eos, PMN, plcells and a few larger, transformed cells positive for CD20/PAX5, some weakly for CD30, MEF2B negative. No hemophagocytosis.

Second BMT is basically very similar, infiltrates are somewhat smaller. No hematophagocytosis.

Spleen shows follicular and MZ hyperplasia, numerous more or less formed granulomas with some PMN and hemophagocytosis. CD30 stains reactive immunoblasts. T markers are equally distributed, with no convincing downregulation. PD1 is stained in reactive pattern. CD20/PAX 5 stain mainly smaller lymphocytes; plasma cells and some larger, transformed cells are MUM1 positive. CD56 stains sprinkled, small lymphocytes in red pulp

Splenic hilar and neck LN show sinus histiocytosis, some small PMN and Eos aggregates

Immunophenotype

In MF section.

Cytogenetics

NA.

Molecular Studies

PCR in spleen was polyclonal for B and T cells.

Genetic testing for autoinflammatory diseases is in progress.

Proposed Diagnosis

Medication – induced HLH: amox-clave/doxycillinerelated DRESS syndrome inducing HLH (forme fruste?).

Interesting Feature(s)

HLH is only rarely induced by medications, most frequently TMP/SMX, lamotrigine, ICI, CART-T. Morphologically, peculiar band-like histiocytic proliferation in the bone marrow looks like lymphomatous infiltration. Poorly formed spleen and bone marrow granulomas - ?amox-clav induced.

Slow, spontaneous recovery with no additional treatment. Medication-induced HLH has relatively favorable outcome in comparison to other etiologies and responds well to steroid monotherapy.

Panel Diagnosis

HLH of undetermined aetiology, possibly due to an unidentified infection

EA4HP24-BMWS-122

Fulminant EBV+ Hemophagocytic Lymphohistiocytosis Mimicking a T-Cell Lymphoma

Dr. Meredith M. Nichols[1], Dr. Fabienne Lucas[2], Dr. Robert Padera[1], Dr. Sam Sadigh[1]

[1] Brigham and Women's Hospital, Department of Pathology, Boston, USA; [2] University of Washington, Department of Laboratory Medicine and Pathology, Seattle, USA

Case Description

A 77-year-old man had night sweats, anorexia, weight loss, dyspnea, and diffuse lymphadenopathy (LAD). Excisional lymph node (LN) biopsy was performed (case 1) and he received steroids with symptom improvement. He underwent bone marrow (BM) biopsy 1 month later (case 2). He presented 2 months later with hypoxia, anemia, thrombocytopenia, splenomegaly, hypertriglyceridemia, hyperferritinemia, renal failure, and increasing LAD. BM biopsy was performed (case 3). The patient decompensated the next day and passed away. An autopsy was performed (case 4).

Biopsy Fixation Details

Cases 1 & 2: Unknown

Case 3: Bouin's solution

Case 4: Formalin

Frozen Tissue Available

-

Details of Microscopic Findings

Case 1: The enlarged lymph node had patent sinuses and a paracortical polymorphous infiltrate of histiocytes, small lymphocytes, immunoblasts, plasma cells, and eosinophils. Areas of increased vasculature and associated nodules of small lymphocytes were present.

Case 2: The aspirate had 18% small lymphocytes. The biopsy was hypercellular with small interstitial and non-paratrabecular lymphoid aggregates.
Case 3: The aspirate was hypocellular. The biopsy was cellular with a polymorphous infiltrate of plasma cells and small to medium-sized lymphocytes and few larger nucleolated cells. There were numerous histiocytes with hemophagocytosis.
Case 4: Diffuse LAD and massive splenomegaly were present. Spleen and lymph nodes showed numerous histiocytes with hemophagocytosis.

Immunophenotype

Case 1: Lymphoid flow cytometry (FC): negative. Small CD20+ B cells were in follicles with focally expanded/distorted CD21+ follicular dendritic cell meshworks. CD3+ T cells had a CD4:CD8 ratio of 2:1 and expressed CD2, CD5, ICOS (subset), and CD7 (subset); they were negative for CD10, PD1, and CXCL13. CD30 highlighted immunoblasts. CD68 showed increased histiocytes. EBER was positive in rare cells.
Case 2: Lymphoid and plasma cell FC: negative. T cells in aggregates were CD4+ with CD5 coexpression and somewhat dim CD2 and CD3. Interstitial CD8+ T cells with intact pan T-cell antigens and TIA1 were increased relative to CD4+ T cells. EBER highlighted rare cells.
Case 3: Lymphoid FC was negative. T cells expressed CD3, CD5, and CD7 with admixed CD4+ and CD8+ cells. Subsets expressed TIA-1, PD1, or CXCL13. There were rare CD56+ cells and rare CD20+ B cells. CD30 highlighted scattered immunoblasts. Plasma cells were polytypic. HHV8 was negative. EBER was positive in numerous cells. CD68, PU.1, and CD163 showed numerous histiocytes.
Case 4: Immunostains showed numerous CD3+, CD4+ T cells and rare CD20+ B cells. CD30 highlighted immunoblasts. CD163 showed a robust histiocytic infiltrate. EBER was positive in numerous small cells.

Cytogenetics

Cases 1, 3, 4: Not performed (NP)
Case 2: 46,X,-Y[5]/46,XY[15]. FISH negative for rearrangements of *MYC*, *BCL6*, or *IGH/BCL2*

Molecular Studies

Case 1: T-cell clonality: Negative
Case 2, 3, 4: NP

Proposed Diagnosis

Fulminant EBV+ hemophagocytic lymphohistiocytosis (HLH) with associated CD4+ T-cell proliferation

Interesting Feature(s)

While the clinical findings, morphologic features in the LN biopsy, and autopsy gross findings were concerning for an evolving angioimmunoblastic T-cell lymphoma, molecular studies failed to show a clonal T-cell population and the T cells lacked immunophenotypic aberrancy. The morphologic and molecular discrepancy underscores the potential utility of lymphoid next-generation sequencing studies. It is unclear whether the fulminant HLH was caused by an undiagnosed lymphoproliferative disorder or by EBV infection; the patient expired before repeat LN biopsy could be performed.

Panel Diagnosis

Nodal T follicular helper cell lymphoma, angioimmunoblastic type (WHO-2022) /

follicular helper T cell lymphoma, angioimmunoblsatic type (ICC), with associated HLH

EA4HP24-BMWS-123

Post-COVID-19 Hemophagocytic Lymphohistiocytosis with Erythroid Dysplasia and Myeloid Hypoplasia

Dr. Joshua E. Lewis, Dr. Olga Pozdnyakova

Brigham and Women's Hospital, Pathology, Boston, USA

Case Description

The patient is an 83-year-old male with a history of follicular lymphoma, transformed to DLBCL, metastatic to the lungs, with complete remission of his disease following Breyanzi CD19 CAR-T therapy. In August 2023, he developed COVID-19 infection with treatment-refractory ARDS, requiring prolonged hospitalization. In November 2023, the patient presented with pancytopenia (WBC 0.98 K/µL, HCT 26.7%, PLT 105 K/µL) with increased ferritin (3,794 µg/L), but without fever or splenomegaly. A bone marrow biopsy was obtained to investigate the cause of his pancytopenia.

Biopsy Fixation Details

The bone marrow biopsy specimen was fixed in Bouin solution.

Frozen Tissue Available

Not available.

Details of Microscopic Findings

The bone marrow biopsy was normocellular for age and showed erythroid predominance with left-shifted and dysplastic erythroid maturation, with myeloid and megakaryocytic hypoplasia. Scattered interstitial histiocytes were present, with a subset showing hemophagocytosis. There was no increase in blasts and only scattered lymphocytes.
The bone marrow aspirate also showed an erythroid predominance (M:E ratio 0.1:1) with erythroid dysplasia and extensive hemophagocytosis of nucleated erythroid elements.
Flow cytometry demonstrated an expanded population of T-large granular lymphocytes, with no evidence of a B-cell lymphoproliferative disorder.

Immunophenotype

CD68 and CD163 immunostains, highlighting histocytes, confirmed the presence of hemophagocytosis. CD34 immunostain confirmed absence of an increased blast population, and PAX5 and CD3 immunostains showed scattered B- and T- cells, respectively. In-situ hybridization for EBV RNA, as well as parvovirus immunostain, were both negative.

Cytogenetics

The obtained karyotype was 46,XY[20].

Molecular Studies

Molecular testing identified variants in ASXL1 (p.Q695*, VAF 9.7%; p.E635Rfs*15, VAF 3.3%) and DNMT3A (p.E477L, VAF 2.7%).

Proposed Diagnosis

The presence of pancytopenia and hyperferritinemia, with hemophagocytosis on bone marrow biopsy, and absence of primary HLH-associated mutations, are suggestive of secondary hemophagocytic lymphohistiocytosis (HLH). This proposed diagnosis is supported by findings of elevated soluble CD25 (1,429.6 pg/mL; ref: 175.3-858.2 pg/mL) and resolution of the patient's pancytopenia with steroids. Additionally, the presence of marked erythroid dysplasia and myeloid hypoplasia, along with variants in ASXL1 and DNMT3A, raised the possibility of an accompanying MDS, but may be associated with the patient's HLH and the presence of CHIP, respectively. The expanded T-LGL population may be secondary to the patient's recent viral infection.

Interesting Feature(s)

While HLH following COVID-19 infection has been previously reported (PMID: 35699822, 33897909), the presence of marked erythroid dysplasia with myeloid hypoplasia has not been previously associated with post-COVID-19 HLH. Dyserythropoiesis has been noted in primary and secondary HLH, with resolution on post-treatment biopsies in two cases (PMID: 11729218). This correlation has been previously associated with inflammatory conditions including Kikuchi-Fujimoto disease (PMID: 27789440), but not for infectious etiologies. Future studies are warranted to determine if the association of HLH with dyserythropoiesis and myeloid hypoplasia is linked to particular causative etiologies, is a general feature of infectious/inflammatory causes of HLH, or is related to the concomitant development of MDS.

Panel Diagnosis

Reactive bone marrow with haemophagocytic activity post-COVID

EA4HP24-BMWS-129

A Cautionary Case of Reactive Increase in Blasts and Megakaryocyte Dysplasia in a Patient with Crohn's Disease Receiving Immunomodulatory Therapies

Dr. Georgi Lukose[1], Dr. Ivo SahBandar[1], Dr. Chandler Sy[1], Dr. Jia Ruan[2], Dr. Julia Geyer[3]

[1] Weill Cornell Medicine/New York-Presbyterian, Pathology and Laboratory Medicine, New York, USA; [2] Weill Cornell Medicine, Medicine, New York, USA; [3] Weill Cornell Medicine, Pathology and Laboratory Medicine, New York, USA

Case Description

Patient was a 73-year-old male with history of long-standing therapy refractory Crohn's disease with extensive perianal fistulae and perirectal abscesses, chronic kidney disease, and gout. The patient was being treated with methotrexate and sulfasalazine. One month prior to presentation, a trial of ustekinumab was started. Ciprofloxacin and metronidazole was also started in the setting of a developing gluteal fistula. Patient presented with two-week history of fatigue, weakness, loss of appetite, intermittent dizziness, increased diarrhea and weight loss. He also complained of worsening oral pain and odynophagia. CBC showed pancytopenia with WBC 1.7 K/mL[3.4-11.2], Hgb 8.9 g/dL[13.3-17.7], MCV 90.2 fL[81-100], PLT 55 K/mL[150-450], neutrophils 20%[47-75], lymphocytes 23%[20-50], monocytes 20%[2-11], eosinophils 29%[0-5], blasts 3%[0].

Biopsy Fixation Details

Bouin

Frozen Tissue Available

No

Details of Microscopic Findings

Marrow cellularity was increased for patient's age (~50%). Myeloid to erythroid ratio was normal. Myeloid maturation was markedly left-shifted with an increase in blasts (12%) and limited maturation to the neutrophils stage. Erythroid maturation was complete. Megakaryocytes were increased in number with small and large dysplastic-appearing forms. Abnormal localization of immature precursors was noted. A reticulin stain showed a mild increase in reticulin fibers.

Immunophenotype

Immunohistochemical stains revealed an increase in CD34+/CD117+ blasts, which formed clusters. CD42b highlighted numerous micromegakaryocytes. Flow cytometry was performed and showed a population of CD34+/CD117+ myeloid blasts (6%) which also expressed CD13, CD33, HLA-DR, and MPO (variable). Immunostains were performed on a clot specimen showing scattered CD20+ B cells and CD3+ T cells, which were not increased. Plasma cells were mildly increased in number (5%) but were polytypic for kappa and lambda immunoglobulin light chains by in situ hybridization. EBV-encoded RNAs by in situ hybridization was negative.

Cytogenetics

Chromosome analysis showed a normal male karyotype in 20 examined metaphase cells. No numerical or structural abnormalities of clinical significance were found in these cells.

Molecular Studies

None

Proposed Diagnosis

Hypercellular bone marrow with megakaryocytic hyperplasia and dysplasia, eosinophilia, myeloid left-shift and an increase in blasts (12%)

Interesting Feature(s)

Bone marrow (BM) dysplasia and increased blasts in conjunction with pancytopenia are concerning for high-grade myelodysplastic syndrome (MDS). However, the patient's complex clinical history of Crohn's disease treated with immunomodulatory agents raised the possibility of therapy induced BM changes. The lack of cytogenetic abnormalities was not supportive of MDS. The patient was initiated on ustekinumab one month prior to presentation with cytopenias. The concurrent use of methotrexate, ciprofloxacin, and metronidazole may have also contributed to cytopenias. Therefore, a descriptive diagnosis was rendered, and close observation and BM re-biopsy were recommended. Clinically, ustekinumab was continued, however cytopenias resolved. A follow-up BM biopsy one month later showed no increased blasts and no dysplasia. Subsequently, the patient has been followed for 7 years and has no evidence of myeloid neoplasm. We hypothesize that pancytopenia and BM findings were due to regenerative changes due to drug-induced myelosuppression. Complete and accurate clinical information, and close communication with the treating hematologist were crucial in avoiding a serious misdiagnosis in this patient.

Panel Diagnosis

Bone marrow with rare dysplastic features and transient increase in blasts in context of treatment for Crohn's disease.

EA4HP24-BMWS-133

A Cautionary Case of Mycobacterial Infection in a Post Bone Marrow Transplant Patient with Cytopenias

Dr. Georgi Lukose[1], Dr. Chandler Sy[1], Dr. Ivo SahBandar[1], Dr. Sebastian Mayer[2], Dr. Tsiporah Shore[2], Dr. Julia Geyer[3]

[1] Weill Cornell Medicine/New York-Presbyterian, Pathology and Laboratory Medicine, New York, USA; [2] Weill Cornell Medicine, Medicine, New York, USA; [3] Weill Cornell Medicine, Pathology and Laboratory Medicine, New York, USA

Case Description

Patient was a 53-year-old female with history of essential thrombocythemia with progression to acute myeloid leukemia (AML). Her treatment history included allogeneic stem cell transplant with relapse of disease, microtransplant cord infusion, followed by haplocord stem cell transplant. Immunosuppression and graft-versus-host disease prophylaxis included anti-thymocyte globulin, mycophenolate mofetil, and tacrolimus. Patient's post-transplant course had been complicated by chronic fatigue and cytopenias. Patient presented (~day 210 post transplant) with worsening dyspnea, diarrhea, recurrent fevers, severe anemia, and severe thrombocytopenia. The previous bone marrow biopsy (~day 180 post transplant) showed no evidence of recurrent disease but atypical megakaryocytes were seen. CBC showed WBC 1.1 K/mL[3.1-11.2], Hgb 7.6 g/dL[11.7-15.3], MCV 102.7 fL[81-100], PLT 8 K/mL[150-450], neutrophils 87%[45-75], and lymphocytes 5%[20-50]. CT of the chest showed new bilateral pulmonary and splenic nodules. Following the bone marrow biopsy, the patient was treated with vancomycin, meropenem, amikacin, and isavuconazole. During her hospital course, she had worsening sepsis and respiratory distress, new cerebellar stroke, and died shortly after presentation.

Biopsy Fixation Details

Bouin

Frozen Tissue Available

No

Details of Microscopic Findings

Bone marrow cellularity was ~60%. The myeloid to erythroid ratio was increased due to increase in granulopoiesis. Myeloid maturation was left-shifted. Erythroid elements were decreased with full maturation. Megakaryocytes were increased in number with small, hypolobated forms. No increase in blasts was seen. The bone marrow core biopsy and the clot section contained many ill-defined aggregates of spindle-shaped histiocytes, resembling non-necrotizing granulomatous inflammation. Acid-Fast Bacilli stain was performed which demonstrated numerous acid-fast bacilli.

Immunophenotype

Immunostains revealed no increase in CD34+/CD117+ blasts. MPO stained myeloid cells. CD71 stained erythroid precursors. CD61 stained numerous megakaryocytes. Flow cytometry was performed and showed no definite evidence of abnormal myeloid blasts.

Cytogenetics

Chromosome analysis revealed a donor male karyotype in 20 examined metaphase cells, 3 of which showed monosomy 7. FISH analysis showed XY cells in 99.6% of 500 interphase nuclei. The remaining 0.4% of cells showed XX cells. Interphase FISH analysis also identified monosomy 7 in 13% of 200 nuclei.

Molecular Studies

Tissue sections of the bone marrow clot section were submitted for PCR based identification of *Mycobacterium* tuberculosis complex and non-tuberculous *Mycobacteria*. *Mycobacterium kansasii* or *Mycobacterium gastri* was detected using 16S rRNA gene primer sets. The testing method did not distinguish between these organisms.

Proposed Diagnosis

Non-Tuberculous Mycobacterial Infection Involving the Bone Marrow

Interesting Feature(s)

In this patient with history of AML who had received a bone marrow transplant, worsening cytopenias may have suggested disease relapse. Instead, subtle ill-defined spindle cell aggregates of histiocytes were found. Well-formed necrotizing or non-necrotizing granulomas were not seen. However, well-formed granulomas are typically absent in immunocompromised patients. Thus, threshold for performing special stains to rule out infectious causes should be low. An AFB stain in our case highlighted numerous acid-fast bacilli. The patient turned out to have disseminated atypical mycobacterial infection and unfortunately passed away soon after the diagnostic bone marrow biopsy.

Panel Diagnosis

Non tuberculous Mycobacterial infection associated with immunosuppression (post alloSCT)

EA4HP24-BMWS-142

HHV-8 associated haemophagocytic syndrome

Dr. Mufaddal Moonim[1], Dr. Rashpal Flora[1], Dr. Ketan Patel[2]

[1] *Imperial College Healthcare Trust, Histopathology, London, UK;* [2] *Imperial College Healthcare Trust, Haematology, London, UK*

Case Description

35/M post renal transplant (22months) for ESKD of unknown aetiology admitted with anaemia, thrombocythaemia & sepsis. Investigations indicated graft failure, elevated ferritin & D dimers, lymphadenopathy not amenable to biopsy, ascites, pleural effusions and subcutaneous oedema. Progressed to multiorgan failure and succumbed. EBV viral load 19500. HHV-8 VL: >2 million copies/L.

Biopsy Fixation Details

AZF

Frozen Tissue Available

No

Details of Microscopic Findings

Hypocellular marrow for age (50%)

Bone marrow oedema.

Reactive changes in haemopoiesis secondary to systemic illness.

Erythroid hyperplasia and dyserythropoiesis secondary to microangiopathic haemoloytic anaemia.

Massive stromal macrophage expansion / activation and haemophagocytosis.

Heterogenous population of HHV-8(+) cells in bone marrow on immunohistochemistry.

No evidence of a co-existing lymphoproliferative disorder (PTLD).

Features of renal osteodystrophy

Immunophenotype

NA

Cytogenetics

NA

Molecular Studies

NA

Proposed Diagnosis

HHV-8 driven haemophagocytic syndrome

Likely HHV-8(+), HIV negative multicentric Castleman's disease (not proven histologically due to inability to biopsy a lymph node).

Interesting Feature(s)

Unique histology as it was possible to identify HHV-8(+) cells by immunohistochemistry within the bone marrow biopsy in this case of haemophagocytic syndrome (HLH).

Morphology of HHV-8(+) cells is heterogenous ranging from small lymphocyte like cells to large cells with lobated nuclei.

Florid features of haemophagocytosis.

Panel Diagnosis

HLH in the setting of immunosuppression (renal transplant) with HHV8 viraemia

EA4HP24-BMWS-144

Hemophagocytic syndrome: the search for the underlaying cause in the bone marrow

PhD/MD Marta Gómez Hernando, PhD/MD Melina Pol, PhD/MD Jose Ramon Álamo Moreno, Dr. Maria Rozman

Hospital Clinic de Barcelona, Unidad de hematopatología, Barcelona, Spain

Case Description

A 60-year-old woman from the Philippines was transferred from another hospital with the diagnosis of hemophagocytic syndrome secondary to an undiagnosed autoimmune disease. She had a 5-month history of fever of unknown origin with multiple associated symptoms (cutaneous erythema, anemia and thrombocytopenia considered as immune-related, hypophysitis). Physical exam showed no lymphadenopathy or hepatosplenomegaly. Laboratory findings were consistent with hemophagocytic syndrome (anemia and thrombocytopenia, hyperferritinemia and hypertriglyceridemia), though an initial bone marrow aspirate did not show any images of hemophagocytosis. She had a skin biopsy initially oriented as vasculitis and a lymph node biopsy that showed only low polyclonal plasma cells. Suspecting an underlaying autoimmune disease, she was treated with high doses of steroids and two doses of rituximab, developing various treatment-related infectious diseases. Her condition worsened and she was finally transferred to our hospital. Once the diagnosis was rendered, the patient was treated with R-CHOP with clinical and laboratory improvement after the first cycle.

Biopsy Fixation Details

B23-40931 A: fixation with formalin 10%, decalcification with formic acid 10%.

Frozen Tissue Available

No.

Details of Microscopic Findings

- Bone marrow aspirate: presence of atypical cells (6%) of medium-big size, irregular nuclei, condensed chromatin with visible nucleoli. Increased macrophages with multiple images of hemophagocytosis.
- Bone marrow biopsy: infiltration by approximately 20% of medium to large cells with round nuclei and condensed chromatin with a predominantly intrasinusoidal and, to a lesser extent, interstitial pattern.
- Lymphadenopathy (surrounding fat) and skin biopsy: on histological re-examination in our institution, intravascular infiltration by atypical B cells was observed.

Immunophenotype

- Flow cytometry: CD19+, CD20+, CD79b+, CD22+, CD5+, CD10+, CD200+, CD23-, CD43+ weak, CD31-, CD305-, CD11c-, CD103-, IgM+, CD38+, CD39+, CD95+, CD27-, CD81+, CD185+, HLA-DR+, CD39+, CD49d-.

- Immunohistochemistry: B-phenotype (CD20+, CD79a+, PAX5+) with coexpression of bcl-2, LMO2, CD5 and partial for C-myc. Kappa/lambda light chains and EBER were negative, and CD10, bcl-6 and MUM1 were not conclusive.

Cytogenetics

FISH for MYC (8q24), BCL2 (18q21) and BCL6 (3q27) were normal.

Molecular Studies

Digital PCR for MYD88 L265P positive in BM (5.17%) and lymphadenopathy (1.55%). NGS studies pending.

Proposed Diagnosis

Intravascular large B-cell lymphoma, hemophagocytic syndrome–associated variant.

Interesting Feature(s)

We present here an uncommon case in which a lymphoma associated with hemophagocytic syndrome was initially suspected by the findings in the bone marrow aspirate. The infiltration pattern seen in the bone marrow biopsy prompted deliberation between intravascular large B-cell lymphoma with an interstitial pattern and DLBCL with an intrasinusoidal component. Interstitial involvement in intravascular lymphomas has been previously described and, in our opinion, the overall clinical profile (ethnicity, fever of unknown origin, hemophagocytic syndrome), immunophenotype (CD5 and CD10 positive), molecular findings (MYD88 mutation), and the reexamination of skin and lymph node biopsies favor the diagnosis of intravascular large B-cell lymphoma.

1. M.Ponzoni, et al *Blood* 2018; 132 (15): 1561–1567.
2. B. Gonzalez-Farre, et al. The American Journal of Surgical Pathology 47(2):p 202-211.
3. A. M. R. Schrader, et al *Blood* 2018; 131 (18): 2086–2089.

Panel Diagnosis

Intravascular large B-cell lymphoma with associated HLH

EA4HP24-BMWS-148

Reactive myelofibrosis with features most suggestive of autoimmune myelofibrosis

April Chiu[1], Melissa Hart[1], **Kaaren Reichard**[1]

[1] Mayo Clinic, Laboratory Medicine and Pathology, Rochester, USA; [2] Mayo Clinic Health System, Pathology, Eau Claire, USA

Case Description

31-year-old male who presented to the ER in May 2023 due to six months history of syncope, night sweat, and fever. CBC showed severe anemia and thrombocytosis. CT showed mild splenomegaly (14cm). Additional workup including vitamin B12/folate, iron studies, Coombs

test, and serum protein electrophoresis were unremarkable. A bone marrow biopsy was performed.

Biopsy Fixation Details

B5 plus

Frozen Tissue Available

No

Details of Microscopic Findings

CBC: Hgb 7.7 g/dL; MCV 91.8 fL; RDW 15.2%; WBC 4.3 x 10 (9)/L; PLT 645 x 10(9)/L.
WBC differential: Neutrophils 65; lymphocytes 27; monocytes 7; basophils 1.
Peripheral Smear: Slight nonspecific anisopoikilocytosis; no cytologic abnormalities otherwise.
Bone marrow differential: Neutrophils 36; metamyelocytes 7; myelocytes 17; eosinophils and precursors 3; blasts 1; normoblasts 18; monocytes 6; lymphocytes 11; plasma cells 1.
Bone marrow aspirate/touch prep quality: Cellular.
Bone marrow biopsy and clot quality: Adequate.
M:E ratio: Normal, 3:1.
Cellularity: Hypercellular, 90%.
Erythroid precursors: Increased. Normal morphology.
Myeloid precursors: Increased . Normal morphology. Blasts not increased (<5%).
Megakaryocytes: Increased . Occasional atypical hyperchromatic forms; no clusters.
Lymphocytes: Benign-appearing lymphoid aggregates.
Plasma cells: Not increased.
Other findings: Dilated sinusoids.
Iron: Increased storage iron; ring sideroblasts not seen.
Reticulin: Reticulin fibers increased, myelofibrosis grade 1 (of 3).

Immunophenotype

Flow cytometric analysis: No monotypic B-cells/plasma cells, increase in blasts or aberrant pattern of myeloid maturation identified.
Immunohistochemistry: The lymphoid aggregate stains admixed small CD3-positive T-cells and CD20-positive B-cells. No increase in CD34-positive blasts.
CD61 highlights increased megakaryopoiesis.

Cytogenetics

Normal. 46,XY[20].

Molecular Studies

JAK2 V617F/exon 12-15, MPL, and CALR mutation analyses, peripheral blood: Negative.
Next generation sequencing (47-gene myeloid neoplasm panel), bone marrow:
Pathogenic Mutations: Not detected.
Variants of Uncertain Significance: None.

Proposed Diagnosis

Reactive myelofibrosis with features most suggestive of autoimmune myelofibrosis.

Interesting Feature(s)

The overall morphologic features, including bone marrow hypercellularity with increased and occasional hyperchromatic megakaryocytes and increased fibrosis, raise the differential diagnosis of autoimmune myelofibrosis vs. primary myelofibrosis. This

differential diagnosis and overlapping morphologic features are well-described in the literature. In our opinion, the overall findings are most suggestive of autoimmune myelofibrosis particularly given the mostly unremarkable megakaryocyte morphology and distribution, absence of a genetic abnormalities (chromosomes and next-generation sequencing), only mild degree of splenomegaly, and young age of the patient. Other potential etiologies could include chronic infection/inflammatory conditions and drug/medication/toxic effects.
The patient was enrolled in the DISC-0974 clinical trial for anemia of inflammation/chronic disease, utilizing monoclonal antibody against hemojuvelin which acts by suppressing hepcidin production, and thereby enhancing iron availability for erythropoiesis. After two cycles of therapy his hemoglobin and platelet levels returned to normal. A repeat biopsy performed in December 2023 showed normocellular bone marrow with resolution of megakaryocytic atypia, sinus dilatation, and fibrosis (MF-0). His symptoms also completely resolved and is currently at his baseline.

Panel Diagnosis
Reactive myelofibrosis, raising the possibility of autoimmune myelofibrosis.

EA4HP24-BMWS-156

Adult Onset Familial Hemophagocytic Lymphohistiocytosis with Heterozygous Mutations of *PRF1* and *STXBP2* following CAR-T Therapy for Diffuse Large B cell Lymphoma

Dr. Shuyu Er[1], Dr. Shaoying Li[1], Dr. Shimin Hu[1], Dr. Ming Guo[2], Dr. Jie Xu[1], Dr. L. Jeffrey Medeiros[1], **Dr. Pei Lin**[1]

[1] UT-MD Anderson Cancer Center, Hematopathology Unit 072, Houston, USA; [2] UT-MD Anderson Cancer Center, Pathology, Houston, USA

Case Description
A 15 yr old boy was diagnosed with DLBCL involving nodal and extranodal sites including a brain mass, treated with R-CHOP and intrathecal MTX, achieved CR. He developed fever, thrombocytopenia, splenomegaly 2 yrs later and underwent splenectomy. At age 18, he was diagnosed with relapsed DLBCL, treated with RICE and CAR-T (Yescarta), and again achieved CR. During the following 4 years, PET/CT showed multifocal lymphadenopathy, waxing and waning. Two lymph node biopsies found no relapsed lymphoma. At age 22, he presented to emergency room (ER) with left facial paralysis, rash and headache. MRI showed leptomeningeal enhancement. CSF revealed lymphocytosis and HHV6 positive. He received IV ganciclovir. CBC at ER visit: WBC: 6.7K/uL (39.9% neutrophils), Hb,13.3 g/dL, Hct: 37.4, Plt 341k/uL. However, neurological symptoms persisted and worsened after HHV6

turned negative. He also developed pancytopenia (neutrophils 17%, Hb 7.7, plt: 93 k/uL) months later. Other labs: sIL-2r: 1603 (normal range:175.3-858.2 pg/mL); Ferritin: 539 (30-400 ng/mL). Lymph node, bone marrow, and skin biopsy still showed extensive CD4+ T cell infiltrate. After extensive lab work up for infection and autoimmune disorders, and several rounds of multidisciplinary consultations, he was finally diagnosed with late onset familial HLH with mainly CNS disease and treated with HLH 2004 protocol. NGS testing on a blood sample found fHLH related mutations. He subsequently received alloSCT and remains in CR.

Biopsy Fixation Details

Fixation: 10% neutral buffered formalin

Frozen Tissue Available

NA

Details of Microscopic Findings

Lymph node needle core biopsy: Effaced nodal architecture with diffuse proliferation of small to medium sized lymphoid cells and scattered histiocytes

Bone marrow: Interstitial and nodular lymphoid aggregates of small to medium sized lymphoid cells with scattered histiocytes

Skin: Superficial and perivascular infiltrate of small to medium sized lymphoid cells

Cerebrospinal fluid (CSF): Increased small lymphocytes

Immunophenotype

Lymph node: Predominantly CD4+ T cells with diminished or absent BCL2 expression. CD8+ T cells and CD20+ B cells are rare. Ki67: 5-10%

Bone marrow: The infiltrating T cells are predominantly CD4+, polytypic for TRBC1, no aberrant expression. Granzyme B stains a small subset. EBER negative

Skin: Superficial and perivascular infiltrate of mainly CD4+ T cells

CSF: 93% CD4+ T cells

Cytogenetics

Bone marrow: 46,XY

Molecular Studies

NGS: 23 genes associated with primary HLH, whole blood

Heterozygous variant:

PRF1: g.72360214C>T, c.445G>A, p.Gly149Ser (p.G149S)

STXBP2: g.7707909C>T, c.1001C>T, p.Pro334Leu (p.P334L)

Bone marrow and lymph node:

An oligoclonal pattern of *TCRG* and *TCRB* gene rearrangements by PCR

Proposed Diagnosis

Adult Onset Familial Hemophagocytic Lymphohistiocytosis with Heterozygous Mutations of *PRF1* and *STXBP2* following CAR-T Therapy for Diffuse Large B cell Lymphoma

Interesting Feature(s)

- Heterozygous comutations of *PRF1* and *STXBP2*, the so called "digenic" inheritance, as predisposing risk factors for development of lymphoma, infection, and late onset familial HLH
- Developed aggressive B cell lymphoma with a brain mass at a young age of 15 yrs

- Marked expansion of CD4+ T cells instead of CD8+ T cells with diminished or absent BCL2 expression involving multiple lymph nodes and extranodal sites after CAR-T therapy, mimicking relapsed lymphoma on imaging studies and CAR-T induced T cell lymphoma by morphology.
- Predominantly neurological symptoms that preceded pancytopenia, accompanied by mildly abnormal values in sIL-2r and ferritin initially, and confounding HHV6+, causing delayed diagnosis

Panel Diagnosis

Adult Onset Familial Hemophagocytic Lymphohistiocytosis with Heterozygous Mutations of PRF1 and STXBP2

EA4HP24-BMWS-170

NK Cell Neoplasms in Hemophagocytic Lymphohistiocytosis (HLH)

Dr. Nadine Demko, Dr. Julia Geyer, Dr. Paul Simonson

Weill Cornell Medicine, Pathology and Laboratory Medicine, Division of Immunopathology, New York City, USA

Case Description

A 44-year-old male with a history of chronic kidney disease presented with pancytopenia with WBC 0.63 K/uL, ANC 0.0882 K/uL, hemoglobin 6.6 g/dL, and platelets 14 K/uL. Other parameters showed increased ferritin (6,836.3 ng/mL), triglycerides (442 mg/dL), soluble CD25/interleukin 2 receptor (260,506.6 pg/mL), CRP (3.9 mg/dL), and LDH (305 U/L) with decreased fibrinogen (148 mg/dL) and normal ESR (4 mm/hr). A PET-CT scan showed diffuse increased FDG avidity in the bone marrow, abdominal lymph nodes, spleen, lung, and cortex.

Biopsy Fixation Details

A bone marrow biopsy was performed, fixed in Bouin's solution, and subsequently decalcified.

Frozen Tissue Available

Yes

Details of Microscopic Findings

Marrow cellularity is hypercellular for the patient's age (>90% cellularity). Hemophagocytosis and increased interstitial lymphocytosis are identified. The myeloid to erythroid ratio is decreased due to an erythroid hyperplasia. Various stages of myeloid maturation are present. Blasts are not increased in number. Erythropoiesis is left-shifted and megaloblastoid (>10%). Dyserythropoietic forms are seen, including nuclear budding and bi-nucleation. Megakaryocytes are adequate in a number with a range of normal morphology.

Immunophenotype

Immunohistochemical studies: CD3 highlights increased T cells (30-40%). CD2, CD5, and CD7 do not demonstrate overt loss. The CD4 to CD8 ratio is ~1:1. CD56 stain highlights 5-10% of cells. CD57 highlights <5% of cells. TIA-1 highlights 30-40% of cells. Perforin and granzyme highlight 10-20% of cells. CD20 highlights rare B cells. CD163 and CD68 highlight numerous histiocytes and marked hemophagocytosis. EBER situ hybridization and LMP are negative. Bone marrow flow cytometry demonstrated an abnormal NK cell population (6.7% of viable cells, 13% of lymphocytes) that co-expresses CD2, CD56, CD16 and CD26 (subset), and is negative for sCD3, CD4, CD5, CD7, CD8, and CD57. Background T cells show no loss of pan-T-cell antigens. The CD4:CD8 T cell ratio is 2.7:1. CD3+, CD57+ large granular lymphocytes are elevated (18% of viable cells, 38% of lymphocytes). Lymphocytes, monocytes, and granulocytes are approximately 47%, 5%, and 0.02% of viable cells, respectively.

Cytogenetics

Conventional karyotyping revealed a complex karyotype:
46,XY,t(1;3)(q32;q29),del(6)(q13q23),del(11)(q21q23),add(15)(p11.2)[1]/92,idemx2[1]/46,XY[18].
FISH results were negative for deletion of 1q21.3, MYB, KMT2A locus, and EVI1 gene rearrangement.

Molecular Studies

T-cell receptor gamma chain results showed a weak clonal population in a polyclonal background by PCR. Next-generation sequencing did not detect any clinically relevant alterations. The following variants of unknown significance were detected: ANKRD26 c.4486G>C (VAF 48.1%), ATM c.1049C>T (VAF 51.1%), BRINP3 c.1511C>T (VAF 20.9%), CTC1 R295C c.883C>T (VAF 5.1%), POT1 c.817C>T (VAF 9.3%), SRP72 c.1030C>A (VAF 31.7%).

Proposed Diagnosis

Hemophagocytosis and abnormal NK cell neoplasm for which the differential includes aggressive NK cell leukemia and extranodal NK/T lymphoma.

Interesting Feature(s)

It has been reported that 6q and 11q losses are among recurrent regions that are characteristic of extranodal NK/T lymphoma, nasal-type group. These losses have been reported as more frequently observed in extranodal NK/T lymphoma compared to aggressive NK cell leukemia. However, it should be noted that prior specimens demonstrated the patient's abnormal NK cells express CD16, more commonly observed in aggressive NK cell leukemia. This case illustrates the importance of considering small involvement by NK cell neoplasms in hemophagocytic lymphohistiocytosis.

Panel Diagnosis

Aggressive NK cell leukemia with associated HLH

EA4HP24-BMWS-174

An interesting case of visceral leishmaniasis in the bone marrow

Dr. Elisa Lin, Dr. Sharon Germans

University Of Texas Southwestern, Hematopathology, Dallas, USA

Case Description

A 50-year-old male in Texas with a history of AIDS (CD4 count 82 cells/mm^3) presented with new-onset pancytopenia (hemoglobin 7.1 g/dL, platelet 71 x 10^9/L, white blood cell 1.75 x 10^9/L), abdominal pain, and no recent travel history. Imaging revealed hepatosplenomegaly and diffuse lymphadenopathy, which raised concern for possible lymphoma. During the course of his hospital admission, he had an acute gastrointestinal bleed with anemia, causing hypotension and coagulopathy. A complete workup, including a bone marrow biopsy, was done to investigate the pancytopenia and to rule out lymphoma.

Biopsy Fixation Details

The bone marrow biopsy was fixed in a formalin fixative.

Frozen Tissue Available

Not applicable

Details of Microscopic Findings

The bone marrow aspirate showed scattered oval- to teardrop-shaped intracellular and extracellular organisms, scattered plasma cells, and small lymphocytes in a background of trilineage hematopoiesis. Bone marrow core biopsy examination revealed a hypercellular marrow (~90%) with intracellular and extracellular organisms that stained negative by Grocott's methenamine silver stain (compared to *Histoplasma*, which stains positive) and periodic acid-Schiff stain, consistent with amastigote forms of *Leishmania*. Also, present was a prominent lymphohistiocytic infiltrate with several polytypic plasma cells and reactive lymphoid aggregates. Touch imprint smears showed several amastigote forms that stained positive by Giemsa stain. While *Histoplasma* is primarily intracellular, *Leishmania* is seen both intracellularly and extracellularly with the presence of a kinetoplast. Since this case displayed both intracellular and extracellular organisms, this also increased suspicion for *Leishmania.* No evidence of hemophagocytosis was noted on the bone marrow exam.

Immunophenotype

Not applicable

Cytogenetics

Not applicable

Molecular Studies

Not applicable

Proposed Diagnosis

The Centers for Disease Control was contacted and the patient was diagnosed with *Leishmania infantum* infection; pancytopenia improved with amphotericin B treatment. IgG antibody directed at *Leishmania* was detected.

Interesting Feature(s)

Leishmania infantum is the causative agent of visceral leishmaniasis in the New World with endemic regions extending from the southern United States to northern Argentina; coinfection with HIV is associated with high infectious burden. Most patients reported previously in non-endemic regions presented with cytopenias, hepatosplenomegaly, or hemophagocytosis, likely due to late diagnosis. In this patient, there was suspicion for hemophagocytic lymphohistiocytosis (HLH), which is a pathologic immune activation syndrome, due to splenomegaly, peripheral blood cytopenia with hemoglobin <9 g/dL and platelet <100 x 10^9/L, bleeding, and lymphohistiocytic infiltrate in the bone marrow. HLH often presents as an acute illness with multiple organ involvement, as was the case in this patient. There was no evidence of hemophagocytosis on the bone marrow exam. The bone marrow examination was crucial to this case, which highlighted the underlying infectious etiology that led to immune activation. This highlights a rare and interesting case of non-endemic visceral leishmaniasis resulting in immune activation.

Panel Diagnosis

Leishmaniasis (in the context of AIDS)

EA4HP24-BMWS-175

An interesting case of MAS/HLH in the setting of autoimmune disease and possible EBV reactivation

Dr. Elisa Lin, Dr. Sharon Germans

University Of Texas Southwestern, Hematopathology, Dallas, USA

Case Description

A 41-year-old male with a history of an autoimmune disease (polyclonal gammopathy with positive results for the following antibodies: ANA with high titer, TIF1-γ, PM/Scl-100, aCL, B2GP1, p-ANCA), splenomegaly, chronic anemia, and cryptococcal pneumonia presented to the emergency room with abdominal pain and melena. He subsequently developed respiratory failure requiring intubation, profound distributive shock and sepsis, and cholestatic liver disease. Laboratory values showed severe refractory thrombocytopenia unresponsive to IVIG/steroids, anemia requiring multiple pRBC units (hemoglobin 8.2 g/dL, platelet 5 x 10^9/L), elevated ferritin (>5,000 ng/mL), significantly elevated soluble CD25 (9615.2 pg/mL, normal 175.3-858.2 pg/mL), elevated CXCL9 (7,133 pg/mL, normal <647 pg/mL), elevated liver enzymes, and evidence of coagulopathy.

His presentation raised suspicion for macrophage activation syndrome/hemophagocytic lymphohistiocytosis (MAS/HLH) and an extensive workup was performed. A quantitative nucleic acid amplification test detected the presence of EBV. IgG antibody directed at EBV was detected (>750.0 U/mL, normal 0-21.9 U/mL). NK cell activity assay showed low NK cell activity at 0.25% NK cells.

Biopsy Fixation Details

The bone marrow biopsy was fixed in formalin fixative.

Frozen Tissue Available

Not applicable

Details of Microscopic Findings

The peripheral blood smear examination revealed mild neutrophilic leukocytosis, normocytic normochromic anemia, and marked thrombocytopenia. The bone marrow aspirate showed several scattered reactive plasma cells, histiocytes (many hemosiderin-laden), and a few cells demonstrating leuko- and erythrophagocytosis. The bone marrow core biopsy examination demonstrated a hypercellular bone marrow (~70%) with trilineage hematopoiesis, increased polytypic plasma cells (~30-35%), histiocytes, and small lymphocytes. Storage iron was increased; there was no increase in IgG4 plasma cells. The bone marrow core biopsy showed negative staining by acid-fast stain, Grocott's methenamine silver stain, and HHV-8 stain. The *in situ* hybridization stain for Epstein-Barr virus (EBV) highlighted rare, scattered positive cells (~1%).

Immunophenotype

Flow cytometric study on the bone marrow aspirate smear revealed polytypic B cells and plasma cells and no definitive immunophenotypic evidence of a hematolymphoid malignancy.

Cytogenetics

Not applicable

Molecular Studies

Not applicable

Proposed Diagnosis

MAS/HLH in the setting of EBV reactivation with underlying unspecified autoimmune disease

Interesting Feature(s)

HLH is a rare, aggressive, and life-threatening syndrome of excessive immune activation. Although it is most often seen in the pediatric population, it can be observed in adults as well. MAS refers to a form of HLH in patients with rheumatologic diseases. The criteria for diagnosis of HLH was met in this patient with splenomegaly, peripheral blood bicytopenia (anemia and thrombocytopenia), hemophagocytosis in bone marrow, low NK cell activity, elevated ferritin, elevated soluble CD25, and elevated CXCL9. Bone marrow pathology can aid diagnosis by providing a clue to the underlying etiology. A diffuse lymphohistiocytic and plasma cell infiltrate is a feature of recurrent antigen stimulation, which can be seen with immunodeficiencies such as HIV. This is an interesting case of MAS/HLH in the setting of possible EBV reactivation with an underlying autoimmune disease.

Panel Diagnosis

MAS/HLH in the setting of EBV reactivation and underlying autoimmune disease

EA4HP24-BMWS-201

Hemophagocytic Lymphohistiocytosis Secondary to Epstein-Barr Virus Infection

Dr. Sandra Sanchez, Dr. Jose Alamo, Robert Albero, Mónica López, Dr. Ece Ozogul, Dr. Nuria Vidal-Robau, Dr. Melina Pol, Dr. Gerard Frigola, Dr. Olga Balagué, Dr. Antonio Martínez

Hospital Clinic, Anatomía Patológica, Barcelona, Spain

Case Description

A 42-year-old male from Morocco presented with a 6-week history of B-symptoms and arthralgia. Physical examination showed hepatosplenomegaly and CBC revealed pancytopenia. Lab tests showed positive IgM+/IgG- for parvovirus and high IgG for R.conorii.

His pancytopenia worsened, with marked thrombocytopenia. He was treated, without response.

Lab tests showed: Hb: 8.5 g/L, WBC: 4400, PTL: 21000, creatinine: 2 mg/dL, AST/ALT: 1045/349 U/L, GGT/FA: 254/942 U/L, bilirubin: 13.6 mg/dL, CRP: 1.47 mg/dL, LDH: 4950 U/L, marked elevation of ferritin (up to 50,000 mg/dL), and soluble CD25 (66,000 pg/mL). These findings were highly suggestive of hemophagocytic syndrome.

Thoracoabdominal CT scan and PET showed diffuse FDG uptake in bone marrow and spleen and pulmonary infiltrates (possibly infectious), splenomegaly. Bronchoscopy showed changes consistent with alveolar hemorrhage.

PCR was positive for EBV (1,000,000 copies) and HSV. Anti-aspergillus antibodies (AGA) were positive. Tests for other microorganisms were negative.

The patient developed secondary pulmonary aspergillosis and sepsis, despite extensive treatment, the patient continued to deteriorate progressively with multiple organ failure and finally passed away.

Biopsy Fixation Details

10% neutral buffered formalin.

Frozen Tissue Available

Yes.

Details of Microscopic Findings

A liver biopsy showed a relatively preserved architecture and no reticulin increase. Hepatocellular and canalicular bilirubinostasis with bile plugs were noted.

Some liver lobules were focally disrupted due to increased intrasinusoidal lymphohistiocytic cells (CD68+), with focal hemophagocytosis. Aggregates of T cells with slight cytological atypia were identified, with normal expression of surface CD3, CD5 and

CD7, without CD56, and normal distribution of beta and delta TCRs. Numerous EBER(+) intrasinusoidal cells were noted. Immunohistochemistry for parvovirus was negative. Isolated B cells were observed.

Immunophenotype

A bone-marrow biopsy revealed a hypercellular marrow (>90%), with normal bony trabeculae and very extensive areas of necrosis (~60% of the core biopsy), with abundant hemosiderin-laden macrophages.

Marked increase in activated M2-type macrophages (CD68-KP1+, CD163+) with focal large cluster formation and prominent hemophagocytosis were seen. The intact marrow particles showed a prominent increase in erythroid precursors (cadherin-E+), with focal megaloblastoid changes. The myeloid series was diminished. Megakaryocytes were normal. Scattered CD20(+) B cells and CD3(+) T cells were detected.

In situ hybridization for EBV-encoded RNA (EBER) was positive in just a few isolated cells. Flow cytometric immunophenotyping showed diminished granulocyte maturation, with no evidence of hematologic malignancy.

Cytogenetics

N/A

Molecular Studies

TCR gamma chain was polyclonal.

Proposed Diagnosis

-Hemophagocytic syndrome with associated bone marrow necrosis secondary to EBV infection and acute hepatitis with marked intrasinusoidal lymphohistiocytosis.

-No evidence of hematologic or metastatic malignancy.

Interesting Feature(s)

Viral infections such as Epstein-Barr virus (EBV) are a well-recognised trigger of Hemophagocytic lymphohistiocytosis (HLH). In our patient primary EBV infection was the cause for secondary HLH. Distinguishing a lymphoma progression from EBV-associated HLH is difficult due to the extensive overlap in the disease characteristics between the two entities. A biopsy is required for a confirmatory diagnosis. This case highlights the potential role of EBV infection in causing fulminant HLH without a lymphoproliferative disorder associated.

Panel Diagnosis

HLH in the setting of EBV reactivation after Parvovirus infection

EA4HP24-BMWS-206

Complicated polycythemia vera: progressive monocytosis with hemophagocytic lymphohistiocytosis

Dr. Giorgio A. Croci[1,2], Dr. Daniele Cattaneo[4,3], Dr. Anna C. Migliorini[3], Dr. Federico Colombo[3], Dr. Cristina Bucelli[3], Dr. Valentina Bellani[3], Dr. Alessandra Iurlo[3], Dr. Carlo Pescia[2]

[1] University of Milan, Department of Pathophysiology and Transplantation, Milano, Italy; [2] Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Ca' Granda Ospedale Maggiore Policlinico, Division of Pathology, Milano, Italy; [3] Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Ca' Granda Ospedale Maggiore Policlinico, Hematology Division, Milano, Italy; [4] University of Milan, Department of Oncology and Hemato-Oncology, Milano, Italy

Case Description

76-year-old male; diagnosed with *JAK2 V617F+* essential thrombocythemia in 2000, managed with antiplatelets.

In 2011: bone marrow biopsy (BMB) performed for increasing leukocyte count and hematocrit, diagnostic for polycythemia vera (PV) with minor reticulin fiber increase (MF-0/1), followed by initiation of hydroxyurea.

Since 2021: detection of monocytosis (680/mmc, 10.1% of WBC), steadily increasing over the months (in Jan 2022, Mo 1.220/mmc, 13.2%).

Aug 2022: admitted to the emergency room due to exertional dyspnea, asthenia, and fever (38°C).Blood tests: anemia (Hb 8.1 g/dL), thrombocytopenia (8.000/mmc), 10.640/mmc WBC with monocytosis (ANC 7.130/mmc; Mo 2.340/mmc, 22%), increased ferritin (1.707 mg/L) and aspartate aminotransferase/SGOT (49 IU/L); triglycerides not measurable. Abdominal sonography reveals splenomegaly (18 cm).

Piperacillin/tazobactam was initiate, but cholestasis escalated and declining blood counts necessitated daily blood transfusions. Chest and abdomen CT scans confirmed splenomegaly; PET scans indicated increased BM and hepato-splenic fixation. To rule out leukemic evolution, a BM aspirate and BMB were performed (see microscopy).

A diagnosis of PV with monocytosis and macrophage activation syndrome / secondary hemophagocytic lymphohistiocytosis (MAS/HLH) was rendered (HLH-2004 diagnostic criteria satisfied).

Shortly after, sudden abdominal pain prompted a contrast CT scan, revealing small bowel thickening and perihepatic effusion. Rapid clinical deterioration ensued, marked by acute abdominal pain, peritonism, fever, and lactacidemia, leading to the patient's demise.

Biopsy Fixation Details

EDTA decalcified; 10% buffered formalin.

Frozen Tissue Available

No

Details of Microscopic Findings

BMB, 2022 (submitted biopsy)

90% cellularity with panmyelosis, dyserythropoiesis, dysgranulopoiesis, MF-1 fibrosis, 6-7% blast count and an increase in monocytes and histiocytes, the latter featuring interstitial and intrasinusoidal aggregation, with aspects of hemophagocytosis.

Autopsy, liver and spleen

Heavy intrasinusoidal accumulation of histiocytes, with features of cytophagocytosis. Red pulp expansion with accumulation of histiocytes and hemophagocytosis; foci of subcapsular infarction and only minimal features of extramedullary hemopoiesis

Immunophenotype

Flow cytometry (Aug 22)

1,3% myeloid blasts (CD34+/CD117+/HLA-DR+)

20% cells with monocytic scatter, including two separate subsets:

- 70% CD14+/CD13+/CD15+(80%)/CD33+(bright)/CD56+/HLA-DR+/CD2-/CD16-(8%)/CD34-/CD117-;
- 30%CD14+/CD13+(bright)/CD15+(dim)/CD16+/CD33+/CD56+/HLA-DR+/CD2-/CD34-/CD117-.

BMB (Aug 22)

6-7% CD34+ blasts; increase in CD14+ monocytes and histiocytes

Cytogenetics

46, XY

Molecular Studies

JAK2 V617F mutation was detected employing allele specific ddPCR

Proposed Diagnosis

PV "progression" with monocytosis and macrophage activation syndrome / secondary hemophagocytic lymphohistiocytosis (MAS/HLH)

Interesting Feature(s)

Monocytosis, can be observed in MPN and particularly in PV as a potential chronic myelomonocytic leukemia (CMML)-like progression. Our patient displayed an initial, steady increase in monocytes, supportive of so-called monocytosic progression of MPN, but his condition abruptly deteriorated within a clinical picture consistent with MAS/HLH. At the time of MAS/HLH, a relative increase in the intermediate type (CD14+/CD16+) monocytes, as compared to classical (CD14+/CD16-), supporting a clinic-pathologic pattern of reactive over a CMML-type monocytosis.

HLH is challenging to diagnose in adult patients and is frequently associated with malignancies, but it is an exceedingly rare complication in MPN.

Panel Diagnosis

PV with CMML-like progression and associated HLH.

EA4HP24-BMWS-210

Unexpected guest in bone marrow biopsy; Leishmania amastigotes –so is it myeloma or not?

Dr. Beril Guler[1], Dr. Busra Cosanay Tekden[1], Prof. Guven Cetin[2]

[1] Bezmialem Vakıf University, Pathology, Istanbul, Turkey; [2] Bezmialem Vakıf University, Hematology, Istanbul, Turkey

Case Description

A 55-year-old male patient, who had B symptoms for a year, was examined for pancytopenia and IgG kappa monoclonal gammopathy. In PET-CT images, FDG uptake in the skeletal system was diffusely increased (SUVmax:6), evident in the vertebral column, pelvic bones, bilateral humerus and proximal femur. No lytic lesion was detected. The craniocaudal dimension of the spleen was measured as 21 cm (SUVmax:2.6) and that of the liver was measured as 24 cm. In biochemistry and hemogram tests; Albumin:2.1, Urea:21, Creatinine:0.74, LDH:348, IgM:677, IgG:4896, IgA:102, Beta-2 microglobulin:10.8, Kappa (urine):380, Lambda (urine):256, WBC:2290, Neutrophil:300, Lymphocyte:1430, Hb:8.85, Hct:27.32, MCV:82.21, Plt:113000. Hepatic viral markers and HIV were negative. M protein was identified in serum. Kappa and lambda light chain were detected in urine immunofixation electrophoresis. Bone marrow aspiration flow cytometric evaluation reported 4.5% abnormal clonal plasma cells. Following histopathological evaluation, Leishmania spp DNA (PCR) was detected positive in the whole blood.

Biopsy Fixation Details

10% neutral buffered formalin - 8 hours decalcification with 10% formic acid.

Frozen Tissue Available

-

Details of Microscopic Findings

-Bone marrow: Ten intertrabecular areas of similar characteristics were hypercellular and fibrotic. A polymorphic cellular population consisting of lymphocytes, plasma cells, and histiocytes with foamy/vacuolated cytoplasm was observed, mixed with the local hematopoietic cells of the bone marrow. No nodule or mass formation was detected, except for scattered small lymphoid aggregates. Plasma cells were interstitially dispersed or perivascularly arranged. Microorganisms compatible with Leishmania amastigotes were detected in the cytoplasm of histiocytes at high magnification.

-Liver: Lymphocytic infiltration rich in T cells was seen in the portal areas. Microorganisms compatible with Leishmania amastigotes were observed in the Kupffer cell cytoplasm.

Immunophenotype

CD138: (Positive in plasma cells), Kappa/Lambda:(Polytypic staining in plasma cells).

Cytogenetics

-

Molecular Studies

-

Proposed Diagnosis

Visceral leishmaniasis.

Interesting Feature(s)

Leishmania is a parasite we are not accustomed to seeing in bone marrow biopsy. The presented case demonstrates that visceral leismaniosis may be a trap for misdiagnosis of plasma cell myeloma.

Panel Diagnosis

Leishmaniasis

EA4HP24-BMWS-220

HHV-8 associated Hemophagocytic Lymphohistiocytosis – an overlap with Kaposi Sarcoma Inflammatory Cytokine Syndrome (KICS)?

Dr. Sarah E. DePew[1], Dr. Gaurav Goyal[2], Dr. Adam C. Wilberger[1], **Dr. Aishwarya Ravindran**[1]

[1] The University of Alabama at Birmingham, Division of Laboratory Medicine-Hematopathology, Birmingham, USA; [2] The University of Alabama at Birmingham, Hematology-Medical Oncology, Birmingham, USA

Case Description

A 48-year-old male with a clinical history of HIV/AIDS and Kaposi sarcoma(KS) s/p treatment with doxorubicin x2 years, presented with fever, chills/rigors, drenching night sweats, shortness of breath, and lower extremity edema. CBC revealed pancytopenia and additional labs showed hyperferritinemia, elevated CD25, hypofibrinogenimia, hyponatremia, hypoalbuminemia, and hypertriglyceridemia, raising concern for hemophagocytic lymphohistiocytosis (HLH). EBV and hepatitis serologies were negative. CT-chest/abdomen revealed diffuse ground glass and nodular opacities throughout bilateral lungs, mild splenomegaly with mildly enlarged portocaval lymph nodes (1.1 cm in greatest dimension), and mild free pelvic fluid collection and anasarca. A bone marrow biopsy showed a hypercellular bone marrow with pan-hyperplasia and associated hemophagocytosis. In addition, given the history of KS, an HHV8 immunostain was performed, which showed scattered HHV8-positive cells, and subsequent peripheral blood HHV8 DNA testing by quantitative PCR revealed 405,248 copies/mL.The bone marrow was otherwise negative for a hematolymphoid neoplasm and KS. The patient subsequently improved on treatment with rituximab and paclitaxel

Biopsy Fixation Details

BM decalcified in hydrochloric acid-EDTA, formalin-fixed and paraffin-embedded

Frozen Tissue Available

None

Details of Microscopic Findings

H&E-stained section shows a hypercellular bone marrow (~70% cellularity) with panhyperplasia. A Wright-Giemsa stained bone marrow aspirate smear shows multiple foci of hemophagocytosis of nucleated erythroid precursors. No lymphoid follicles were noted.

Immunophenotype

Polytypic plasmacytosis was present, and CD163 marked foci of hemophagocytosis. Scattered HHV-8 positive cells (also known as viroblasts) were present.

Cytogenetics

Chromosome analysis, bone marrow: 46,XY[20]

FISH analysis, bone marrow: The following abnormalities were tested and 'not detected:'

-5/5q-, -7/7q-, +8, 17p-, and 20q-

Molecular Studies

B-cell immunoglobulin Gene Rearrangement by PCR, bone marrow aspirate: Negative

Proposed Diagnosis

Hemophagocytic Lymphohistiocytosis secondary to Kaposi's Sarcoma-associated Herpes Virus (KSHV), cannot exclude Kaposi Sarcoma inflammatory cytokine syndrome (KICS)

Interesting Feature(s)

1. KICS is a rare syndrome that is exclusively diagnosed in HIV patients with concurrent HHV8 infection and has overlapping features with HLH and multicentric Castleman's disease (MCD). The lack of significant lymphadenopathy in conjunction with bone marrow morphology (absent lymphoid follicles) essentially excludes MCD in this case (Bacon *et al.*, *Br J Haematol.* 2004, PMID: 15566362)2. KICS refers to a constellation of clinical features (fever, edema, respiratory symptoms, altered mental status), radiologic findings (lymphadenopathy, hepatosplenomegaly, body cavity effusions), and laboratory abnormalities (cytopenia, hypoalbuminemia, hyponatremia, elevated KSHV viral load >1000 copies/mL) (Cantos *et al., Open Forum Infect Dis.* 2017, PMID: 29766014)
3. While some of the above features overlap with HLH, the findings in this case (hyperferritinemia, elevated soluble IL2 receptors (CD25), hypertriglyceridemia, hemophagocytosis in bone marrow, cytopenias) are more favorable towards HLH secondary to HHV8 over KICS; nevertheless, these appear to exist in a spectrum and result in significant morbidity/mortality rates, necessitating prompt diagnosis and management
4. This case underscores the importance of HHV8 testing in HIV cases in the setting of acute clinical presentation with pancytopenia

Panel Diagnosis

HLH in the setting of immunosuppression (HIV) with HHV-8 viraemia

EA4HP24-BMWS-277

Postpartum Presentation of Anaplastic Large Cell Lymphoma (ALCL), ALK-positive with Associated Hemophagocytic Lymphohistiocytosis (HLH)

Dr. Parnaz Daneshpajouhnejad, Dr. Gabriel C. Caponetti

Hospital of the University of Pennsylvania, Department of Pathology and Laboratory Medicine, Philadelphia, USA

Case Description

A 26-year-old female with no significant past medical history and an uneventful cesarean section a month before admission presented with fever and tachycardia, which raised a concern for pulmonary embolism (PE). During her hospitalization, a PE was not found, although other findings were suspicious for retained products of conception and presumed endometritis. A total abdominal hysterectomy was performed, although an infectious process was not identified. CT angiogram revealed hepatosplenomegaly, and diffuse lymphadenopathy. Laboratory studies showed elevated ferritin (11400 ng/mL) and soluble IL-2 receptor (241900 pg/mL), low fibrinogen (106 mg/dl), anemia, and thrombocytopenia. The serum EBV viral load was <975 copies/mL. NK functional studies were normal. The findings were compatible with HLH. Bone marrow (BM) studies and a left supraclavicular lymph node (LN) biopsy were performed, and the diagnoses of HLH and ALCL, ALK+were established. The patient was treated with HyperCVED and CHOEP, achieved complete response and sustained remission.

Biopsy Fixation Details

Neutral buffered formalin

Frozen Tissue Available

-

Details of Microscopic Findings

BM: Wright-Giemsa-stained slides of the aspirate smears and touch preparations revealed numerous hemophagocytic macrophages. No dysplastic or malignant cells, and no increased proportion of blasts were noted. H&E-stained sections of the BM biopsy revealed normocellular marrow (80%) with unremarkable trilineage hematopoiesis, and a few hemophagocytic histiocytes.

LN: H&E-stained sections display fragments of lymphoid tissue composed of a mixture of frequent small to large atypical lymphoid cells with variably irregular nuclei (including ”hallmark” forms and "donut-like" nuclei), few conspicuous nucleoli, vesicular chromatin, and moderate cytoplasm.

Immunophenotype

BM: Not performed.

LN: Immunohistochemistry show that atypical lymphoid cells are positive for CD30 (strong, uniform), ALK, CD2, CD25, TIA-1, perforin, and granzyme B, partially positive for CD3, CD4, CD5, and BCL6, and negative for CD8, CD20, PAX5, CD10, CD56, BCL2, PD1, TCR-gamma and

EBER(ISH). A minor subset is weakly positive for TCR-betaF1. Ki67 proliferative index is ~30%. Limited flow cytometric evaluation revealed 92.5% lymphocytes that included CD19+ CD5- CD10- polytypic B cells (5% of total events), CD5+ putative T cells (49% of total events), and CD19- CD5- events (42% of total) which could not be further characterized.

Cytogenetics

BM aspirate: FISH: nuc ish(ALKx2)[200]

Karyotype (ISCN Nomenclature): 46,XX[20]

LN: not performed

Molecular Studies

BM: Gene sequencing studies (68 gene panel) showed two toxicity risk variants (but no disease-associated):

TMPT: c.460G>A p.A154T: VAF=55%

TMPT: c.719A>G p.Y240C: VAF=49%

LN: not performed

Proposed Diagnosis

BM: Frequent hemophagocytic macrophages, compatible with HLH in appropriate clinical and laboratory setting. No evidence of malignancy.

LN: ALCL, ALK+

Interesting Feature(s)

This is an unusual case of ALCL-ALK+ with HLH arising in the postpartum setting. Sporadic HLH is typically triggered by infection, autoimmune disease, immunosuppression, malignancy, or immune-activating therapies. HLH occurring during or following pregnancy is a rare entity associated with high morbidity and mortality. Similarly, malignancy-associated HLH is associated with poor prognosis. HLH is underdiagnosed due to its rarity and a variable presentation with nonspecific clinical features. The limited literature on HLH developing during or following pregnancy underscores the diagnostic challenges of HLH in these women and urges heightened awareness among healthcare providers.

Panel Diagnosis

ALK-positive anaplastic large cell lymphoma with associated HLH

EA4HP24-BMWS-289

Visceral leishmaniasis discovered on bone marrow biopsy

Dr. Marie Donzel[1,2], Dr. Alexis Trecourt[1,2], Prof. Meja Rabodonirina[3,2], Dr. Juliette Fontaine[1], Dr. Lucile Baseggio[4], Prof. Alexandra Traverse Glehen[1,2]

[1] Hospices Civils de Lyon, Pathology, Lyon, France; [2] Université Lyon 1, Centre International de Recherche en Infectiologie (CIRI), Institut national de la santé et de la recherche médicale (INSERM) U1111, Centre national de la recherche scientifique (CNRS), UMR5308, Ecole normale supérieure de Lyon, Lyon, France; [3] Hospices Civils de Lyon, Institut des agents infectieux, Lyon, France; [4] Hospices Civils de Lyon, Hematology-biology, Lyon, France

Case Description

A 42-year-old man without any relevant medical history for altered general condition, unusual asthenia, and night sweats for several months. There has been a weight loss of 4kg since 5 months. He reported digestive symptomatology of epigastric heaviness, bloating, and loose stools. PET-TDM (SUV MAX 6) showed splenomegaly, without lymphadenopathy or hepatomegaly.

Blood count was the following: hemoglobin 122 g/L, neutrophil count 1,32 G/L, lymphocyte count 0,96 G/L, monocyte count 0,30 G/L, and platelets count 158 G/L.

Serum LDH level was normal, but β2 microglobulin and CRP were elevated (6,2 mg/L and 60 mg/L, respectively). He also presented hyperferremia (1170 µg/l), hepatic cytolysis (1.5x average), and polyclonal hypergammaglobulinemia. Serologies (VIH, VHC, VHB, brucellose, bartonella, coxiella) were negative.

He had several regular trips to the south of France, most recently this summer.

Biopsy Fixation Details

Neutral buffered formalin and EDTA decalcification

Frozen Tissue Available

No

Details of Microscopic Findings

The bone marrow biopsy measured 25 mm. Cellularity was elevated for the age (80%). Megakaryocytes (CD61+) were numerous but well dispersed, with sometimes moderately hypersegmented nuclei. The erythroblastic (glycophorin) lineage was hyperplasic but present at all stages of maturation without dyserythropoiesis. The granular lineage (MPO) was present in regular proportions and at all stages of maturation.

Giemsa staining reveals clusters of large cells with clear cytoplasm occupied by micrograins compatible with Leishman bodies. They are also weakly stained with PAS.

Bone marrow smears showed normal hematopoiesis with reactional lymphoid cells admixed with plasma cells. However, there were numerous histiocytes with intracytoplasmic leishmania. Extrahistiocytic leishmania bodies alone were also observed in BM smears.

Immunophenotype
CD3 immunohistochemistry revealed a moderate and interstitial lymphocytic infiltrate. CD20+ B-cells were scarce. T lymphocytes were 60% CD4 and 40% CD8, with no loss of CD2, CD5 or CD7 expression. In situ hybridization for EBER RNA was negative.
Cytogenetics
No
Molecular Studies
Clonality study (BIOMED-2) did not show any monoclonal T-cell population.
Leishmaniasis PCR on venous blood and DNA extracted from bone marrow biopsy were positive for *Leishmania infantum* (>500 parasites/mL).
Proposed Diagnosis
Visceral leishmaniasis
Interesting Feature(s)
This case illustrates bone marrow changes linked to leishmaniosis. In the absence of skin lesions, symptoms are not specific. With decreasing numbers of visceral manifestations and the sometimes very long incubation time (up to 10 years) the disease can be difficult to diagnose. Leishmaniasis should be considered in patients with fever and pancytopenia with a compatible clinical syndrome. In this patient, there was no notion of travel; it is, therefore, essential to consider this diagnosis even outside the context of foreign travel. Definitive diagnosis requires PCR identification of Leishmania in peripheral blood and/or after bone marrow DNA extraction; it is therefore essential for pathologists to look for the parasite, particularly on the Giemsa stain. Bone marrow aspiration/biopsy could have a cornerstone place in the analysis of fever without bacterial or viral infection. Performing a bone marrow assessment is relatively simple in light of this potentially fatal but curable disease.

Panel Diagnosis
Leishmaniasis

EA4HP24-BMWS-294

Hemophagocytic Lymphohistiocytosis (HLH) in a patient with Rheumatoid Arthritis

Dr. Sucheta Malik, Dr. Nourhan Ibrahim, Dr. Swati Gite, Dr. Brenda Mai

UT Health Houston, Pathology & Laboratory Medicine, Houston, USA

Case Description
Patient is a 59-year-old male with a history of rheumatoid arthritis who presented with fever, backache, decreased appetite, and night sweats of one week duration. Ferritin levels

were 923 micrograms per liter. Fasting triglycerides levels were 340 mg/dL. EBV and CMV were negative by serology; no other infectious diseases were detected.

Biopsy Fixation Details

Formalin fixed formic acid-decalcified paraffin embedded tissue

Frozen Tissue Available

No

Details of Microscopic Findings

Peripheral blood examination showed normocytic anemia (hemoglobin 10.6 g/dl) and thrombocytopenia (platelet count 99,000 per ml).

Bone marrow examination showed normocellular marrow and multiple macrophages with ingested hematopoietic cells (principally erythroid cells), consistent with hemophagocytes in a background of trilineage hematopoiesis with adequate maturation.

Prussian blue iron stain was performed and showed decreased iron storage in the bone marrow.

Immunophenotype

Flow cytometry performed on peripheral blood and bone marrow aspirate showed no abnormal immunophenotype.

Cytogenetics

Cytogenetics study showed normal male karyotype 46,XY [20]

Molecular Studies

No molecular studies were performed.

Proposed Diagnosis

Hemophagocytic lymphohistiocytosis (HLH)

Interesting Feature(s)

Hemophagocytic lymphohistiocytosis (HLH) is a pathologic immune activation syndrome in which there is either congenital or acquired defective natural killer (NK)/ T cell function which leads to accentuated T-helper cell response causing high levels of TNF alpha and TNF gamma. Such uncontrolled hypercytokinemia causes pancytopenia and end organ damage. Familial HLH is an inherited autosomal recessive disease involving mutations in perforin and other genes. Secondary HLH can occur in various conditions including viral infections (EBV, CMV, severe COVID-19), malignancy (NK/T cell neoplasms), autoimmune diseases (rheumatoid arthritis), immunosuppression and pregnancy. The diagnosis of HLH requires the presence of at least 5 of 8 criteria:

1. Fever
2. Splenomegaly
3. Cytopenias (affecting at least 2 lineages in the peripheral blood)

- Hemoglobin levels < 9 g/dL
- Platelets < 100 x 10^9/L
- Neutrophils < 1.0 x 10^9/L

4. Hypertriglyceridemia or hypofibrinogenemia

•

Fasting triglycerides ≥ 265 mg/dL

•

Fibrinogen ≤ 1.5 g/L

5. Documented hemophagocytosis in the bone marrow, spleen or lymph nodes

6. Low or absent NK cell activity

7. Ferritin ≥ 500 µg/L

8. Soluble CD25 ≥ 2,400 U/mLAlthough there are eight criteria, only five are needed for diagnosis. In this particular case, the patient did not meet criteria based off serum studies and the bone marrow examination was crucial for the diagnosis. Interestingly, HLH is generally caused by secondary infections or malignancy, however, this patient's symptoms were due to exacerbation of his autoimmune disease. The patient made a full recovery after one month of high dose steroids and proper Methotrexate dosing without Etoposide treatment.

Panel Diagnosis

MAS/HLH in the setting of autoimmune disease (rheumatoid arthritis)

EA4HP24-BMWS-302

Fever, cytopenia and bone marrow granulomas one year after bladder instillation of BCG-therapy.

Dr. Juliette Fontaine, Dr. Marie Donzel, Dr. Alexis Trécourt, Prof. Alexandra Traverse Glehen

Hospices Civils de Lyon, Pathology, Pierre Bénite, Germany

Case Description

A 77-year-old man presented with altered general condition, fever and night sweats. He had a previous history of urothelial carcinoma treated with intravesical BCG instillation one year earlier, and adenocarcinoma from ampullary origin treated with radiochemotherapy this year. A complete blood count revealed anemia (8.3 g/dL), thrombopenia (107 x 10^9/L) and lymphopenia (0.26 x 10^9/L). CT scan showed no evidence of cancer relapse but splenomegaly. CRP = 58 mg/L. Broad infectious workup was negative. Myelogram showed stigmata of hemophagocytosis, with a bone marrow inflammatory in appearance, without tumoral infiltration nor pathogens. A bone marrow was performed to look for a lymphoma, a mycobacterial infection +/- BCG infection, or systemic granulomatosis.

Biopsy Fixation Details

Neutral buffered formalin fixation, EDTA decalcification

Frozen Tissue Available

No

Details of Microscopic Findings

Bone marrow was hypercellular. Epithelioid and gigantocellular granulomas were observed, with focal discussion of necrosis. Small lymphocytes were present at the rim of the granulomas. No typical Hodgkin or Reed Sternberg cells were seen. Hematopoietic cells of all three lineages were present between these granulomas without obvious morphological abnormalities. No haemophagocytosis was observed. There was no obvious carcinomatous infiltration or obvious pathogens on standard or special stains (Grocott, PAS, Ziehl, Giemsa).

Silver-stained section showed a grade 1 fibrosis.

Immunophenotype

The lymphoid infiltrate was predominantly composed of T cells, with very rare small B cells. T lymphocytes appeared predominantly small, with a few medium-sized cells. They expressed CD3, CD2, CD5 and CD7 and were predominantly CD4 compared with CD8. The anti PD1 antibody weakly highlighted rare cells, while the anti CXCL13 and ICOS antibodies were negative. Cytotoxic markers were negative. The anti-CD30 antibody highlighted rare cells in these areas, which tended to be small to medium-sized, without Hodgkin or Reed Sternberg cell appearance nor PAX5 or CD15 expression. Eber RNA *in situ* hybridization was negative.

Cytogenetics

No

Molecular Studies

The clonality study was non-contributory (DNA degraded to 100 pb). An NGS analysis revealed a probably pathogenic variant (classe 4) for DNMT3A (VAF = 6.4%). *Mycobacterium tuberculosis* complex PCR on medullary blood and parrafin-embedded bone marrow was negative.

Proposed Diagnosis

The diagnosis was difficult, but suggested a reactive process (tuberculosis ? infection after bladder instillation of BCG-therapy ?) rather than a T-cell lymphoma which cannot be formally ruled out, a fortiori in the presence of the *DNMT3A* variant difficult to interpret (clonal hematopoiesis of indeterminate potential ? T-cell lymphoma ?).

After 1.5 months, an hemoculture became finally positive for *Mycobcaterium bovis* BCG and the final diagnosis was tuberculosis after bladder instillation of BCG. In retrospect, a careful examination of the Ziehl staining showed two Ziehl positive elements within granulomas, whose morphology was suggestive of acido-alcoolo-resistant bacilli. Patient was treated with anti-tuberculosis quadritherapy, with a good evolution.

Interesting Feature(s)

The difficult differential diagnosis between infection and T-cell lymphoma, both clinico-biologically and histologically

The sometimes misleading nature of NGS

The difficulty sometimes encountered in proving *Mycobacterium* infections

Panel Diagnosis

Mycobacterial bovis infection (after BCG therapy for urothelial carcinoma)

EA4HP24-BMWS-310

A patient with CLL/SLL who developed HLH/MAS post CAR-T-cell infusion

Dr. Xueyan Chen[1,2], Dr. Teresa Hyun[1,2], Dr. Lianqun Qiu[2], Dr. Kikkeri Naresh[1,2]

[1] Fred Hutchinson Cancer Center, Translational Science and Therapeutics Division, Seattle, USA; [2] University of Washington, Laboratory Medicine and Pathology, Seattle, USA

Case Description

A 66 year old woman was diagnosed with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) in 2013. In 2019, she developed progressive leukocytosis and adenopathy. She started ibrutinib on 10/18/2019. Overall, she tolerated this treatment well but began having progressive leukocytosis and recurrent adenopathy in 2021. She next went on to have venetoclax and received rituximab (7 cycles). In April 2022, she developed progressive lymphadenopathy and chest swelling. CT scan on 4/18/2022 showed disease progression with extensive adenopathy. Venetoclax was discontinued at the time when she began treatment with Duvelisib (25 mg twice daily) starting on 4/29/2022. A repeat CT scan on 5/25/2022 showed decreased size in the thoracic, abdominal and pelvic lymphadenopathy.

A bone marrow biopsy performed on 6/22/2022 showed 70-80% involvement by CLL/SLL, further characterized by 17p deletion and a complex karyotype. She received anti-CD20 CAR-T-cell infusion on 7/20/2022. She developed immune effector cell-associated neurotoxicity syndrome (ICANS) and possible hemophagocytic lymphohistiocytosis (HLH) with fever, pancytopenia and elevated ferritin (max 15685 ng/mL), triglyceride (333 mg/dL), and soluble IL-2 receptor (46472 U/mL). A follow up bone marrow biopsy was performed on day 30 post CAR-T-cell infusion.

Biopsy Fixation Details

The bone marrow aspirate smears were Wright Giemsa stained and the bone marrow biopsy was decalcified, fixed and paraffin embedded.

Frozen Tissue Available

NA

Details of Microscopic Findings

Aspirate smears are aspiculate and hemodilute. The biopsy is markedly hypercellular for age (90-100%) and shows a diffuse infiltrate of small atypical lymphocytes with mildly irregular nuclei, clumped chromatin, and scant cytoplasm. Trilineage hematopoiesis is markedly decreased. Increased histiocytes are noted. A few forms have suggestion of hemophagocytosis.

Immunophenotype

By immunohistochemistry, the atypical lymphocytes are positive for PAX5 and CD5 and negative for cyclin D1. CD163 stain shows increased histiocytes.

Flow cytometry: An abnormal mature B cell population is identified by flow cytometry representing 51% of the total white cells and having abnormal expression of CD5, CD19 (mildly decreased), CD20 (decreased to absent) and kappa light chain restriction (decreased to absent) with normal expression of CD38, CD45 and CD200 without CD3, CD10 or CD56.

Cytogenetics

41~42,XX,del(1)(q42),-2,-3,add(3)(q29),-6,-8,+9,add(9)(p12),add(10)(q22),12,add(13)(q22), add(14)(q24),add(15)(p11.2),der(17;18)(q10;q10),-21,add(22)(p11.2),+1~3mar[cp18]/46,XX[2]

Molecular Studies

NA

Proposed Diagnosis

Extensive involvement by CLL/SLL with increased histiocytes, supportive of HLH/MAS post CAR-T-cell therapy.

Interesting Feature(s)

- Here we present a patient who developed fever, pancytopenia, hypertriglyceridemia, hemophagocytosis in bone marrow, elevated ferritin, and sIL-2R HLH after anti-CD20 CAR-T-cell infusion. The findings support a diagnosis of HLH/MAS post CAR-T-cell therapy.
- Cytokine-release syndrome (CRS) is the most commonly observed toxicity post CAR-T-cell immunotherapy. Patients who develop CRS after CAR-T-cell therapy have clinical and laboratory features that overlap with those of HLH/MAS. Rarely, severe CRS can evolve into fulminant HLH, requiring additional therapy.
- The traditional diagnostic criteria for HLH/MAS are not specific, and the findings may be present in low grade CRS. Therefore, new criteria are needed for the diagnosis of HLH/MAS in patients post CAR-T-cell therapy. Recognition of HLH is crucial to optimize treatment and improve patient outcomes.

Panel Diagnosis

Extensive involvement by CLL/SLL with HLH post CAR-T-cell therapy

EA4HP24-BMWS-324

Smoldering myeloma-associated pure red cell aplasia

Dr. You-Wen Qian[1], Dr. Malak Alharbi[2], Dr. Sawyer Bawek[2], Dr. Sean Glenn[1], Dr. Jens Hillengass[2]

[1] Roswell Park Cancer Center, Pathology, Buffalo, USA; [2] Roswell Park Cancer Center, Medicine, Buffalo, USA

Case Description

A 30-year-old female presented in February 2023 with severe anemia necessitating blood transfusions. An initial external bone marrow evaluation revealed 10-15% lambda-predominant plasma cells with left-shifted erythroid. The patient initiated care at our hospital on 4/27/2023. As part of the anemia workup, parvovirus IgM was negative. HIV, Hepatitis B and C, hemolytic anemia, and autoimmune workup were all negative. Creatinine and calcium levels, and nutritional studies (B12, folate, iron, and copper) were within normal ranges. CBC indicated WBC: 4.1x10^9/L; Hgb: 6.3 g/L; Platelets: 268x10^9/L; Neut: 2.20x10^9/L; Lymph: 1.60 x10^9/L. M-Spike: 1.87 g/dL; IgG: 2338 g/L; Kappa Free Light Chain: 14.0 mg/L; Lambda Free Light Chain: 774.9 mg/L; Free Light Chain Ratio: 0.02. A PET scan showed no hypermetabolic or individually destructive lesions.

Biopsy Fixation Details

A bone marrow trephine biopsy was formalin-fixed, paraffin-embedded, and sectioned for routine H&E staining.

Frozen Tissue Available

No

Details of Microscopic Findings

Blood smear displayed normocytic, normochromic red blood cells without Rouleaux formation. No increased schistocytes. The bone marrow biopsy revealed hypercellular marrow (80% cellularity) with essentially no erythroid islands. The marrow aspirate smears displayed many atypical plasma cells with occasional bi-nucleation. Myeloid series showed progressive maturation without overt dysgranulopoiesis, while erythroid hypoplasia/aplasia was noted.

Immunophenotype

Immunohistochemistry indicated approximately 10-15% CD138 positive lambda monoclonal plasma cells. Flow cytometry identified an abnormal plasma cell population expressing CD38, CD138, CD56, CD81(dim), CD27(dim), CD45(dim), cytoplasmic lambda light chain, CD86(dim), and CD200.

Cytogenetics

Conventional cytogenetics revealed a normal female karyotype. FISH for CD138+ enriched plasma cells was unsuccessful.

Molecular Studies

Next-generation sequencing (NGS) detected a CREBBP S302N gene mutation with a Variant Allele Frequency (VAF) of 47.3%.

Proposed Diagnosis

Smoldering myeloma-associated pure red cell aplasia

Interesting Feature(s)

Red cell aplasia associated with plasma cell neoplasm (smoldering myeloma in this case) is a rare condition with unknown mechanisms.

CREBBP/EP300 gene mutation has been described in association with myeloid neoplasm. The CREBBP S302N mutation detected in this patient is intriguing. The relationship between this gene mutation and the severe anemia, along with smoldering myeloma is unclear. ClinVar interpretation is benign or likely benign.

In the context of severe anemia, myelodysplastic neoplasm was initially considered in the differential diagnosis since myelodysplastic neoplasm may present with red cell aplasia. The differential diagnosis between myelodysplastic neoplasm and smoldering myeloma-associated anemia can be challenging.

The patient's anemia is recovered after a single agent of daratumumab for 5 months without further transfusion therapy or other medications. The IgG decreased to 720 g/L, Kappa Free Light Chain decreased to 5.2 mg/L, Lambda Free Light Chain reduced 10.2 mg/L and his Hg level gradually recovered to Hgb 14.2 (11/15/2023).

Panel Diagnosis

Smouldering multiple myeloma with red cell hypoplasia

EA4HP24-BMWS-347

Compensatory erythroid hyperplasia with marked left shift, mimicking acute erythroid leukemia, in a patient with TP53 mutated myeloid neoplasm and BK virus associated hemorrhagic cystitis.

Dr. Matthew Shapiro, Dr. Christian Salib, PhD/MD Dalia Abdel Azim, Dr. Bruce Petersen, Dr. Alina Dulau-Florea, PhD/MD Amy Duffield, **Dr. Shafinaz Hussein**

Mount Sinai Hospital, Icahn School of Medicine, Pathology, Molecular and Cell Based Medicine, New York, USA

Case Description

This is a 61 y/o man with a h/o colon cancer metastatic to the liver (s/p colectomy, liver resection, and chemotherapy) and therapy related myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML) (s/p allogeneic stem cell transplant (SCT)). The diagnostic bone marrow (BM) biopsy, performed at an outside institution, showed therapy related MDS/AML with multilineage dysplasia and 10-15% blasts. The chromosome analysis revealed 45,XY,-3,add(5)(q11.2),-7,+8,-12,+mar[12]/45,sl,add(10)(q22)[5]/45,sdl,del(1)(p22p13)[3]. Within a month a peripheral blood (PB) flow cytometry analysis showed AML with 40% blasts. The blasts were CD45

dim/moderate, CD34+, CD117+, HLA-DR+, CD13+, CD33+, CD7 partial (dim), myeloperoxidase partial (dim), TdT-, CD15-, cytoplasmic CD3-, CD19-, CD79a-, and CD22-. A targeted NGS analysis of the PB identified: *SMC3* splice site c.2645-1G>T (23.1%), *TP53* F341Y (23%), *TP53* E326* (22.1%), and *NRAS* G12C (6.8%).

He was admitted to the hospital on post-transplant day 70 for worsening hematuria and anemia. The patient reported onset of hematuria shortly after SCT. A bladder irrigation with cystoscopy revealed severe hemorrhagic cystitis with oozing blood vessels. Labs showed WBC 6.6K/uL, HGB 5.7g/dL, MCV 98fL, PLT 46K/uL. Urine and plasma samples were positive for BK virus by quantitative PCR. A BM biopsy was performed.

Biopsy Fixation Details

BM biopsy fixed in B-Plus fixative. BM aspirate smears were air dried.

Frozen Tissue Available

N/A

Details of Microscopic Findings

Hypercellular BM, ~80%, with sheet-like proliferation of immature mononuclear cells resembling early erythroid cells. They show round nuclei, fine chromatin and small nucleoli. There are admixed mature erythroid precursors. Mature myeloid forms are decreased. Megakaryocytes are not readily appreciated.

Aspirate smears disclose increased pronormoblastic cells (intermediate in size, fine chromatin, variably distinct nucleoli and basophilic cytoplasm with cytoplasmic blebs). Some cells show cytoplasmic vacuoles. The remaining nucleated red cells include frequent dysplastic forms (megaloblastoid changes, irregular nuclear contours). Myeloid cells display maturation with no significant atypia. Megakaryocytes show normal morphology. A differential count is as follows: 1% blasts, 3% promyelocytes, 5% myelocytes, 1% metamyelocytes, 20% bands/neutrophils, 2% monocytes, 33% pronormoblasts, 30% remaining erythroid precursors, 5% lymphocytes.

Immunophenotype

IHC shows sheet-like erythroid cells with the following immunophenotype: CD71+, Ecadherin+, p53+ (extensive), CD117 weak, CD34-, and CD61-.

Cytogenetics

N/A

Molecular Studies

Post transplant engraftment studies, performed on BM aspirate using whole blood, identified 79% donor DNA.

Proposed Diagnosis

Hypercellular marrow with predominance of erythroid precursors showing marked left shift and extensive p53-positivity, consistent with recurrent myeloid neoplasm.

Interesting Feature(s)

- Extensive p53-positivity supports a diagnosis of recurrent myeloid neoplasm.
- Marked expansion of erythroid precursors, substantial pronormoblasts and extensive p53 raise a consideration for an acute erythroid leukemia (AEL), which is an aggressive subtype of AML.
- Severe BK virus associated hemorrhagic cystitis with active bleeding may be a driving force in compensatory erythroid hyperplasia with marked left shift. This process adds

another layer of complexity in the evaluation/diagnosis of myeloid neoplasms with *TP53* mutation resulting in a BM morphology that can mimic AEL.
- BK virus related hemorrhagic cystitis in the post-SCT setting is associated with increased morbidity and mortality (PMID: 30711618).

Panel Diagnosis

Recurrence of myeloid neoplasm with TP53 mutation

EA4HP24-BMWS-355

Post COVID long term fever and granulomatosis

PhD/MD Pascale Cervera[1,2], Dr. Adrien Pecriaux[1], Dr. Romain Héritier[1], Dr. Lucian Craciun[1], Dr. Etienne Ghrenassia[3]

[1] APHP Sorbonne Université, Pathologie, PARIS, France; [2] APHP Sorbonne Université, Génétique, PARIS, France; [3] APHP Sorbonne Université, Médecine Interne, PARIS, France; [4] Hôpital St Antoine, 75012, Paris, France

Case Description

A 53 year-old woman is cared since 2014, for a breast cancer in remission, treated chemotherapy, mastectomy and hormonotherapy.
In 2022, she presents a pauci-symptomatic COVID, followed by a persistent fever, with asthenia and moderate weight loss. Headaches, myalgia are spontaneously reversible.
Complete blood count is normal with moderate lymphopenia (600/mm3) with normal blood smear.
Myelogram : Marrow of average richness, balanced, without megakaryocytes observed despite the absence of thrombocytopenia. The granular and erythroblastic lineages are well represented without signs of dysplasia with balanced maturation. Absence of pathological infiltrate (no lymphoma cells and no metastatic cells). In total, no cytological argument for hemopathy.
CRP was between 38 and 89mg/L, Albuminemia, Procalcitonin, LDH, anti-nuclear antibodies are absent or normal.
The viral, bacterial, parasitic fungal infectious assessment is negative.
TDM TAP: finds only a 13 cm splenomegaly, that was already known.
FDG PET reveals: diffuse and symmetrical osteomedullary hyperactivity axially and in the proximal part of the femurs and humerus (SUV max 5.1), hepatosplenomegaly.

Biopsy Fixation Details

EDTA decalcification. 4% buffered formalin

Frozen Tissue Available

No

Details of Microscopic Findings

The distribution of marrow content is homogeneous; with a 90% repletion. In this rich marrow, numerous rounded epithelioid granulomas, sometimes confluent, very well

defined (with a clear border), are surrounded by a little lymphocytic infiltrate without central necrosis. These granulomas are scattered throughout the osteomedullary spaces. The three hematopoietic lineages are represented. Erythroblastic colonies appear dislocated, poorly defined, partly para-trabecular, with preserved erythroblast/granular ratio; The megakaryocytes are quite numerous, without atypia, without grouping, even if they are sometimes found paratrabecularly.
Reticulin staining highlights diffuse grade I fibrosis; it strengthens and densifies around the epithelioid granulomas and the lymphoid clusters.

Immunophenotype

Rare small CD20+ lymphocytes are scattered in the marrow spaces. - numerous small CD5+ lymphocytes are scattered in the osteomedullary spaces as well as the presence of small lymphoid clusters often in contact with epithelioid granulomas or surrounding them. - CD61 confirms the absence of megakaryocyte grouping. - Glycophorin highlights erythroblastic islets, in a poorly defined network, partly para-trabecular and dislocated, with a preserved erythroblast/granulocytes ratio. - The immature granulocytes move intramedullary. - CD34 does not reveal blasts.

Cytogenetics

None

Molecular Studies

JAK2 V617F 58%, NF1 27%, DNMT3A 2%.

Proposed Diagnosis

Post COVID bone marrow granulomatosis and masked Vaquez disease

Interesting Feature(s)

Background: patient's mother presents with Vaquez disease.
The clinical presentation with a normal complete blood count and the appearance of the bone marrow with major granulomatosis are unusual ,

Panel Diagnosis

Ring granulomas, possibly related to the recent COVID infection

EA4HP24-BMWS-357

Acquired resistance to targeted IDH inhibitor therapy

Dr. Emily F. Mason

Vanderbilt University Medical Center, Department of Pathology, Microbiology, and Immunology, Nashville, USA

Case Description

A 56 year-old female was diagnosed with *NPM1*-mutated AML with *IDH1* R132C and *FLT3*-ITD co-mutations. She underwent standard induction and consolidation chemotherapy and achieved morphologic remission with persistent cytopenias and persistent *IDH1*-

mutated clonal hematopoiesis, for which she was observed. She presented for follow up 3.5 years after original diagnosis and was found to have relapsed AML, with 27% circulating myeloblasts. Next generation sequencing at relapse identified *NPM1*, *IDH1* R132C, and *SRSF2* mutations. She achieved a second morphologic remission after two months of treatment with a targeted inhibitor of mutant IDH1. She presented for follow up after 12 months on IDH inhibitor therapy and was found to have severe neutropenia (WBC 1.5 k/microL, ANC 0.55 k/microL, Hgb 10.9 g/dL, Plt-Ct 93 k/microL). A bone marrow biopsy was performed.

Biopsy Fixation Details

Bone marrow particle preparation: B+. Bone marrow core biopsy: Decal Stat followed by B+.

Frozen Tissue Available

None

Details of Microscopic Findings

The peripheral blood smear showed pancytopenia. The aspirate smears were cellular and showed a prominent population of immature-appearing mononuclear cells with round to oval nuclei, dispersed chromatin, prominent nucleoli, and prominent cytoplasmic granulation, comprising approximately 35% of marrow cellularity. Mature neutrophils were markedly decreased. Erythroid elements and megakaryocytes showed no significant atypia. The core biopsy was limited, but the particle preparation showed a normocellular marrow for age with left-shifted myeloid maturation, consistent with the aspirate findings.

Immunophenotype

Flow cytometry identified an abnormal myeloid population, comprising 31% of total cells, with the following phenotype: positive for CD4, CD13 (heterogeneous), CD15, CD33 (bright), CD38 (bright), CD45 (dim), CD117 (heterogeneous), and HLA-DR (dim); and negative for CD2, CD7, CD11b, CD14, CD34, CD56, and CD64.

Cytogenetics

46,XX[20]

Molecular Studies

Next generation sequencing identified the following pathogenic variants:*NPM1* (p.Trp288Cysfs*12; 9%), *IDH1* (p.Arg132Cys; 12%), *SRSF2* (p.Pro95Arg; 40%), *RUNX1* (p.Ala338Argfs*256; 10%), *TET2* (p.Arg1161Glyp; 7%), and *TET2* (p.Tyr234Phefs*17; 4%).

Proposed Diagnosis

Acquired resistance to targeted IDH inhibitor therapy via acquired *TET2* mutations

Interesting Feature(s)

The bone marrow biopsy showed essentially arrested myeloid maturation, with a large population of mononuclear cells with prominent cytoplasmic granulation, which was not present on the prior bone marrow biopsy. The findings were concerning for relapsed AML; however, the arrested myeloid population appeared morphologically to have differentiated past the blast stage, resembling abnormal promyelocytes. We have recognized this morphology as a feature of blast differentiation, indicative of treatment response, in patients with *IDH*-mutated AML who are initiated on IDH inhibitor therapy (Mason *et al*. *Blood Adv* 2021;5:2279). However, in the patient presented here, this immature myeloid population proliferated in the context of 12 months of continued IDH inhibitor therapy, after the patient had previously achieved morphologic remission. The molecular results

confirmed that this population represented relapsed *NPM1*-mutated AML. The unusual morphology was taken as evidence of limited blast differentiation in response to the IDH inhibitor. However, the acquisition of two *TET2* mutations, which act directly downstream of the IDH inhibitor, allowed for expansion of the patient's abnormal clone despite continued IDH inhibitor therapy.

Panel Diagnosis

Promyelocytic differentiation NPM1-mutated AML with IDH1 R132C and FLT3-ITD co-mutations

EA4HP24-BMWS-372

Bone marrow aplasia and severe pancytopenia developed as a late event to post CAR-T infusion in a patient with R/R large B-cell lymphoma

Dr. Dina S. Soliman[1,3], Dr. Afaf Al Battah[4], Dr. Ruba Yasin[4], Dr. Safaa Al-Azewi[4], Dr. Sara Elkourashy[4]

[1] Hamad medical corporation, Department of laboratory medicine and pathology, Doha, Qatar; [2] National Cancer Institute, Clinical Pathology department, Cairo, Egypt; [3] Weill Cornell Medicine-Qatar, Doha, Qatar; [4] Hamad medical corporation, Department of hematology, Doha, Qatar

Case Description

60-year-old male patient presented in March 2022 with upper abdominal pain diagnosed as large B-Cell lymphoma stage IV with translocation of both MYC& BCL-6. treated with R-CHOP. In Feb 2023, he developed large cerebral mass, biopsy confirmed disease relapse (with the same biology). He received 3 cycles of salvage chemotherapy with R-ICE protocol from March-May 2023. By end of June 2023, the patient received CAR-T cell therapy with Axicabtagene ciloleucel (axi-cel) with unremarkable course, PET CT imaging in July & Sept 2023 confirmed maintained CMR. In Oct 2023, the patient suffered from recurrent infections with marked pancytopenia (WBC: 0.8 x10^3/ul, ANC:0.2 x10^3/ul, Hgb: 9.4 gm/dL, Platelet: 111 x10^3/uL, Retics:52x10^3/ul). In Jan 2024, his condition then worsened with progressive pancytopenia required Erythropoietin replacement. No Filgrastim given. The patient developed drowsiness with 6th cranial nerve palsy, PET CT imaging confirmed systemic relapse in liver & osseous skeleton and CNS relapse. Ferritin was high at 887.0 ug/L, coagulopathy with prolonged PT and APTT and hypofibrinogenemia. His condition continued to deteriorate, severe pancytopenia (WBC: 0.1 x10^3/ul, Hgb: 8.7 gm/dL, Platelet:3 x10^3/uL),later developed septic shock by end of Jan 2024 and unfortunately expired.

Biopsy Fixation Details

AZF

Frozen Tissue Available

N/A

Details of Microscopic Findings

At the time of BM examination, peripheral blood showed mild anemia, mild thrombocytopenia and severe neutropenia. The BM aspirate smears show enhanced erythropoiesis (EPO response) and dyserythropoiesis. Granulopoiesis is decreased with decreased M/E ratio, marked left shifted maturation (partial maturation arrest), hypergranulation/toxic granulation and 1% blasts. The BM biopsy shows marked hypoplasia (estimated cellularity is 5-25%) with relatively increased erythropoiesis, decreased granulopoiesis and megakaryopoiesis (with uneven distribution). Megakaryocytes include some small hypolobated forms.

Immunophenotype

IHC: B-cells are depleted as shown by CD20, PAX-5 and CD79A immunostains. The provided material shows no evidence of BM involvement by B-cell neoplasm.

Flow cytometry: on BM shows approximately 8% T-cells. No mature B-cells detected.

Cytogenetics

FISH: positive for cMYC and BCL-6

Molecular Studies

Not done

Proposed Diagnosis

BM hypoplasia as a late event of post CART therapy

Interesting Feature(s)

Increasing use of chimeric antigen receptor T-cell therapy (CAR-T) has uncovered diverse toxicities warranting specific recognition and management. BM hypoplasia/aplasia is rarely reported as a late event in patients receiving CAR-T therapy. Variable etiologies of these cytopenias, some of which remain incompletely understood, create clinical challenges and uncertainties about optimal management strategies. Proposed causes of prolonged cytopenia (> 90 days post–CAR T-cell therapy) include cytokine release syndrome (CRS) with a high tumor burden and elevated inflammatory markers (T-helper dysfunction) providing a potential mechanistic explanation of CAR-T-mediated aplasia.

Immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome (IEC-HS) is a newly described entity that can manifest following CAR-T. BM aplasia could represent an uncommon manifestation of IEC-HS reported after CAR-T-cell therapy.

Pancytopenia in our case persisted for more than 6 months with progressive deterioration. The most affected parameter in this case was the granulocytes which were markedly suppressed with partial maturation arrest and abnormal hypergranulation/toxic granulation like what is seen in patients on Filgrastim.

Panel Diagnosis

Post-CAR-T cell therapy bone marrow hypoplasia

EA4HP24-BMWS-383

Hemophagocytic lymphohistiocytosis in the setting of HIV infection and disseminated histoplasmosis

Dr. Jason Love[1], Dr. Alireza Torabi[1], Dr. Kikkeri Naresh[2]

[1] University of Washington, Department of Hematopathology, Seattle, USA; [2] Fred Hutchinson Cancer Center, Department of Hematopathology, Seattle, USA

Case Description

A 46 year old male with a history of polysubstance abuse and newly diagnosed HIV was admitted to our hospital with pancytopenia, chronic progressive weight loss, fatigue, and recurrent epistaxis. Imaging showed extensive scattered ground glass opacities thought to represent atypical/viral pneumonia along with multiple sub-4 mm solid pulmonary nodules thought to be either infectious or inflammatory. Splenomegaly was noted. Laboratory studies showed elevated ferritin (13,908 ng/mL), elevated AST (202U/L), pancytopenia, and moderately elevated triglycerides (314 mg/dL). This spectrum of clinical and laboratory findings were consistent with hemophagocytic lymphohistiocytosis. Urine histoplasmosis studies were positive and a bone marrow biopsy was performed to rule out disseminated histoplasmosis

Biopsy Fixation Details

Formalin with decalcification.

Frozen Tissue Available

No frozen tissue

Details of Microscopic Findings

H&E core biopsies showed hypercellular marrow for age with complete trilineage hematopoiesis and increased numbers of macrophages, highlighted by CD68R immunohistochemical staining. Hemophagocytosis was present, best demonstrated on the aspirate smears. GMS and PAS stains demonstrated scattered fungal forms compatible with histoplasmosis.

Immunophenotype

There was no immunophenotypic evidence myeloid neoplasm or non-Hodgkin lymphoma by flow cytometry. Plasma cells were polyclonal. The T cells showed a reversed CD4:CD8 ratio of 1:13.

Cytogenetics

No performed

Molecular Studies

Myeloid NGS studies showed no clinically significant variants were detected in the targeted genes.

Genes tested include: ABL1, ANKRD26, ASXL1, BCOR, BCORL1, BRAF, CBL, CBLB, CSF3R, CXCR4, DDX41, DNMT3A, ETV6, ETNK1, EZH2, FBXW7, FGFR1, FLT3, GATA1, GATA2, HRAS, IDH1, IDH2, JAK2, KIT, KMT2A (MLL), KRAS, MAP2K1, MPL, MYD88, NF1, NOTCH1, NPM1, NRAS, PDGFRA, PDGFRB, PHF6, PPM1D, PTEN, PTPN11, RAD21, RB1, RUNX1, SAMD9L, SAMD9,

SETBP1, SF3B1, SH2B3, SMC1A, SMC3, SRSF2, STAG2, STAT3, STAT5B, TET2, TP53, U2AF1, WT1, ZRSR2.

Proposed Diagnosis

Histoplasmosis associated hemophagocytic lymphohistiocytosis in the setting of HIV

Interesting Feature(s)

This is an excellent example of histoplasmosis associated hemophagocytic lymphohistiocytosis which is a well known but uncommon complication of HIV infection. The lack of granulomatous response makes recognition of the fungal forms difficult without a high index of clinical suspicion and the aid of GMS or PAS stains.

Panel Diagnosis

Histoplasmosis with associated HLH (in the setting of immunocompromise due to HIV)

EA4HP24-BMWS-387

Pediatric case of therapy-related myeloid neoplasm: Juvenile myelomonocytic leukemia, therapy-related

Dr. Aida I. Richardson[1,2], Dr. Shunyou Gong[1,2]

[1] Ann & Robert H. Lurie Children's Hospital of Chicago, Department of pathology, Chicago, USA; [2] Northwestern University Feinberg School of Medicine, Department of pathology, Chicago, USA

Case Description

A 5-year-old female with a history of atypical teratoid rhabdoid tumor (ATRT), s/p chemotherapy and radiation therapy developed anemia, thrombocytopenia and monocytosis for which bone marrow procedure was performed 6 months after completion of treatment. Bone marrow was mildly hypocellular and showed progressive granulocytic and erythroid hematopoiesis, reduced megakaryocytes and increased mature monocytes, with no evidence of leukemia; however cytogenetic analysis detected abnormal mosaic female karyotype with t(1;10)(p10;p10),t(4;6)(q12;p25) in 2 cells. The patient was closely followed. Due to the progression of monocytosis and thrombocytopenia, another bone marrow procedure was performed. Abdomen ultrasound revealed no splenomegaly.

Biopsy Fixation Details

Bone marrow biopsy was decalcified and fixed in formalin.

Frozen Tissue Available

No

Details of Microscopic Findings

Peripheral blood smears show anemia (Hb: 9 K/uL), thrombocytopenia (PLT: 22) and leukocytosis (WBC: 28.8 K/uL) with absolute monocytes (19.9 K/uL). A review of the peripheral blood smear revealed majority of cells are mature monocytes (69%), mild

neutrophil left shift, occasional pseudo-Pelger Huet and hypogranular neutrophils, and few nucleated RBCs. The bone marrow aspirate smears contained 2% blasts, 5% promonocytes (blast equivalent; overall about 7% blasts) as well as 31.4% monocytes. The bone marrow core biopsy had about 30% cellularity, predominately comprised of monocytes.

Immunophenotype

Flow cytometry of bone marrow aspirate revealed approximately 51% of the viable nucleated cells are immunophenotypically atypical monocytes that are left-shifted and partially expressing CD56.

Cytogenetics

Bone marrow: normal 46,XX[20]

Molecular Studies

Next-generation sequencing (includes comprehensive analysis of RNA, DNA, and copy number variants) on bone marrow aspirate detected one pathogenic variant in KRAS gene: Tier 2 variant of potential clinical significance: KRAS; Chr12:25398284; NM_033360.3; exon 2; c.35G>A; p.Gly12Asp; AF: 39.2%

Proposed Diagnosis

Juvenile myelomonocytic leukemia (JMML), therapy-related

Interesting Feature(s)

Pediatric therapy-related myeloid neoplasm is a very rare disease with an incidence of approximately 0.5-1.0%.

Atypical presentation as in this case, in a child without splenomegaly and at an older age makes diagnosis challenging. While splenomegaly was required for the diagnosis of juvenile myelomonocytic leukemia (JMML) per the 4th edition of the WHO classification (WHO-HAEM4); this has been recently changed per International Consensus Classification (ICC) and the 5th edition of the WHO classification (WHO-HAEM5). However, per the literature review, pediatric cases of therapy-related myeloid neoplasms are either MDS or AML. Thus, not much is known about JMML cases that are therapy-related.

Notably, the genomic landscape of pediatric myeloid neoplasms that are therapy-related shows that genes involving the RAS/MAPK pathway are the most frequent somatic drivers in these patients, with a mutation in KRAS being the most common one.

Children with therapy-related myeloid neoplasm have a significantly worse prognosis when compared to de-novo myeloid neoplasm. Additionally, the rarity of this disease makes the choice for chemo treatment challenging. Patients who undergo stem cell transplant have a better prognosis.

Panel Diagnosis

JMML post cytotoxic therapy (WHO-2022) / JMML, therapy-related (ICC)

EA4HP24-BMWS-392

Hemophagocytic lymphohistiocytosis in a diagnostically challenging case

Dr. Feryal Ibrahim[1], PhD/MD Samah Kohla[1,2], Dr. Hasan Rizvi[3], Dr. Dina Soliman[1,2], Dr. Aliaa Amer[1], Dr. Amna Gameil[4,2], Dr. Ruba Taha[4], Dr. Feryal Ibrahim acts as the principal author and is accountable for diagnosing the case and drafting the abstract. Dr. Samah Kohla contributed to and submitted the abstract, while Dr. Hassan Rizvi assisted in the diagnosis. Dr. Dina Soliman and Dr. Aliaa Amer reviewed the manuscript. Dr. Amana Gamil and Dr. Ruba Taha contributed to the clinical aspects and reviewed the manuscript.

[1] Hamad Medical Corporation, Lab Medicine and Pathology Department, Hematology Divsion, NCCCR, Doha, Qatar; [2] Weill Cornell Medicine Qatar, Doha, Qatar; [3] Hamad Medical Corporation, Lab Medicine and Pathology Department, Anatomic Pathology Division, HGH, Doha, Qatar; [4] Hamad Medical Corporation, Clinical Hematology Department, NCCCR, Doha, Qatar

Case Description

A-24 years male patient with no known past medical history, presented with 5 months spiking fever cough, excessive sweating and weight loss. PET/CT revealed multiple FDG avid lymph nodes above and below the diaphragm, FDG avid enlarged spleen and multiple foci in bones and bilateral pleural effusion. His CBC revealed pancytopenia (WBCs 1.7x10^3/ul, Hgb 9.1 gm/d and Platelets 43x10^3/u). EBV by PCR, 436,000.0 IU/mL, high Ferritin, 7,963.0 ug/L, high triglyceride, 3.5 mmol/L with positive DAT (IgG1+).

Biopsy Fixation Details

BM biopsy fixed with AZF

Frozen Tissue Available

NA

Details of Microscopic Findings

Peripheral blood showed pancytopenia with red cells agglutination. Bone marrow (BM) aspirate showed increased erythroid precursors with reduced granulocytic cells and some histiocytes with haemophagocytosis. Histiocytic proliferation and haemophagocytosis were more evidently noted in touch imprints. BM biopsy was hypercellular with multiple areas of abnormal infiltrate composed mostly of lymphocytes (predominantly T-cells), histiocytes/macrophages, few plasma cells and some abnormal large mononuclear cells with single or multiple inclusion like nucleolus (Hodgkin's cells morphology) with rare binucleated cells (R-S cell morphology) and few cells with multilobated nucleus.

Immunophenotype

Immunohistochemistry: The large cells were positive for CD30 with characteristic membrane & paranuclear dot-like positivity, few appear weakly positive for PAX5 and MUM1. CD15 was difficult to interpret. EBER positive in rare large cells. CD68/CD163 showed increased histiocytes and some with haemophagocytosis. CD3/CD5/CD2 highlighted increase in T-cells, mix of CD4 and CD8.

Flow cytometry: on BM aspirate revealed 80% T-cells with increased CD4:CD8 ratio (6.6) and no phenotypic aberrancies, 5% B-cells and 2% plasma cells with no immunophenotypic evidence of monotypic B-cell or plasma population.

Cytogenetics

Not done

Molecular Studies

Not done

Proposed Diagnosis

BM involvement by Hodgkin Lymphoma with haemophagocytic. lymphohistiocytosis (HLH). Tissue biopsy was recommended for confirmation

Interesting Feature(s)

Interesting feature(s) of submitted case: Although Hodgkin lymphoma with HLH was the diagnosis proposed from BM, lymph node biopsy which was done later revealed Epstein-Barr virus-associated lymphoproliferative disorder. This case illustrates a critical challenge in diagnosis, and through light on the ambiguous boundary between lymphoma and EBV-associated proliferation encountered in some case, particularly when immunological dysregulation or deficiency is not a recognized underlying setting.

Panel Diagnosis

EBV+ lymphoproliferative disease, favour EBV+ DLBCL

EA4HP24-BMWS-403

Fulminant Multisystem granulomatous disease in a young female patient with fatal course

Dr. Dina S. Soliman[1,3], Dr. Ahmad Al-Sabbagh[1], Dr. Firyal Ibrahim[1]

[1] Hamad medical corporation, Department of laboratory medicine and pathology, Doha, Qatar; [2] National Cancer Institute, Clinical Pathology department, Cairo, Egypt; [3] Weill Cornell Medicine-Qatar, Doha, Qatar

Case Description

In November 2012, a 25-year-old female patient, previously healthy presented to emergency with fever, gross hepatosplenomegaly and lymphadenopathy, elevated liver enzymes and high C-reactive protein. All virology work-up including CMV, EBV, adenovirus, HIV and Hepatitis were negative with negative Leishmania and TB. Autoimmune panel was negative apart from positive Antinuclear antibody (ANA) is positive at a titer of 1:160 (homogenous). Anti ds DNA was negative. The patient unfortunately expired shortly on 12th December, that is almost one month after the first presentation with no specific diagnosis reached.

Biopsy Fixation Details

Paraffin embedded,AZF fixed.

Frozen Tissue Available

N/A

Details of Microscopic Findings

Peripheral smear shows moderate microcytic hypochromic anemia with moderate neutropenia and thrombocytopenia (WBC:1.7, Hb:8.3g/dl, Platelets: 69x10 ^{3}/UL). Bone marrow aspirate is significantly hemodilute (dry tap). The BM biopsy is cellular with maturing trilineage hematopoiesis. Megakaryopoiesis is enhanced with no significant atypia noted.

There are evident bone changes with increased bone remodeling well demarcated by evident osteoblastic activity.

The BM is infiltrated with well-defined epithelioid granulomas composed almost entirely with clusters of histiocytes highlighted by CD68 and including multinucleated giant cells, mixed with some small lymphocytes and few eosinophils. No increase in CD34-positive precursors and no increase in mast cells as highlighted by mast cell tryptase and CD117. No lymphoid aggregates seen. The BM shows stromal changes with dilated marrow vasculature, increased marrow fibrosis (accentuated within the granulomas) and areas of fibrinoid necrosis.

Liver biopsy was done and revealed multifocal portal and lobular non-caseating granulomas, periportal fibrosis, Z/N negative. No malignancy was identified.

Immunophenotype

Flow cytometry shows no evidence of monotypic B-cells. No T-cell aberrancies. IHC: The granulomas are negative for CD163, Langerin, CD1a, S100, CD30, CD15, Ziel-Neelsen and fungal stains are negative. CKAE1-AE3: Negative

Cytogenetics

Not done

Molecular Studies

Not done

Proposed Diagnosis

Multisystemgranulomas of unknown cause, probably autoimmune or idiopathic

Interesting Feature(s)

The diagnosis of this case was quite challenging as the cause of multisystem granulomas could not be confidently concluded. The differential diagnosis usually includes sarcoidosis, TB, autoimmune disorder, drugs, etc. Extensive laboratory work-up came negative for all the common causes. Systemic lupus erythematosus (SLE) was suspected given the patients' age and gender and positive ANA, however definitive diagnosis cannot be made due to progressive deterioration of the patient's condition with very short survival.

SLE with fulminant course and systemic granulomas is very rare and could be attributed to defective clearance of apoptotic bodies by the complement system which may stimulate granuloma formation.

This case could also be the entity of Granulomatous Lesions of Unknown Significance (GLUS Syndrome), a syndrome which was described with prolonged fever, epithelioid granulomata in the liver, bone marrow, spleen, and lymph nodes. Unlike sarcoidosis, pulmonary granulomatous involvement is rare. Although, it has been argued that the GLUS syndrome is a form of extrapulmonary sarcoidosis. This syndrome is extremely rare, having

been rarely reported during this millennium. However, this syndrome is reported to have a benign course, and a tendency for recurrence.

Panel Diagnosis

Granulomatous Inflammation

EA4HP24-BMWS-410

Pancytopenia associated with parvovirus B19 infection in an AIDS patient

Dr. Luis Veloza, **Prof. Laurence de Leval**

Lausanne University Hospital and Lausanne University, Institute of Pathology, Department of Laboratory Medicine and Pathology, Lausanne, Switzerland

Case Description

The patient is a sixty-year-old man with HIV infection since 2004 and previous history of a high-grade B-cell lymphoma in 2009, treated by HPSCT. He was admitted to the emergency department with weight loss, asthenia and diarrhea. He had interrupted antiretroviral treatment a couple of months ago. Laboratory tests showed a low CD4+ T-cell count (59 cells/mm^3) and an HIV viral load of 2.4×10^3 copies/ml. Full blood count showed pancytopenia: Hb 93 g/l, MCV 91 fl, leukocytes 3.7×10^9/L, lymphocytes 0.7×10^9/L and platelets 57×10^9/L. Blood smear analysis reported anisocytosis and moderate poikilocytosis with elliptocytes (++). A bone marrow biopsy (BMB) was performed (submitted).

The patient had no splenomegaly or lymphadenopathy. In addition, he was diagnosed with pneumocystis jirovecii pneumonia, CMV reactivation, and a cryptosporidiosis gastrointestinal infection. Hematological parameters improved within 2 months of the reintroduction of antiretroviral treatment and antimicrobial treatment, while PCR for parvovirus B19 (PVB19) in blood remained positive until one month after diagnosis.

Biopsy Fixation Details

Acetic acid–zinc–formalin fixative.

Frozen Tissue Available

No.

Details of Microscopic Findings

BMB showed an hypercellular bone marrow (65%), with a marked left-shifted, erythroid hyperplasia with giant erytroblasts harboring eosinophilic intranuclear inclusions, suggestive of viral inclusions. The more mature forms showed mild dyserythropoies. There was slight megakaryocytic hyperplasia. The granulocytic lineage was left-shifted with preserved terminal maturation. A moderate nodular and interstitial lymphocytosis and a mild plasmacytosis were observed. No granuloma or necrosis were seen. Silver staining showed mild reticulin fibrosis (MF-1). Special stains revealed no germs.

Immunophenotype

CD71 highlighted the erythroid hyperplasia and MPO stained the granulocytic lineage (M:E ratio of 1-2:1). Remarkably, PVB19 immunostaining revealed numerous giant erytroblasts with intranuclear inclusions positive for PVB19. CD42b and CD61 stained a spectrum of megakaryocytes and showed no micromegakaryocytes. CD34 did not reveal excess of blasts. CD20, PAX5, CD3, and CD5 demonstrated a polytypic nodular and interstitial lymphocytosis, admixed with polytypic kappa and lambda-positive plasma cells. CMV and HHV8 IHC were negative. EBER-ISH for EBV revealed few scattered small nuclei.

Cytogenetics

Not performed.

Molecular Studies

Not performed.

Proposed Diagnosis

Hypercellular bone marrow (65%) with:

-Trilinear left-shifted, hematopoiesis with erythroid hyperplasia, mild dyserythropoiesis, and extensive PVB19 infection;

-Reactive lymphocytosis and polytypic plasma cells;

-Special stains, EBV, CMV and HHV8 negative.

Interesting Feature(s)

This is an interesting case of an AIDS patient and pancytopenia related to acute PVB19 infection, which was strongly suspected by morphology and confirmed with PVB19 IHC.

In immunocompetent individuals, PVB19 infection commonly produces a marked erythroid hypoplasia with rare erythroblasts showing viral inclusions. In contrast, in immunocompromised patients with PVB19 infection, bone marrow may be hypercellular with erythroid hyperplasia and numerous giant erythroblasts with intranuclear inclusions. Immunoglobulin G antiparvovirus antibodies may be negative in these patients.

Thus, PVB19 infection should be considered in immunodeficient patients presenting with pancytopenia. Morphological evaluation of BMB and confirmation by PVB19 IHC can be important in the evaluation of cytopenias in immunodeficient patients as serological studies for PVB19 are commonly negative.

Panel Diagnosis

Parvovirus B19 infection in the context of AIDS

EA4HP24-BMWS-417

Bone Marrow with Hemophagocytosis likely secondary to *Klebsiella Pneumoniae* infection in a patient with chronic immunosuppression

Dr. Priya Skaria[1], Dr. Muhammad Salyana[1], Dr. Raghad Abdul-Karim[2], Dr. Brent Bedke[1], Dr. Ryan Swapp[1], Dr. Dennis O'Malley[3]

[1] Yuma Regional Medical Center, Pathology, Yuma, USA; [2] Yuma Regional Medical Center, Hematology and Oncology, Yuma, USA; [3] M.D Anderson Cancer Center, Pathology, Houston, USA

Case Description

48 year old woman who presents in 12/2023 with fever, shortness of breath, vomiting and hypotension. Clinical diagnosis of sepsis due to urinary tract infection by *Klebsiella pneumoniae*.

Past medical history of Adult onset Still's disease (diagnosed in 2002) on chronic immunosuppression with steroids and methotrexate. Flow cytometry on peripheral blood in 08/2023 revealed non- CLL type monoclonal B cell lymphocytosis and aberrant T cells with loss of CD5 in 10/2023.

Admission in 12/2023: CBC: WBC: 53.0, RBC: 4.0, Hemoglobin: 11.9, Hematocrit: 36.3, MCV: 90.5, MCH: 29.4, Platelets: 334, RDW-CV: 20.4

CT abdomen & pelvis: Multiple prominent inguinal, retroperitoneal and pelvic lymph nodes, hepatic steatosis and splenic atrophy.

Other relevant labs: Creatinine 0.7, AST 61, ALT <7, Albumin 2.5, Ferritin >15,000, Triglycerides 159, Fibrinogen 280, Sodium 125, Serum soluble IL-2 receptor 2968 (normal <858), EBV VCA IgM negative, EBV VCA IgG positive, EBV Nuclear Ag Ab positive

Clinical concern for HLH.

Biopsy Fixation Details

Bone marrow core biopsy (suboptimal for evaluation; 10% formalin and immunocompatible decalcification) and bone marrow clot (suboptimal for evaluation; 10% formalin)

Frozen Tissue Available

Not applicable

Details of Microscopic Findings

Bone marrow: Mildly hypercellular bone marrow, preserved trilineage hematopoiesis and maturation, myeloid hyperplasia, no increase in blasts or significant dysplasia, histiocytic hyperplasia, few histiocytes with 'intact' ingested red blood cells, neutrophils and fewer lymphocytes consistent with hemophagocytosis.

Peripheral blood: Moderate normocytic normochromic anemia with rare circulating schistocytes, marked leukocytosis with absolute neutrophilia, few circulating immature myeloid precursors and rare blast (1%), few circulating monocytoid lymphocytes and rare reactive/ atypical lymphocytes.

Immunophenotype
CD34 (2-3% blasts), CD163 and CD68 highlighted the histiocytes, ISH EBV negative
Cytogenetics
46,XX[20]
Molecular Studies
Normal Comprehensive NGS panel for myeloid and lymphoid malignancies (302 genes)
Proposed Diagnosis
Mildly hypercellular bone marrow with trilineage hematopoiesis, myeloid hyperplasia. No evidence of increase in blasts, significant dysplasia or a lymphoproliferative disorder. Foci of hemophagocytosis.
Interesting Feature(s)
Bone marrow with hemophagocytosis likely secondary to *Klebsiella Pneumoniae* infection in a patient with chronic immunosuppression

Panel Diagnosis
Haemophagocytosis in the setting of Klebsiella Pneumoniae infection and (immunosuppressed) adult-onset Still's disease

EA4HP24-BMWS-437

EBV-positive aggressive B-cell lymphoma with associated secondary hemophagocytic lymphohistiocytosis

Dr. Marcello P. Toscano, **Dr. Anna B. Owczarczyk**

Cleveland Clinic, Robert J. Tomsich Pathology and Laboratory Medicine, Cleveland, USA

Case Description
A 77-year-old male with a medical history significant for polymyalgia rheumatica treated with hydroxychloroquine, presented with to the ED with a month-long history of fever, upper respiratory symptoms, abdominal pain, weight loss, and night sweats. Antibiotics prescribed by his primary care physician did not help his symptoms. E*3127*D labs revealed thrombocytopenia, normocytic anemia, and an elevated total bilirubin. Peripheral blood smear showed leukocytosis with neutrophilic left shift and occasional atypical lymphoid cells. CT of the chest, abdomen and pelvis demonstrated splenomegaly and extensive lymphadenopathy. The patient required ICU care, where infectious causes were excluded and a bedside bone marrow biopsy with aspiration and lymph node biopsy were urgently performed. Dexamethasone and etoposide were considered given the preliminary aspirate smear read of a large cell lymphoma with secondary hemophagocytic lymphohistiocytosis (HLH). Unfortunately, the patient passed away within 12 hours of the biopsy procedures.

Biopsy Fixation Details

Formalin.

Frozen Tissue Available

No.

Details of Microscopic Findings

Core biopsy, clot section, and aspirate smear demonstrate multifocal nodules of large, atypical lymphocytes and histiocytes with extensive hemophagocytosis. The atypical lymphoid cells account for 20-40% of the marrow cellularity and show prominent nucleoli, vesicular chromatin, irregular nuclear contours, and vacuolated basophilic cytoplasm. Frequent atypical mitotic activity and apoptotic debris are noted. Background bone marrow is hypercellular with maturing trilineage hematopoiesis.

Immunophenotype

Lymphoma cells: positive for CD20, BCL2, BCL6 (dim), MUM1, CD30, and chromogenic in situ hybridization for EBV-encoded RNA (EBER-CISH) with a Ki-67 proliferation index >90%; negative for CD5 and CD10. Background T-cells are positive for CD3, while CD163 marks histiocytes. Flow cytometry performed on the peripheral blood, lymph node biopsy (entirely submitted for flow), and bone marrow aspirate showed no evidence of a lymphoproliferative disorder.

Cytogenetics

45,X,-Y[3]/47,XY,+Y[2]/46,XY[15].

Molecular Studies

Not performed.

Proposed Diagnosis

EBV-positive aggressive B-cell lymphoma, non-germinal center immunophenotype, with secondary HLH.

Interesting Feature(s)

The findings are consistent with an aggressive B-cell lymphoma, with a differential diagnosis of an EBV-positive diffuse large B-cell lymphoma versus iatrogenic immunodeficiency-associated lymphoproliferative disorder secondary to hydroxychloroquine use. The short time frame from symptoms to death are rather astonishing – the patient was in usual health up until his symptoms began. At the time of the bone marrow biopsy, HLH was not on the clinical differential, and likely contributed to the patient's rapid demise. Additional molecular and cytogenetic studies to further subclassify the lymphoma were not pursued given the patient's passing.

Panel Diagnosis

EBV-positive diffuse large B-cell lymphoma with associated HLH

EA4HP24-BMWS-443

T-cell/Histiocyte-rich large B-cell lymphoma complicated by disseminated Histoplasmosis and Hemophagocytic Lymphohistiocytosis (HLH)

Dr. Marcello P. Toscano, **Dr. Kelly A. Bowers**

Cleveland Clinic, Robert J. Tomsich Pathology & Laboratory Medicine Institute, Cleveland, USA

Case Description

The patient is a 59-year-old male with T-cell/Histiocyte-rich Large B cell lymphoma status post autologous stem cell transplant with post-transplant relapse treated with Epcoritamab, presenting with pancytopenia, fever, tachycardia, and perioral swelling/mass and admitted for cytokine activation syndrome and tumor lysis syndrome. A bone marrow biopsy revealed disseminated fungal infection, morphologically consistent with *Histoplasma capsulatum*, as well as hemophagocytosis. The patient fulfilled diagnostic criteria for hemophagocytic lymphohistiocytosis (HLH), including fever, pancytopenia, splenomegaly, hypertriglyceridemia (201), hypofibrinogenemia (73), and elevated serum ferritin (135,732). An oral biopsy showed similar findings with numerous fungal yeasts. Microbiologic cultures remain pending. Urine quantitative *Histoplasma* antigen was positive. The patient was started on antibiotics, antifungals, and high dose steroids. Unfortunately, his clinical condition progressively deteriorated, and he passed away twelve days after admission.

Biopsy Fixation Details

Formalin

Frozen Tissue Available

No

Details of Microscopic Findings

Peripheral blood smear showed pancytopenia and rare intracellular fungal yeasts. Bone marrow aspirate smears and core biopsy demonstrate cellular marrow (10-40%) with maturing trilineage hematopoiesis, increased histiocytes containing hemophagocytosis as well as intracellular fungal yeasts.

Immunophenotype

Fungal yeasts were positive for GMS and PAS-F stains. There was no evidence of lymphoma within the bone marrow by immunohistochemical stains or flow cytometry.

Cytogenetics

Pending

Molecular Studies

Not performed

Proposed Diagnosis

Disseminated histoplasmosis associated with hemophagocytosis.

Interesting Feature(s)

The interesting feature of this case is the cytologic findings, which were diagnostic for this patient and showed both the triggering agent (disseminated fungal yeasts) and resultant illness (hemophagocytosis) within the same cells (histiocytes). The fungal etiology of this case is also unique, since Hemophagocytic Lymphohistiocytosis (HLH) is more commonly caused by viruses. The differential diagnosis includes *Candida*, which can be morphologically similar to *Histoplasma*. The presence of germ tubes, more basophilic staining on H&E, and rapid growth in culture are features seen in *Candida* infection and can be used to help aid in the distinction from *Histoplasma*. The patient had multiple contributing factors, including an immunocompromised state from bone marrow transplant, chemotherapy and immunotherapy, which predisposed him to the opportunistic infection triggering HLH. His history of lymphoma confounded the clinical picture, where his symptoms including pancytopenia, fever, and oral swelling/mass, can be caused by both lymphoma as well as a disseminated infection. Mucocutaneous lesions have been documented in disseminated histoplasmosis and could offer an clue to this diagnosis. In conclusion, this case highlights that especially when HLH is caused by slow growing fungal organisms, a pathologist's identification of yeasts, in conjunction with serologic and antigen testing, are some of the most important tools for rapid diagnosis in this high mortality disease.

Panel Diagnosis

Histoplasmosis with associated HLH (in the setting of immunocompromise due to bone marrow transplant, chemotherapy and immunotherapy)

EA4HP24-BMWS-451

Histoplasma associated HLH

PhD/MD Roos Leguit[1], PhD/MD Nils 't Hart[2]

[1] *University medical center Utrecht, Pathology, Utrecht, Netherlands;* [2] *Isala, Pathology, Zwolle, Netherlands*

Case Description

This 54 year-old male had been admitted (Nov 2022) with fever, night sweats, weight loss and malaise. He was later referred to an academic centre (Feb 2023) because of the development of HLH (strongly elevated ferritin 185600 µg/L, elevated triglycerides 3.2 mmol/L (416 µg/dL), elevated liver enzymes, pancytopenia, lowered fibrinogen 1.7g/L and increased sol IL-2 receptor 36850 pg/mL). At the time he was COVID19 positive. He had been treated for his seronegative rheumatoid arthritis with methotrexate (2021) and adalimumab (until Nov 2022). In Jan 2023, he had been treated with prednisone for a pneumonitis. This is the bone marrow biopsy that had been taken when he was still in the outside hospital.

PET-CT:
1. Metabolic active splenomegaly
2. Diffuse increase in FDG in bone marrow
3. FDG avide lymph nodes mesenterial and right axilla
4. FDG avide lesion apex right lung
5. Metabolic active thickend adrenal glands

Biopsy Fixation Details

Formalin fixation, EDTA decalcification

Frozen Tissue Available

No

Details of Microscopic Findings

Slightly hypercellular marrow (cellularity approx. 60%) with a hyperplastic and slightly left-shifted erythropoiesis with nuclear abnormalities, left-shifted granulopoiesis and megakaryocytes with overall normal lobulation. There are multiple granulomas made up of histiocytes admixed with fibrin. In the marrow, especially within the granulomas, there are round to oval yeasts of 2-6 µm. These yeast are positive in the PAS stain and negative in the alcian blue and ZN stain. They stain positive in the GMS (Grocott Methenamine Silver) stain, but this stain gave a lot of background staining so that the organisms were hard to see. They were, however, well seen in the reticulin (silver) stain. This showed the yeast were budding with a so-called narrow-neck.

Bone marrow aspirate showed a marked increase in erythropoiesis (86%) with many nuclear abnormalities (karyorrhexis, double nuclei, slightly megalobastoïd). No lymphoma, no increase in blast (<5%)

Immunophenotype

Immunohistochemistry: MPO, Glycophine C and CD61 highlighted the hematopoietic cell lineages and confirmed the erythroid hyperplasia. There was no increase in blasts in the CD34 stain. CD79a show a normal amount of scattered small B-cells and plasma cells. CD3 showed many small T-cells in the granulomas.

Immunophenotyping of the aspirate showed a polyclonal B-cell population, a T-cell population with a normal CD4/CD8 ration and 0.2% CD45weak+ CD34+ cells. No evidence for PNH.

Cytogenetics

Not performed

Molecular Studies

PCR studies on urine and bone marrow were positive for Histoplasma capsulatum var. capsulatum.

Proposed Diagnosis

Histoplasma associated hemophagocytic lymphohistiocytosis (HLH)

Interesting Feature(s)

This is an example of disseminated Histoplasmosis infection in an immunocompromised patient, causing HLH. Although hemophagocytosis was difficult to see in the bone marrow, clinical criteria of HLH were met. Detection of Histoplasma yeast in the bone marrow biopsy led to start of treatment with Amphotericin B, with the infection being later confirmed by PCR. Unfortunately patient also suffered from a COVID-19 infection, Pseudomonas and

Enterococcus bacteremia, diffuse intravascular coagulation, gastro-intestinal blood loss and a HSV-1 stomatitis and eventually died.
Histoplasmosis is usually seen in an immunocompromised patient. This patient had been treated for his arthritis with methotrexate and later adalumimab followed by prednisolon.

Panel Diagnosis

Histoplasmosis with associated HLH (in the setting of immunocompromise due to immunosuppression for rheumatoid arthritis)

EA4HP24-BMWS-456

A case of reactive bone marrow changes with macrophage activation in Sickle cell disease

Dr. Hanine M. Medani[1,2], Prof. Maria Calaminici[1,2], Dr. Frederick Chen[1], Dr. Matthew Smith[1]

[1] Barts Health London, Cellular pathology/ SIHMDS, London, UK; [2] NHS East and South East London Pathology Partnership, Barts Health NHS Trust, SIHMDS, London, United Kingdom, Cellular Pathology, London, UK

Case Description

This is 57 year old female with sickle cell anaemia (HbSS), a history of stable ITP and ANA positive connective tissue disorder .
September 2022 she presented with progressive anaemia and neutropenia with Hb of 45 g/dl, WCC 5.5x10~9, Neut 1.1x10~9 and platelets 197x10~9. Bone marrow examination revealed trilineage dysplasia and excess blasts. Blasts were 9% on aspirate morphology and 5-7% on marrow histology. Myeloid NGS revealed several mutations with an adverse genetic risk. A diagnosis of Myelodysplastic syndrome with excess blasts-1 was established. She was referred for a bone marrow allogenic transplant, and while being worked up was receiving regular blood transfusions for symptomatic relief, with subsequent iron overload and rising ferritin levels. To bridge the gap to transplant she was started on Azacitadine therapy, planned for 6 cycles, to improve counts and reduce transfusion requirements. This was frequently interrupted by recurrent cutaneous infections and a neutropenic sepsis episode, during which she received several courses of antibiotics and GCSF support. She eventually completed chemotherapy but ongoing fevers and infections continued causing delays in transplantation. A Pre transplant bone marrow trephine biopsy was done and showed interesting features.

Biopsy Fixation Details

Decalcification immersion in **10% formic acid** at **room temperature** for **4–5** hours

Frozen Tissue Available

None

Details of Microscopic Findings

The marrow is hypercellular with 90-100% cellularity. There is marked erythroid hyperplasia with large confluent colonies and left shifted maturation.

Widespread activation of macrophages with marked erythrophagocytosis and focal haemophagocytosis is seen. Many hemosiderin laden macrophages are present. Granulopoiesis is reduced with few neutrophils seen. It is disordered with increased MPO+ precursors in the intertrabecular space with some clustering. Megakaryocytes are reduced but unevenly distributed with large, loose clusters and dysplastic morphology present.

There is a general increase in reactive lymphocytes with focal small aggregates and plasma cells.

Reticulin fibrosis is increased (MF1).

Immunophenotype

CD117 and CD34 staining is positive in <1% of the nucleated cells. CD68 and CD163 highlight erythrophagocytosis and focal haemophagocytosis. CD3+ T cells and CD20+ B cells are mildly increased.

Cytogenetics

No abnormal clone was detected in the 20 G-banded metaphase cells examined.

Molecular Studies

Myeloid gene panel performed for MDS: mutations in *ASXL1*, *STAG2*, *TET2* and *SRSF2*.

Proposed Diagnosis

Myelodysplastic syndrome with excess blasts -1

Extensive marrow macrophage expansion and erythrophagocytosis due to macrophage activation. This is likely multi-factorial, but includes proinflammatory properties in Sickle cell disease, aggravated by regular blood transfusions, sepsis, and chemotherapy.

Interesting Feature(s)

Multi-factorial cause for macrophage activation in this case. Whilst HLH can develop with Myelodysplasia, it is rare and therefore unlikely the causative factor.

Similar findings are noted in post-transfusion hyperhaemolysis syndrome (PTHS), reported mostly in SCD.

Also highlights the reported increased incidence of haematological malignancies in SCD. It is postulated that, lifelong haemolysis and compensatory increase in erythroid cell turnover causes proliferative pressure on Haemopoietic Stem Cells that may lead to early HSC senescence and development of leukaemogenic mutations.

Panel Diagnosis

Haemophagocytosis in the setting of unspecified autoimmune disease, sickle cell disease, sepsis and MDS on azacitidine

EA4HP24-BMWS-468

Bone Marrow Infiltration in Langerhans Cell Histiocytosis, case report.

Dr. Paulina E. Santana Vargas[1,3], Dr. Marlon A. Arias Intriago[3], Dr. Manuel A. Granja Morán[2,4]

[1] Universidad Central del Ecuador, Pathology, Quito, Ecuador; [2] Universidad Central del Ecuador, Internal Medicine, Quito, Ecuador; [3] Hospital Carlos Andrade Marín, Anatomical Pathology, Quito, Ecuador; [4] Hospital Carlos Andrade Marín, Hemathology, Quito, Ecuador

Case Description

A retrospective analysis of bone marrow examination reports from the Anatomic Pathology department was carried out between January to June 2023. A total of 2 patients had bone marrow (BM) infiltration out of 5 patients diagnosed with Langerhans Cell Histiocytosis (LCH) during the described period.

Case 1

A 26-month-old boy was admitted with a diagnosis of Pancytopenia, Neutropenic fever, and Pneumonia. The initial suspicion was Leukemia. Bone marrow analysis initially suspected non-Hodgkin lymphoma due to atypical paratrabecular lymphoid aggregates with S100 and CD1a negative. After a second core bone marrow biopsy, CD1a and S100 were found to be positive, confirming the diagnosis of BM infiltration with Langerhans Cell Histiocytosis and concluding that the atypical paratrabecular lymphoid aggregates were reactive. The patient received chemotherapy with cyclosporine to improve his basal condition.

Biopsy Fixation Details

The BM core biopsy was:

- Fixated in formalin 10% buffered during 7-8 hours approximately.
- Decalcificated with Osteomoll during 2-3 hours.

Frozen Tissue Available

- No available.

Details of Microscopic Findings

Case **1**

Bone marrow with adécuate 12 intratrabecular spaces, BM cellularity was hypocellular, with diffuse Histiocytes aggregates, accompanying by occasional eosinophils and diffuse fibrosis, cellular background was characterized by atypical lymphoid aggregates, atypic megakaryocytes and altered myeloid erythroid relation with M:E 1:1.

Immunophenotype

- CD20 Showing membranous positivity in lymphoid aggregates.
- PAX-5 Showing nuclear positivity in lymphoid aggregates.
- CD19 Showing membranous positivity in lymphoid aggregates.
- CD3 Showing membranous positivity in lymphoid aggregates.

- CD5 Showing membranous positivity in lymphoid aggregates.
- CD10 Negative in neoplastic cells.
- CD34 Negative in neoplastic cells.
- CD117 Negative in neoplastic cells.
- CD21 Negative in neoplastic cells.
- CD23 Negative in neoplastic cells.
- Anti-pan Cytokeratin Negative in neoplastic cells.
- MPO Showing cytoplasmatic positivity in myeloid lineage.
- CD68 Showing membranous positivity in tumor cells.
- S100 Showing cytoplasmatic positivity in tumor cells.
- CD1A Showing cytoplasmatic positivity in tumor cells.
- CICLINA D1 Showing nuclear positivity in tumor cells.

Cytogenetics

- No available.

Molecular Studies

- No available.

Proposed Diagnosis

Langerhans Cell Histiocytosis with bone marrow infiltration.

Interesting Feature(s)

Langerhans cell histiocytosis (LCH) is an uncommon histiocytic neoplasm characterized by the proliferation of abnormal Langerhans cells driven by sporadic activating mutations in the MAPK pathway. Our team diagnosed two cases in two Ecuadorian children between January and June 2023. In Case 1, the LCH diagnosis was made via bone marrow biopsy. Involvement of the bone marrow, liver, spleen, skeleton, and lungs are related to high-risk disease and poor survival. Bone marrow involvement is particularly unusual. The aim of this report is to highlight the clinical, hematological, and bone marrow findings of two cases of LCH with bone marrow infiltration, which may help guide detection in suspected patients.

Panel Diagnosis

Langerhans cell histiocytosis

EA4HP24-BMWS-469

Bone Marrow Infiltration in Langerhans Cell Histiocytosis, case report.

Dr. Paulina E. Santana Vargas[1,2], Dr. Marlon A. Arias Intriago[2], Dr. Manuel A. Granja Morán[3,4]

[1] Universidad Central del Ecuador, Pathology, Quito, Ecuador; [2] Hospital Carlos Andrade Marín, Anatomical Pathology, Quito, Ecuador; [3] Universidad Central del Ecuador, Internal Medicine, Quito, Ecuador; [4] Hospital Carlos Andrade Marín, Hemathology, Quito, Ecuador

Case Description

Case A 17-month-old female was admitted with anemia, fever, and cervical adenopathy. A lymph node biopsy was performed, showing positivity for CD1a, CD68, and S100, leading to the diagnosis of Langerhans Cell Histiocytosis. The suspicion of bone marrow infiltration due to severe anemia required confirmation with a bone marrow biopsy, which also showed positivity for CD1a, CD68, and S100. Chemotherapy was initiated with vinblastine and prednisone, resulting in a good response.

Biopsy Fixation Details

The BM core biopsy was:

- Fixated in formalin 10% buffered during 7-8 hours approximately.
- Decalcificated with Osteomoll during 2-3 hours.

Frozen Tissue Available

No available.

Details of Microscopic Findings

Case **2**

Bone marrow with adequate 10 intertrabecular spaces, BM cellularity was normocellular, with patchy histiocytes aggregates, accompanying by eosinophils and patchy fibrosis, cellular background was characterized by megakaryocytes, and normal myeloid erythroid relation with M:E 3:1.

Immunophenotype

- CD30 Negative in neoplastic cells.
- CD21 Negative in neoplastic cells.
- CD23 Negative in neoplastic cells.
- CD68 Showing membranous positivity in tumor cells.
- S100 Showing cytoplasmatic positivity in tumor cells.
- CD1A Showing cytoplasmatic positivity in tumor cells.

Cytogenetics

No available.

Molecular Studies

- No available.

Proposed Diagnosis

Langerhans cell histiocytosis with bone marrow infiltration.

Interesting Feature(s)

Langerhans cell histiocytosis (LCH) is an uncommon histiocytic neoplasm characterized by the proliferation of abnormal Langerhans cells driven by sporadic activating mutations in the MAPK pathway. Our team diagnosed two cases in two Ecuadorian children between January and June 2023. In Case 1, the LCH diagnosis was made via bone marrow biopsy. Involvement of the bone marrow, liver, spleen, skeleton, and lungs are related to high-risk disease and poor survival. Bone marrow involvement is particularly unusual. The aim of this report is to highlight the clinical, hematological, and bone marrow findings of two cases of LCH with bone marrow infiltration, which may help guide detection in suspected patients.

Panel Diagnosis (correct)

Langerhans cell histiocytosis

BONE MARROW WORKSHOP PART II: Myeloid neoplasms with evidence of the additive and/or cumulative effect of molecular genetic alterations that have “paved the way” to a specific disease category, subcategory or entity

Oral Presentations

EA4HP24-BMWS-230	Myeloproliferative Neoplasm NOS with CML-like features, novel NUP214::POMT1, PRRC2B::ABL1 and BCR::PRRC2B fusions and complete response after imatinib therapy
EA4HP24-BMWS-182	A young adult with Myelodysplastic/myeloproliferative neoplasm (MDS/MPN) with isolated iso17q, overt myelofibrosis and very short survival
EA4HP24-BMWS-353	HiJAKed by MPL: Genotypic and phenotypic evolution of a myeloid neoplasm
EA4HP24-BMWS-195	Myelodysplastic Syndrome with Excess Blasts and Concurrent Smoldering Myeloma in a VEXAS Patient
EA4HP24-BMWS-292	AML with NPM1 mutation

EA4HP24-BMWS-230

Myeloproliferative Neoplasm NOS with CML-like features, novel *NUP214::POMT1*, *PRRC2B::ABL1* and *BCR::PRRC2B* fusions and complete response after imatinib therapy

Dr. Humberto C. Carneiro[1], PhD/MD Marianne D.C. Goncalves[1], Dr. Rodrigo D.A. Natal[1], Dr. Luan B. Furtado[1], Dr. Abel D.C. Neto[2]

[1] Rede D'Or, Pathology, Sao Paulo, Brazil; [2] Rede D'Or, Hematology, Sao Paulo, Brazil

Case Description

In September of 2023, a 47-year-old male with previous normal blood cell count and history of hepatitis B was referred to hematology service due to leukocytosis (WBC 25.1x109/L) with neutrophilia (19.1x109/L), eosinophilia (1.0x109/L) and basophilia (1.6x109/L) and thrombocytosis (447.0x109/L), detected by the time of surgical treatment for diverticulitis.

He underwent bone marrow evaluation, and the aspirate showed a marked granulocytic hypercellularity with left-shift, accumulation of myelocytes and frequent eosinophilic precursors; blast count was less than 5%. There were only few erythroid precursors. Megakaryocytes were predominantly atypical, “dwarf-like”. Bone marrow biopsy cellularity was 100% with corresponding findings.

Considering a Chronic Myeloid Leukemia (CML) as the main differential, search for BCR::ABL1 by quantitative rtPCR was done in a peripheral blood sample, which rendered a very low level of BCR::AB1L p190 (0.0052%). That unexpected result led to more thorough investigation. NGS fusion panel revealed three fusions: NUP214::POMT1, of unknown significance; and PRRC2B::ABL1 and BCR::PRRC2B, both described in B-Acute Lymphoblastic Leukemia. The findings may reflect a more complex cytogenetic event, such as an intrachromosomal inversion or deletion on chromosome 9, followed by translocation with chromosome 22, since BCR::ABL1 was detected by rtPCR but with very low expression.

Despite molecular results, hematology team decided to initiate treatment with imatinib, with excellent response after 1 month, the patient reaching normal complete blood count with no side effects. The is no evidence of recurrence or progression until this time (4-month follow-up).

Biopsy Fixation Details

Lymph node and bone marrow biopsy fixed in buffered neutral formalin; trephine decalcified in EDTA.

Frozen Tissue Available

No.

Details of Microscopic Findings

BM aspirate: M:E ratio>10. Erythroid series: marked hypocellular, normoblastic with no dysplastic features. Granulocytic series: hypercellular with left-shift: 2% myeloblasts, 8%

promyelocytes, 22% myelocytes, 28% of metamyelocytes, 23% of band cells and 17% segmented forms; 15% of eosinophilic precursors. Megakaryocytic series: hypercellular, with predominance of small monolobated megakaryocytes. Lymphoplasmacytic series: 1% of lymphocytes. Other findings: 1% of monocytes.
BM biopsy: cellularity: increased for patient`s age (100%), with marked increase of M:E ratio. Decreased erythroid cellularity with normal maturation. Granulocytic hypercellularity with left-shift and 10-15% of eosinophils precursors. Hypercellular megakaryocytic series with abnormal monolobated forms. Reticulin fibrosis: absent. Iron deposits (Perls): not found.

Immunophenotype

Flow cytometry: Not performed. Blast count less than 1%, assessed by CD34 stain performed on bone marrow biopsy.

Cytogenetics

46,XY, del(3)(p21p13)[11]/46, XY[9].

Molecular Studies

Peripheral blood:
rtPCR: *BCR::ABL* 1p190 detected (0,0052%)
NGS DNA panel (38 genes): no variants of pathogenic significance were detected.
NGS fusion panel: *NUP21::POMT1, PRRC2B::ABL1* and *BCR::PRRC2B* detected.

Proposed Diagnosis

Myeloproliferative Neoplasm, NOS, with CML-like features and novel *NUP214::POMT1, PRRC2B::ABL1* and *BCR::PRRC2B* fusions.

Interesting Feature(s)

This case highlights the importance of combination of techniques to correctly classifying myeloid neoplasms. Moreover, it describes novel fusions in a Myeloproliferative Neoplasm that could function similarly to a variant of Philadelphia Chromosome, with clinical and morphological aspects typical of CML and complete response after imatinib therapy.

Panel Diagnosis

Myeloid/lymphoid neoplasms with eosinophilia and other tyrosine kinase gene fusions (WHO-5) / Myeloproliferative neoplasm, unclassifiable (ICC 2022)

EA4HP24-BMWS-182

A young adult with Myelodysplastic/myeloproliferative neoplasm (MDS/MPN) with isolated iso17q, overt myelofibrosis and very short survival

Dr. Dina S. Soliman[1,2], Dr. Hasan Rizvi[1], Dr. Shehab Fareed[3], Dr. Susanna Akiki[1], Dr. Feryal Ibrahim[1], Dr. Samah Kohla[1]

[1] Hamad medical corporation, Department of laboratory medicine and pathology, Doha, Qatar; [2] Weill Cornell Medicine-Qatar, Department of laboratory medicine and pathology, Doha, Qatar; [3] Hamad medical Corporation, Department of hematology/oncology, Doha, Qatar; [4] National Cancer Institute, Department of clinical pathology, Cairo, Egypt

Case Description

A 32-year-old Bangladeshi patient, with no previous medical illness, presented to ED with left upper abdominal pain, weight loss (10-15 kg in 9 months), loss of appetite, chest pain and tongue ulcer. US abdomen showed Gross hepatosplenomegaly (liver 18 cm, spleen 25.9 cm). BM examination done.

Biopsy Fixation Details

AZF

Frozen Tissue Available

N/A

Details of Microscopic Findings

Peripheral smear shows moderate thrombocytopenia, moderate macrocytic anemia with poikylocytosis, few nucleated red blood cells and few tear-drop cells.

The peripheral blood is infiltrated with ~13% blasts, medium in size with fine nuclear chromatin including few blasts with megakaryoblasts morphology. There is basophilia (~5%) with shift to left and dysgranulopoiesis. No monocytosis.

BM aspirate is hemodilute and shows trilineage hematopoiesis with increased blasts ~13%, significant dyserythropoiesis, dysgranulopoiesis (hypersegmented neutrophils, forms with hyposegmentation and hypogranulation), 3% basophils and increased monocytic cells. No ring sideroblasts seen. The trephine sample shows predominance of bone with many areas of crush artefact, but where morphology is preserved, there is unequivocal evidence of osteosclerosis and collagen fibrosis with very few entrapped haematopoietic cells. No viable well preserved intertrabecular spaces are noted that preclude further morphological assessment. Repeat BM biopsy showed similar findings with sclerotic BM.

Immunophenotype

Flow cytometry on BM aspirate shows 13% blasts expressing CD34, CD117 (majority), CD33 and HLA-DR. CD13 expressed on the minority of blasts (~23%). There is aberrant expression of CD56 (on majority) and partial CD9. Basophils comprise 2%. There are less than 0.5% cells with bright CD123 (may represent plasmacytoid dendritic cells). IHC is non-informative.

Cytogenetics

An extra signal for *RARA* probe on chromosome 17 observed in ~23 % of the cells analyzed, indicating either Trisomy 17 or 17q rearrangement. Karyotype revealed iso 17 q :
46,XY,i(17)(q10)[22]/46,XY,i(21)(q10)[5]/46,XY[38]
BCR::ABL-1 is negative.

Molecular Studies

NRAS (36%), TP53 (24%), SETBP1 (45%), CALR (52%), ASXL1 (44%), EZH2 (48%)

Proposed Diagnosis

Myelodysplastic/ Myeloproliferative Neoplasm (MDS/MPN) with Isolated Isochromosome 17q (ICC, 2022, provisional entity).

Interesting Feature(s)

This case showed overlapping features between MDS and MPN at the time of diagnosis but did not meet the criteria for any of the specific subtypes. Increased blasts and myelodysplasia coupled with cytogenetics finding of iso17q, necessitates consideration of the recently included provisional entity of MDS/MPN-U with Isolated Isochromosome 17q. The inclusion criteria of this entity: (1) 2016 WHO diagnosis of MDS/MPN-U; (2) adequate karyotype at baseline showing i(17q) as an isolated abnormality or with 1 additional abnormality (non-complex karyotype) and negative for *BCR/ABL1* rearrangement. Similar to what is published in the literature, MDS/MPN-U with isolated i17q has distinct features compared to MDS/MPN-U cases. Our patient presented at a younger age (33 years old), had significant splenomegaly with high number of circulating blasts, significant fibrosis (osteosclerosis) and very short survival. And hence, MDS/MPN-i(17q) deserves recognition as a distinct subtype within the MDS/MPN-U category based on its unique clinicobiologic features and uniformly poor prognosis.

Panel Diagnosis

Myelodysplastic/myeloproliferative neoplasm with isolated isochromosome (17q) (ICC 2022) / Myelodysplastic/myeloproliferative neoplasm, not otherwise specified (WHO-5)

EA4HP24-BMWS-353

Hi*JAK*ed by *MPL*: Genotypic and phenotypic evolution of a myeloid neoplasm.

Dr. Matthew Shapiro, Dr. Christian Salib, PhD/MD Dalia Abdel Azim, Dr. Bruce Petersen, PhD/MD Amy Duffield, Dr. Alina Dulau-Florea, **Dr. Shafinaz Hussein**

Mount Sinai Hospital, Icahn School of Medicine, Pathology, Molecular and Cell Based Medicine, New York, USA

Case Description

64 yo man p/w abdominal bloating. Labs were WBC 110K/uL (neutrophils 70%, bands 15%, metamyelocytes 5%, myelocytes 4%, promyelocytes 1%, blasts 3%, monocytes 7%), Hgb 8.9g/dL, MCV 75.3fL, Plt 53K/uL, serum iron 12mcg/dL, and transferrin sat 4%. EPO levels were not measured. The LDH was 644U/L. Ultrasound revealed splenomegaly (27cm). On admission, the patient underwent a bone marrow biopsy (BM1) and was treated with hydroxyurea and allopurinol. Decitabine and ruxolitinib were added. There was recovery of platelets (196K/uL), and reduction of leukocytosis (0.7K/uL) and spleen size (5cm). A 2nd BM biopsy (BM2) was taken 12 months later. A 3rd BM biopsy (BM3) was taken, 21 months after initial presentation, when the patient presented with abdominal pain and labs showed WBC 31.4K/uL, Hgb 12.9g/dL, and Plt 827K/uL. Spleen size increased to 11cm.

Biopsy Fixation Details

B-Plus fixative.

Frozen Tissue Available

N/A

Details of Microscopic Findings

BM1: >95% cellularity; left-shifted myeloid hyperplasia; decreased erythroid precursors and megakaryocytes. Megakaryocytes with atypical hypolobated/hyperchromatic nucleus. Neutrophils with hypogranularity and hypolobated nucleus, in subset.

BM2: Aspirated marrow with intact areas showing hypercellularity. Megakaryocytic hyperplasia in loose clusters with atypical large hyperlobated and smaller hypolobated forms with hyperchromatic nuclei. Decreased erythroid precursors. Myeloid forms show maturation.

BM3: >95% cellularity with megakaryocytic and granulocytic hyperplasia. Megakaryocytes with atypia (larger hyperlobated forms and smaller hypolobated forms with hyperchromatic nuclei).

Immunophenotype

All BM: <1% CD34+ blasts.

BM1: MF1 reticulin fibrosis (RF).

BM3: MF2 RF.

Cytogenetics

BM1: 46,XY[20]; Microarray CNLOH in 1p & 4q.

BM2: CNLOH 1p & 4q.

BM3: 46,XY[20], CNLOH 1p & 4q.

Molecular Studies

BM1: Mutations in *SRSF2*p.P95H (49.2%), *TET2*p.C1135Y (46.3%), *TET2*p.R1261H (46.6%), *JAK2*p.E543_D544del (31.4%), *RUNX1*p.L56Vfs*82 (12.8%).

BM2: New mutation in *MPL*(p.Y591D, 41%) and persistence of previous mutations, but reduced allele frequencies (AF), *SRSF2*p.P95H (29.5%), *TET2*p.C1135Y (29.4%), *TET2*p.R1261H (28.5%), and *JAK2*p.E543_D544del (3.7%). *RUNX1* mutationundetected.

BM3: Increase in AF of *MPL*(p.Y591D, 71.7%), new mutation in *ASXL1*(p.E635Rfs*15, 11.5%), and persistence with increased AF of *SRSF2*p.P95H 42.4%, *TET2*p.C1135Y 48%, *TET2*p.R1261H 46.7%, *JAK2*p.E543_D544del 21.8%. *RUNX1* remains undetectable.

Proposed Diagnosis

BM1- MDS/MPN, unclassifiable

BM2- Persistent myeloid neoplasm, no increase in blasts.

BM3- Myeloid neoplasm with primary myelofibrosis-like features

Interesting Feature(s)

- A constellation of mutations with varying AF indicates the presence of different clonal populations. Initial clinical and BM features were not entirely specific, but the emergence and dominance of *MPL*(p.Y591D) was associated with megakaryocytic hyperplasia with atypia, MF2 reticulin fibrosis, and thrombocytosis; features resembling primary myelofibrosis.
- Reports of *MPL*(p.Y591D) is uncommon in the literature, but has been described in PV and ET; in vitro studies showed that cells with this alteration have a proliferative advantage, increased sensitivity to TPO and upregulation of the JAK-STAT5 and ERK signaling (PMID: **26423830)**
- JAK2 exon 12 mutations are typically described in PV and cases of isolated erythrocytosis. Our case did not show characteristic clinico-pathologic features of PV or erythrocytosis, but the presence of iron deficiency may be a confounding factor (PMID: **36774789)**
- Co-occurrence of two driver mutations, *JAK2* exon 12 (p.E543_D544del) and *MPL*(p.Y591D), is uncommon.

Panel Diagnosis

Myelodysplastic/myeloproliferative neoplasm, NOS (ICC 2022 / WHO-5) with myeloproliferative evolution

EA4HP24-BMWS-195

Myelodysplastic Syndrome with Excess Blasts and Concurrent Smoldering Myeloma in a VEXAS Patient

Dr. Yanna Ding[1,2], Dr. Raul C. Braylan[1], Dr. Irina Irina Maric[1], Dr. Nisha Patel[1], Dr. Marcela Ferrada[3], Dr. Emma Groarke[4], Dr. Bhavisha Patel[5], Dr. Neal Young[5], Dr. Peter Grayson[3], Dr. Katherine R. Calvo[1]

[1] NIH Clinical Center, Laboratory Medicine, Bethesda, USA; [2] NIH/NCI, Laboratory of Pathology, Bethesda, USA; [3] NIH, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, USA; [4] NIH, National Heart, Lung, and Blood Institute, Bethesda, USA; [5] NIH, National Institute of Allergy and Infectious Diseases, Bethesda, USA

Case Description

60-year-old man with history of progressive pancytopenia, severe inflammation, fevers, night sweats, weight loss, arthralgias, and erythematous skin lesions. SPEP and immunofixation identified monoclonal IgA lambda protein. Skeletal survey was negative. Outside BM biopsy showed hypercellular BM with 5% blasts, and lambda-restricted plasma cell neoplasm (PCN;10-20%). NGS identified *UBA1* mutation and subclonal *EZH2* and *KDM6A* mutations. At our institution CBC showed pancytopenia (WBC 3.05 K/mcL, Hgb 8.4 g/dL, MCV 112.3 fL, Plt 38K/mcL, ANC 2.23K/mcL, ALC 0.67K/mcL, AMC 0.03 K/mcL) and other abnormal labs: CRP 37.9mg/L, ESR>140, IgA (Lambda) 1212 mg/dL, K:L ratio 0.10, ferritin 808, and elevated TNF, IL-18, IL6, IL10. A BM biopsy was performed. The patient underwent HSCT which was curative.

Biopsy Fixation Details

10% neutral buffered formalin fixed, decalcification, paraffin embedded sections

Frozen Tissue Available

NA

Details of Microscopic Findings

Peripheral blood smear:

Marked thrombocytopenia.

Macrocytic anemia, anisopoikilocytosis with polychromasia and rouleaux.

Leukopenia with absolute lymphopenia and monocytopenia. Hypogranular and pelgeroid neutrophils.

BM aspirate:

Increased blasts (7%) with fine chromatin, prominent nucleoli and high N/C ratio, and frequent cytoplasmic vacuoles.

Myeloid lineage: cytoplasmic vacuoles in early precursors, left shifted, frequent hypogranular forms

Erythroid lineage: cytoplasmic vacuoles in early precursors, occasional binucleate forms

Megakaryocytes: marked dysplasia, frequent small hypolobated forms, micromegakaryocytes.

Abnormal plasma cells: large nuclei, prominent nucleoli; some binucleation, rare multinucleated forms.

BM biopsy:

Hypercellular for age (90%) with granulocytic hyperplasia.

Increased megakaryocytes with frequent hypolobate forms and micromegakaryocytes.

Marked increased atypical plasma cells (20-30% of all cells) with large nuclei, prominent nucleoli, abundant cytoplasm, and some binucleation, forming small clusters or cords.

Immunophenotype

IHC:

Blasts: positive for CD34 (7% of all cells)

Megakaryocytes: positive for CD61

Atypical plasma cells: positive for CD138, CD56, Lambda ISH

MPO and CD71 showed M:E ratio of 4:1

Flow cytometry:

Blasts: positive for CD34, CD45, CD117, variable CD13, bright CD33, bright HLA-DR, dim CD38, dim partial CD2 and negative for CD56

Abnormal plasma cells (large cells): positive for CD138, CD38, CD56, downregulated CD81 and CD27 and negative for CD45, CD19, CD117

Cytogenetics

Karyotype: 46,XY,del(20)(q11.2q13.3)[2]/46,XY[18]

FISH: t(4;14) positive

MDS FISH: negative

Molecular Studies

NGS: UBA1 (c.118-1G>C)(splice acceptor variant) (VAF 34.5%), EZH2 (p.Thr350 AspfsTer17, VAF 2.29%), EZH2 (p.Pro563Gln, VAF 1.85%), CTCF (p.Ile96_Leu98del, VAF 16.3%), ARID1A (p.Leu2199Pro, VAF 1.66%).

Proposed Diagnosis

Myelodysplastic syndrome-excess blasts and concurrent smoldering myelomain setting of VEXAS syndrome

Interesting Feature(s)

VEXAS is a newly discovered disease with acquired *UBA1* mutations and severe autoinflammation predisposing to MDS and PCNs. The initial BM biopsy in this VEXAS patient showed hypercellularity and 5% blasts, concurrent PCN (10-20% clonal plasma cells), and MDS and myeloma-associated genetic abnormalities. Several months later the marrow showed disease progression with MDS-EB, 20-30% abnormal plasma cells and additional acquired mutations by NGS. MDS and PCN are not uncommon in VEXAS patients. However, high grade MDS and concurrent high risk PCN is unusual. In this case, progressive disease manifestations were accompanied by cumulative genetic alterations. Bone marrow transplantation was curative.

Panel Diagnosis

Myelodysplastic syndrome with excess blasts (ICC 2022) / increased blasts 1 (WHO-5) and plasma cell neoplasm in the setting of VEXAS

EA4HP24-BMWS-292

AML with *NPM*1 mutation

Dr. Hira Qadir, Dr. Yulei Shen, Dr. Sharmila Ghosh, Dr. Wei Liu, Dr. Juan Gomez-Gelvez, **Dr. Kedar Inamdar**

Henry Ford Health, Pathology, Detroit, USA

Case Description

81-y.o male with Type II DM, heart failure, chronic kidney disease, hypertension, presented due to worsening chest pain. CBC at admission revealed pancytopenia- white blood cell count (WBC) of 2.2 x 10^3/uL, hemoglobin (Hb) level of 7.3 g/dl, and a platelet count of 51K/uL. Six months ago, the patient's blood counts was significant for only anemia (Hb 10 g/dl). Laboratory screening for HIV, acute hepatitis, and hemolysis, was negative. A bone marrow exam was performed in December 2023.

Biopsy Fixation Details

Formalin for 1 hour at room temperature followed by 30 minutes at 50° C, and subsequently subjected to 4 hours of EDTA heated decalcification at 50° C.

Frozen Tissue Available

No

Details of Microscopic Findings

Core biopsy reveals >50% cellularity, featuring trilineage hematopoiesis, left-shifted myelopoiesis and significant (>10%) dysmegakaryopoiesis. Aspirates reveal dysgranulopoiesis and increasedblasts up to 5%. No morphologic or immunophenotypic evidence of B- or T-lymphoid or plasma cell neoplasm.

Immunophenotype

CD34: Negative in the blasts

Factor 8: positive in megakaryocytes with frequent hypo/monolobated forms

Cytogenetics

FISH:

Deletion of 5q. No anomalies of chromosome 7, 8, 11, 13, 17 or 20

Karyotype:

46, XY [20] with two related cell populations - del(5)(q13q33) in 18/20 (90%) and del 12p in 2/20 (10%) metaphases examined.

Molecular Studies

Myeloid Plus NGS showed the following variants of strong or potential clinical significance

*NPM*1 p. Trp288Cysfs*12) VAF 22.0%

TP53 p. (Val216Met) VAF 93.9%;

DNMT3A p. (Thr691Ile) VAF 47.3%;

PHF6 p. (Gly291*) VAF 18.4%;

PHF6 p. (Arg274*) VAF 7.7% and

TET2 p. (Gln1466Profs*13) VAF 4.1%

Proposed Diagnosis

AML with *NPM*1 mutation (WHO-HAEM5 classification)

MDS with mutated *TP53* (ICC classification)

Interesting Feature(s)

According to the WHO-HAEM5, this case can be categorized AML with *NPM*1 mutation, There are however, unusual findings including the presence of MDS-related cytogenetic abnormalities and a concurrent *TP53* mutation that complicate the diagnosis. Majority of *NPM*1-mutated AML cases display normal cytogenetics and *TP53* mutations are mutually exclusive with *NPM*1 mutation.

According to ICC classification, the blast count is below the recommended threshold of 10%, thus not compatible with AML with mutated *NPM*1. Presence of *TP53* with high VAF (93.9%), suggesting a likely homozygous *TP53* mutation, possibly through the loss of the normal allele or copy-neutral loss of heterozygosity (cnLOH) at the *TP53* locus (17p13.1) and therefore, supports MDS with mutated *TP53* using ICC guidelines. A *TP53* VAF ≥ 50% may be regarded as presumptive evidence of copy loss on the trans allele or copy neutral loss of heterozygosity. The presence of *NPM*1 mutation however, poses a diagnostic conundrum. The blast percentage in the marrow is below ICC recommended threshold for MDS/AML or AML with mutated *NPM*1 or *TP53*.

The high VAF of *TP53* raises the likelihood of germline *TP53* mutation. Germline (inherited) *TP53* mutations are linked to Li-Fraumeni syndrome. Germline testing could not performed as the patient died within one week of diagnosis. The clinical phenotype of our patient however, did not, indicate inherited Li-Fraumeni syndrome.

Given the presence of monolobated megakaryocytes and del(5q)(q31q33), it is tempting to speculate that the current disease state in our patient may represent progression of MDS with isolated deletion of chromosome 5 (MDS-del5q) secondary to additive and/or cumulative effect of molecular genetic alterations (*TP53* and *NPM*1 mutations) paving the way to a specific disease category, AML with *NPM*1 mutation (WHO-HAEM5).

Panel Diagnosis

Myelodysplastic syndrome with mutated TP53 (ICC 2022) / Acute myeloid leukemia with NPM1 mutation (WHO-5)

Cases Discussed by the Panel

EA4HP24-BMWS-14	A microscopic look into the clairvoyant's sphere: MDS/MPN, NOS with *PHF6, ASXL1, ETV6, U2AF1* and *JAK2* mutations
EA4HP24-BMWS-25	AML with TP53 Mutations Presenting with APL-like Features
EA4HP24-BMWS-30	Progression to chronic neutrophilic leukemia (CNL) from CCUS/low risk MDS (NOS)
EA4HP24-BMWS-57	Indolent systemic mastocytosis with progression to acute myeloid leukemia, associated with multiple new genetic alterations.
EA4HP24-BMWS-58	Myeloid/lymphoid neoplasm with eosinophilia and novel *ETV6::ACSL6* fusion
EA4HP24-BMWS-80	A myeloid neoplasm with del(5q) and mutations in *MPL*, *ASXL1* and *TP53*
EA4HP24-BMWS-84	Myeloid Neoplasm with a rare *PDGFRB* rearrangement
EA4HP24-BMWS-98	The boundaries between primary myelofibrosis and MDS/MPN with SF3B1 mutation and thrombocytosis.
EA4HP24-BMWS-120	Erdheim-Chester disease with associated hematological neoplasm
EA4HP24-BMWS-125	Pediatric Acute Leukemia with *NFIA::CBFA2T2* Fusion and Erythroid Differentiation
EA4HP24-BMWS-127	A vexxing diagnosis
EA4HP24-BMWS-137	Acute myeloid leukemia (AML) with *BCR::ABL1*fusion in a patient with thrombocytopenia -absent radius (TAR) syndrome
EA4HP24-BMWS-139	Chronic myelomonocytic leukemia with ET type megakaryocytes - modulation of phenotype & morphology by genotype
EA4HP24-BMWS-151	Acute Promyelocytic Leukemia with a Variant Translocation
EA4HP24-BMWS-168	Isolated CNS Erythroblastic Sarcoma with *NFIA-RUNX1T1* fusion
EA4HP24-BMWS-198	Myeloid/lymphoid neoplasm with *BCR::JAK2* rearrangement
EA4HP24-BMWS-223	Acute myeloid leukemia with NPM1 mutation and associated myeloid sarcoma in a 1-year-old child
EA4HP24-BMWS-242	Myeloid Neoplasm in VEXAS Syndrome
EA4HP24-BMWS-244	Clonal and disease evolution of a myeloid neoplasm with germline *DDX41* mutation
EA4HP24-BMWS-259	A Case of Myeloid Neoplasm with Germline DDX41 Mutation and Normal Karyotype
EA4HP24-BMWS-263	Chronic myelomonocytic leukemia evolving into acute myeloid leukemia after acquisition of an NPM1 mutation
EA4HP24-BMWS-264	Aggressive systemic mastocytosis associated with later-onset chronic myeloid leukemia
EA4HP24-BMWS-272	"Paving the way" to Systemic Mastocytosis
EA4HP24-BMWS-279	t(9,15)(q22;q24.1) *PML::SYK*: A new recurrent translocation in myeloid neoplasm with marked eosinophilia
EA4HP24-BMWS-283	Leukocytosis in an infant associated with an NRAS mutation

EA4HP24-BMWS-306	Myeloid/erythroid Sarcoma and Pure Erythroid Leukemia with *ZMYND8::RELA* Fusion
EA4HP24-BMWS-307	Myelodysplastic syndrome with multiple truncating *GATA2* mutations
EA4HP24-BMWS-308	Acute myeloid leukemia with *DDX41* mutation
EA4HP24-BMWS-320	Divergent evolution from a founding clonal hematopoiesis clone to initial acute myeloid leukemia with *NPM1* mutation and later AML with mutated *TP53* after relapse
EA4HP24-BMWS-335	An unusual myelodysplastic progression of essential thrombocytemia, with subsequent short-term blastic transformation
EA4HP24-BMWS-343	Acute B-lymphoblastic leukemia with previous history of ET and follicular lymphoma
EA4HP24-BMWS-382	Exploring the Significance of Positive JAK2 V617F in a 5q Deletion Myeloid Neoplasm with Myelodysplastic and Myeloproliferative Overlap Features: A Case Report"
EA4HP24-BMWS-391	Ras-associated autoimmune leukoproliferative disorder (RALD): A patient with large cell transformation of mycosis fungoides, plasma cell neoplasm and persistent monocytosis
EA4HP24-BMWS-395	A rare case of simultaneous coexistence of chronic lymphocytic (CLL) and chronic myeloid leukemia (CML)
EA4HP24-BMWS-401	An unusual orbital granulation tissue
EA4HP24-BMWS-405	Myelodysplastic/myeloproliferative neoplasm with *NPM1*, *DNMT3A* and *NOTCH3* mutations evolving into acute myeloid leukemia in an adolescent patient
EA4HP24-BMWS-411	MDS/MPN - using mutation analysis
EA4HP24-BMWS-428	The concurrent presence of plasma cell myeloma and a JAK2 V617F-positive hypoplastic fibrotic myeloid neoplasm with dysmegakaryopoiesis presents a diagnostic challenge.
EA4HP24-BMWS-429	Bone marrow findings of chronic myeloid leukemia with concurrent *BCR-ABL* fussion and *JAK2* V617F mutation at diagnosis
EA4HP24-BMWS-432	CALR mutated myeloid neoplasm with features of myeloproliferative neoplasm and myelodysplastic syndrome (neoplasm)
EA4HP24-BMWS-442	Progression of MPL-mutated myeloproliferative neoplasm with bi-allelic *TP53* mutations
EA4HP24-BMWS-444	A patient with Rosai-Dorfman disease (RDD), Ras-associated autoimmune leukoproliferative disorder (RALD) progressing to chronic myelomonocytic leukemia (CMML) and a clonal plasma cell proliferation
EA4HP24-BMWS-457	Transformation of a JAK2 positive Myeloproliferative neoplasm with 2 early/undifferentiated cell populations
EA4HP24-BMWS-472	Myelodysplastic syndrome/myeloproliferative neoplasm with concurrent JAK2 V617K mutation and del(5q)
EA4HP24-BMWS-475	A case of triple-negative MPN with alternative mutations affecting JAK-STAT signaling and epigenetic regulation

EA4HP24-BMWS-14

A microscopic look into the clairvoyant's sphere: MDS/MPN, NOS with *PHF6, ASXL1, ETV6, U2AF1* and *JAK2* mutations

Prof. Alexandar Tzankov

University Hospital Basel, Pathology, Basel, Switzerland

Case Description

M, 78 with unremarkable past clinical history presented to his general practitioner and thereafter to the hematology department due to fatigue.

His PBC/CBC at the time of presentation were:

- RBC 2.6 x 10^{12}/L
- Hb 79 g/L
- PLT 106 x 10^{9}/L
- WBC 13.47 x 10^{9}/L
- Neutro 84%
- Lymph 9.7%
- Mono 5.8%
- Eos 0.4%
- Baso 0.1%
- No peripheral blasts

Biopsy Fixation Details

FFPE, EDTA decalcified

Frozen Tissue Available

No

Details of Microscopic Findings

Hypercellular bone marrow with erythroid hypoplasia, megakaryocytic clustering and dysmegakaryopoiesis, and diffuse myelofibrosis grade 3. No increased blasts.

Immunophenotype

Increased microvascular density but no increase of blasts (CD34-stain). Presence of nuclear pSTAT5+ and pSTAT5- megakaryocytes, suggesting the acquired subclonal nature of a/the *JAK2* mutation; nuclear pSTAT5+ megakaryocytes being highly predictive of *JAK2* mutation (AJSP 2007;31:233).

Cytogenetics

46,XY

no *BCR::ABL1*-fusion, no t(3;3), no inv(3), no del(5q)

Molecular Studies

- *PHF6* R342* (VAF 84%)
- *ASXL1* D820Vfs*4 (VAF 42%)
- *U2AF1* Q157P (VAF 42%)

- *ETV6* T161Dfs*8 (VAF33%)
- *JAK2* V617F (VAF 19%)
- *ETV6* D101Rfs*22 (VAF 9%)

Proposed Diagnosis

Myelodysplastic/myeloproliferative neoplasm (MDS/MPN), NOS

Interesting Feature(s)

The mutational composition almost perfectly explains the clinical presentation and the morphologic appearance of the case:

- *PHF6, ASXL1**, and *ETV6* – linked to myelodysplasia and *platelet decrease
- *U2AF1* (and *ASXL1*) – linked to severe anemia
- *JAK2* – linked to myelofibrosis and megakaryocytic clustering

  The case generally addresses the question whether the time is ripe for a genetic classification of chronic myeloid neoplasms.

References:

- AJSP 2007;31:233
- Am J Hematol 2010;85:866;
- Blood 2013;122:3616;
- Blood 2017;130:1125;
- Blood 2020;136:2249;
- Blood Cancer J 2016;6:e415;
- Br J Haematol 2019;186:396;
- Clin Lymphoma Myeloma Leuk 2020;20:324;
- JCO 2021;39:1223.

Panel Diagnosis

Myelodysplastic/myeloproliferative neoplasm, not otherwise specified (ICC 2022 / WHO-5)

EA4HP24-BMWS-25

AML with TP53 Mutations Presenting with APL-like Features

Dr. Jeremiah Karrs, Dr. Prabhjot Kaur, Dr. Wahab Khan

Dartmouth Hitchcock, Pathology, Lebanon, USA

Case Description70 year old male found have leukocytosis with anemia and thrombocytopenia at an outside institution. Clinically, the patient appeared stable without coagulopathy on laboratory studies.

WBC 27.2 x10(3)/mcL (Ref. Range 4.0 - 9.5)
RBC 3.12 x10(6)/mcL (Ref. Range 4.58 - 5.54)
Hgb 9.5 g/dL (Ref. Range 13.7 - 16.5)
Hct 30.3 % (Ref. Range 40.5 - 48.5)
MCV 97.1 fL (Ref. Range 82.9 - 93.1)
MCH 30.4 pg (Ref. Range 27.5 - 32.1)
MCHC 31.4 g/dL (Ref. Range 32.0 - 35.7)
RDWSD 87.9 fL (Ref. Range 36.0 - 45.0)
RDWCV 25.3 % (Ref. Range 11.4 - 13.8)
Platelet 85 x10(3)/mcL (Ref. Range 145 - 357)
MPV 9.4 fL (Ref. Range 7.6 - 12.9)
Reticulocyte % 3.4 % (Ref. Range 0.7 - 2.6)
Retic Abs # 0.110 x10(6)/mcL (Ref. Range 0.030 - 0.120)
Immature Retic% 35.3 % (Ref. Range 0.0 - 15.6)
Reticulated Hgb 34.2 pg (Ref. Range 31.3 - 40.2)
NRBC% auto 1.7 %
Segs Man 10 %
Lymph Man 19 %
Monocyte Man 3 %
Eos Man 1 %
Meta Man 4 %
Myelo Man 31 %
Promyelo Man 26 %
Blasts Man 6 %
ANC 2.72 x10(3)/mcL (Ref. Range 1.70 - 6.10)
Lymph Abs Man 5.2 x10(3)/mcL (Ref. Range 0.9 - 3.2)
Monocyte Abs Man 0.8 x10(3)/mcL (Ref. Range 0.3 - 0.9)

Biopsy Fixation Details

10% buffered formalin

Frozen Tissue Available

No

Details of Microscopic Findings

Peripheral blood and aspirate smears show immature appearing cells with promyelocytic features. Core biopsy was limited but showed predominately immature appearing myeloid cells.

Immunophenotype

By flow cytometry, the blast gate was expanded and cells were positive for (c)MPO, CD117 (variable), CD33, CD64, CD13 (variable), while negative for CD34, HLA-DR, CD3 (surface and cytoplasmic), CD19, (c)CD79a, CD10, CD7, CD14, (n)TdT, CD56. Immunostains on the core showed immature appearing mononuclear cells were positive for MPO, CD117 and negative for CD34 and E-cadherin.

Cytogenetics

- The karyotype was abnormal showing 44,X,-Y,del(5)(q13q33),-9,del(17)(p11.2),14~53dmin[cp15]/88,slx2[cp5]

- FISH for PML-RARA was negative for rearrangement, although it did show low level extra copies; loss of NUP214 and ABL1 loci were also seen
- Optical genome mapping showed gains and losses comparable to the karyotype while also showing at the gene level amplification of MYC

Molecular Studies

- RT-qPCR for PML-RARA was negative.
- NGS (next-generation sequencing) showed a pathogenic TP53 mutation (p.S241C) at 79.60% VAF.

Proposed Diagnosis

AML with mutated TP53

Interesting Feature(s)

- The neoplastic cells in this case show unusual morphologic and immunophenotypic features initially suggesting APL
- Genomic work-up ruled out APL (negative FISH, PCR and OGM) while providing findings (dmin MYC amplification) that have been reported in APL-like acute myeloid leukemia cases
- Additionally, NGS and cytogenetic studies support a diagnosis of an AML with mutated TP53, which appears to underlie the other findings

Panel Diagnosis

Acute myeloid leukemia with mutated TP53 (ICC 2022) / Acute myeloid leukemia, myelodysplasia related (WHO-5)

EA4HP24-BMWS-30

Progression to chronic neutrophilic leukemia (CNL) from CCUS/low risk MDS (NOS)

Kai Zhang

Geisinger Health System, Geisinger Medical Laboratory and Department of Pathology, Danville, Germany

Case Description

74 M with Afib, HTN and OSA with mild anemia since 2015 with Hb (12.5-13.5), later developed thrombocytopenia associated with mild neutrophilia in 07/2021. Intial clinical and lab workup failed to reveal causes for abnormal CBC. On 4/7/2022, bm biopsy was performed. PB smear showed mild normochromic normocytic anemia (Hb 11.6 and MCV 88.9), thrombocytopenia (90), borderline high WBC (10.6) with neu 67%, myelo 2% ,Meta 5%, eos 1% baso 1%, mono 4% and lym 20%. Rare blasts were observed. Leukocytes showed no dysplasia. BM aspirate and bx were adequate, showing markedly increased cellularity at 80% for age with increased myeloid element with M:E ratio of 6:1. Diffs: blasts 1%, pro1%,

myelo/meta 34%, bands/neut 37%, mono 4%, eos 0%, lym 3% and nRBCs18%. No RS was seen. Trilineage hematopoietic cells showed orderly maturation except there were a few midly dyspoietic nucleated erythroid as well as megakaryocytes. Flow study showed no significant abnormalities with no EB. Karyotype was normal with XY/46 [20]. NGS revealed multiple Tier 2 gene mutations which included ASXL1, EZH2, TET2 (x2), and U2AF1. All mutated genes have allele frequency (AF) ranging from 45% to 48%. Diagnosis of CCUs or evolving low risk low blast MDS (NOS) was considered.
On 9/2023, the patient presented with marked worsening leukocytosis. WBC 36.12, HGB 11.0, MCV 90.1 and PLT 84. Neut 84%, meta 2%, myelo 3%, eos 1%, mono 4%, and lym 6%. Pb smear showed marked leukocytosis with predominantly neutrophilia and other findings were similar to the pb smear in 2022. Repeat of BMbx biopsy showed overall similar differentials and findings to the bmbx in 2022, which included marked hypercellularity at 90% with predominantly increased neutrophilic elements, and the presence of a few midly dyspoietic nucleated erythroid as well as megakaryocytes. Flow study was normal with no EB. Karyotype was normal with XY/46 [20]. NGS revealed newly emerging Tier 1 CSF3R gene mutation with AF at 18% in addition to previously identified gene mutations (ASXL1, EZH2, TET2 x2, and U2AF1) with similar allele frequency ranging from 45% to 48%. Diagnosis of progression to chronic neutrophilic leukemia (CNL) from CCUS/low risk MDS (NOS) was made.

Biopsy Fixation Details

10% buffet formaline with brief decal.

Frozen Tissue Available

NA

Details of Microscopic Findings

See case description.

Immunophenotype

Flow study: Normal.

Cytogenetics

2022. Karyotype was normal with XY/46 [20].
2023: Karyotype was normal with XY/46 [20].

Molecular Studies

2022: NGS revealed multiple Tier 2 gene mutations which included ASXL1, EZH2, TET2 (x2), and U2AF1. All mutated genes have high allele frequency (AF) ranging from 45% to 48%. NGS revealed newly emerging Tier 2023. NGS reveals CSF3R gene mutation with AF at 18% in addition to previously identified gene mutations (ASXL1, EZH2, TET2 x2, and U2AF1 with similar high allele frequency ranging from 45% to 48%).
2023: NGS revealed newly emerging Tier 1 CSF3R gene mutation with AF at 18% in addition to previously identified multiple Tier 2 gene mutations (ASXL1, EZH2, TET2 x2, and U2AF1) with similar high allele frequency ranging from 45% to 48%.
FISH MPN panel: Norma for probes (9;22, PDGFRA, PDGFRB and FGFR1).

Proposed Diagnosis

Progression to chronic neutrophilic leukemia (CNL) from CCUS/low risk MDS (NOS)

Interesting Feature(s)

Newly accumulated or acquired CSF3R gene mutation in the background of multiple Tier 2 gene mutations results in progression to CNL from CCUS /low MDS.

Panel Diagnosis

Myelodysplastic neoplasm with low blasts (WHO-5) / Myelodysplastic syndrome, NOS with multilineage dysplasia (ICC 2022) with CNL-like progression

EA4HP24-BMWS-57

Indolent systemic mastocytosis with progression to acute myeloid leukemia, associated with multiple new genetic alterations.

Dr. Chandler Sy[1], Dr. Georgi Lukose[1], Dr. Ivo Sahbandar[1], Dr. Sanjay Patel[1], Dr. Madhu Ouseph[1], Dr. Paul Simonson[1], Dr. Pinkal Desai[2], Dr. Julia Geyer[1]

[1] Weill Cornell Medicine / New York Presbyterian Hospital, Pathology and Laboratory Medicine, New York, USA; [2] Weill Cornell Medicine / New York Presbyterian Hospital, Medicine, New York, USA

Case Description

The patient is a 67-year-old man with a history of urticaria and a markedly elevated eosinophil count, which was discovered on routine lab testing 6 years ago. A bone marrow (BM) biopsy performed 5 years ago revealed systemic mastocytosis (SM). NGS showed genetic alterations in *SF3B1* (c.1998G>C), *STAT5B* (c.1924A>C), and *TET2* (c.4189A>G, c.688_689delCT); BM PCR was positive for the *KIT* D816V mutation. He has been asymptomatic and did not require therapy. He presented for a routine follow-up and a BM biopsy was performed. CBC showed normal values of WBC 6.5 K/µL, Hgb 13.2 g/dL, MCV 99.2 fL, PLT 157 K/µL, with persistent eosinophilia (27%, 1.76 K/µL). Serum tryptase was 16.6 µg/L (RR ≤10.9 µg/L).

Biopsy Fixation Details

Bouin

Frozen Tissue Available

None

Details of Microscopic Findings

The BM cellularity was 50-60%. The M:E ratio was decreased due to erythroid hyperplasia and decreased granulopoiesis. The myeloid elements were left-shifted with increased blasts. Megakaryocytes showed some small hypolobated forms. A reticulin stain revealed no fibrosis. BM aspirate demonstrated 14% blasts. Subtle dysgranulopoiesis was present with occasional hypogranulation and abnormal segmentation. Erythropoiesis appeared megaloblastoid. Eosinophilia was present with increased immature forms. Scattered mast cells were seen.

Immunophenotype

Immunostains revealed increased CD34, CD117+ blasts, occurring singly and in occasional clusters (10-20%). Mast cell tryptase and CD117 highlighted scattered CD25+ spindle-shaped mast cells (<5%). CD61 marked occasional micromegakaryocytes. CD123 highlighted increased plasmacytoid dendritic cells. Flow cytometry detected abnormal myeloid blasts with aberrant TdT and partial CD7 expression.

Cytogenetics

Cytogenetic analysis showed a normal karyotype.

Molecular Studies

NGS demonstrated genetic alterations in the following genes: *SF3B1* (c.1998G>C, 45% VAF) of strong clinical significance; 8 *TET2* (c.688_689delCT, 6%; c.4189A>G, 45%; c.2218C>T, 2%; c.3383_3384dupAT, 4%; c.2290delC, 1%; c.3308_3315del8, 4%; c.3385G>T, 4%; c.4642C>T, 1%), *CBL* (c.1211G>A, 24%), *STAT5B* (c.1924A>C, 22%), 3 *RUNX1* (c.502G>T, 20%; c.472T>C, 8%; c.422C>G, 12%), and *FH* (c.1462G>A, 25%) of potential clinical significance. A very low *KIT* D816V mutation was identified (below reporting threshold).

Proposed Diagnosis

Systemic mastocytosis with associated evolving acute myeloid leukemia

Interesting Feature(s)

This previously untreated patient with known indolent SM and genetic alterations in *SF3B1*, *TET2*, *STAT5B*, and *KIT* presented for routine BM evaluation and was found to have increased CD34, CD117+ blasts (14% on aspirate). At the time he was asymptomatic with normal CBC values except for persistent eosinophilia. Repeat molecular testing revealed multiple genetic alterations including those previously reported as well as additional alterations in *TET2* (6 new variants), *CBL*, *RUNX1* (3 variants), and *FH*. A month later, repeat BM aspirate revealed a blast count of 41%. We believe that 5 years after being diagnosed with SM, the patient developed an associated acute myeloid leukemia (SM-AML) as evidenced by the cumulative genetic alterations. These findings are consistent with a prior study which reported that *RUNX1* and *TET2* are among the most frequent mutations in patients with SM-AML (Jawhar 2017). The patient was treated with daunorubicin/cytarabine for secondary AML with evidence of minimal residual disease, followed by venetoclax/azacitidine. A BM transplant is planned.

Panel Diagnosis

SM-AMN / AHN (ICC 2022 / WHO-5): ISM and AML with myelodysplasia-related gene mutations (ICC 2022) / myelodysplasia-related (WHO-5)

EA4HP24-BMWS-58

Myeloid/lymphoid neoplasm with eosinophilia and novel *ETV6::ACSL6* fusion

Dr. Ashley K. Volaric, Dr. Juli-Anne Gardner, Dr. Katherine A. Devitt, Dr. Joanna L. Conant

University of Vermont Medical Center, Pathology and Laboratory Medicine, Burlington, USA

Case Description

76-year-old female with a past medical history of hypertension and chronic hyponatremia, presents with new altered mental status found to have multiple watershed infarcts, COVID-19+, cardiomyopathy, and peripheral blood eosinophilia.

Laboratory findings were notable for leukocytosis (WBC 21,670/cmm; reference range of 4,000-12,400/cmm) with absolute eosinophilia (10,290/cmm; 30-610/cmm), absolute basophilia (1,650/cmm; 10-110/cmm), 2% blasts, and microcytic anemia (Hgb 7.4 gm/dL (11.6-15.2 gm/dL), MCV 76 fL (81-98 fL)). Infectious work-up for viral, bacterial, and parasitic causes were negative with the exception of a positive COVID-19 antigen test.

Biopsy Fixation Details

Neutral-buffered formalin fixation

Frozen Tissue Available

No

Details of Microscopic Findings

The peripheral blood smear was notable for increased eosinophils with left shift, dysplastic (hypo-granular and hypo-lobated) neutrophils, and circulating blasts. The blasts demonstrated monocytoid features including convoluted nuclei, fine chromatin, prominent nucleoli, and abundant amphophilic cytoplasm with prominent vacuoles. Bone marrow biopsy demonstrated multi-lineage dysplasia including hypo-lobated megakaryocytes, myeloid hyperplasia, and increased blasts (9%).

Immunophenotype

Flow cytometry of the peripheral blood revealed a small population of myeloid blasts (1% of CD45+ events) expressing CD33, CD117, and CD34. Blasts also highlighted by CD34 immunohistochemistry in bone marrow core biopsy.

Cytogenetics

Complex abnormal female karyotype including deletion of long arm of chromosome 5 (del(5q) in four related cell lines and t(5;12) in one of the four cell lines. FISH negative for *CBFB* rearrangement, *RUNX1::RUNX1T1* fusion,and deletion 17p.

46,XX,del(5)(q13q33)[11]/46,idem,t(5;12)(q31;p13)[12]/46,idem,-X,-2,11,+3mar[4]/45,idem,add(1)(p32),-7,-8,-19,+2mar[2]/46,XX[1].

Eosinophilia FISH panel was abnormal with one intact copy of the *PDGFRB* gene region at 5q32 but negative for *PDGFRB::ETV6* gene fusion.

Molecular Studies

Next generation sequencing (NGS) of the bone marrow for a panel of pathogenic genes and RNA fusion transcripts identified a novel *ETV6::ACSL6* fusion, which resulted in an in-frame fusion of *ETV6* exon 2 with *ACSL6* exon 2. In addition, several pathogenic variants were identified: *IDH1* (p.R132C, c.394C>T, 2.9% variant allele frequency (VAF)), *TP53* (p.E298Afs*6, c.893_897del5, 3.3% VAF), *TP53* (p.S240R, c.720T>G, 69.6% VAF), *TP53* (p.H168R, c.503A>G, 3.6% VAF), *PPM1D* (p.L484*, c.1451T>A, 3.5% VAF).

Proposed Diagnosis

Myeloid/lymphoid neoplasm with eosinophilia and *ETV6::ACSL6* fusion

Interesting Feature(s)

- Novel pathogenic fusion of *ETV6::ACSL6* was detected by molecular NGS assay and presented as a deletion of *PDGFRB* gene region at chromosome 5 by FISH and t(5;12) on karyotype analysis.
- Deletion(5q) with t(5;12) subclone by karyotype analysis suggests the possibility of an underlying myelodysplastic neoplasm with del(5q) with evolution to a myeloid/lymphoid neoplasm with eosinophilia and *ETV6::ACSL6* fusion.
- The *ETV6::ACSL6* fusion, although rare in myeloid/lymphoid neoplasms, has been reported in the literature and is associated with poor prognosis as the fusion protein typically is not susceptible to tyrosine kinase inhibition.

Panel Diagnosis

Myeloid/lymphoid neoplasms with eosinophilia and other tyrosine kinase gene fusions (WHO-5) / Chronic eosinophilic leukemia (ICC 2022)

EA4HP24-BMWS-80

A myeloid neoplasm with del(5q) and mutations in *MPL*, *ASXL1* and *TP53*

PhD/MD Maria Rozman, PhD/MD Irene Lopez-Oreja, Dr. Monica Lopez-Guerra, PhD/MD Marina Diaz-Beya, Dr. Silvia Bea

Hospital Clilic Barcelona, Hematopathology & Hematology Department, IDIBAPS, BARCELONA, Spain

Case Description

A 82 year-old male with pluripathology consulted in April 2022 in another center for asthenia. The hemogram showed anemia, for which he received iron, folic acid and several blood transfusions and was diagnosed with MDS with del(5q). In October 2023 he was sent to our hospital, where the blood count revealed Hb 71 g/L, MCV 113 fL, platelets 145x10^9/L, leukocytes 7190 x10^9/L (neutrophils 68%, bands 2 %, eosinophils 1%, basophils 1%, lymphocytes 11%, monocytes 16%, myelocytes 1%). The examination showed only mild splenomegaly (14 cm). The biochemistry including LDH was unremarkable. The patient was

treated with Lenalidomide, and after 6 cycles the need of transfusions lowered significantly (1 every 3 months) and the del(5q) by FISH became negative; the bone marrow features and the molecular profile remained unaltered.

Biopsy Fixation Details

B23-105 A: fixation with formalin 10%, decalcification with formic acid 10%; B23-105 B: fixation with Bouin solution, decalcification with hydrochloric acid 1-5% decalcifier (bone marrow decalcifier Casa Alvarez).

Frozen Tissue Available

No

Details of Microscopic Findings

Bone marrow aspirate and biopsy were hypercellular, with increased myeloid and erythroid precursors irregularly distributed but with preserved M/E ratio. 88% dysgranulopoiesis (hypogranulation and abnormal segmentation) and 45% dyserythropoiesis (megaloblastic changes, irregular nuclei) were seen. Megakaryocytes were preserved and normally distributed, many of them without morphologic abnormalities but occasional hypolobated or non-lobated forms were seen. Blasts were <5%. Monocyte count in the aspirate was 13%. Iron staining showed decreased iron deposits, with 10% sideroblasts and 2% ring sideroblasts. Reticulin fibrosis was MF1.

Immunophenotype

Flow cytometry of peripheral blood showed mild monocytosis with >94% classical monocytes, half of them CD56 positive.

Cytogenetics

46,XY,del(5)(q21q33)[4]/46,XY[17]

Molecular Studies

NGS showed the following mutations: *MPL* p.Ser204Pro (41%) pathogenic, *ASXL1* p.Gln588Ter (34%) pathogenic and *TP53* p.Cys141Gly (36%) probably pathogenic. *BCR::ABL1* by PCR was negative.

Proposed Diagnosis

Chronic myelomonocytic leukemia, myelodysplasic (CMML-MDS).

Interesting Feature(s)

Our case describes a myeloid neoplasm with del(5q) and mutations in *MPL*, *ASXL1* and *TP53*. The clinical picture with anemia, slight splenomegaly and monocytosis, together with the mutational profile raised two main possible diagnoses: chronic myelomonocytic leukemia (CMML) or primary myelofibrosis (PMF) with monocytosis. The *MPL* mutation, although it was not the commonest *MPL*W515L/S505 found in myeloproliferative neoplasms, has been described in triple-negative ET[1], but the histopathology of the bone marrow biopsy ruled out PMF. The del(5q) in a patient with anemia let to an erroneous diagnosis of MDS with del(5q) in another center, but this alteration was subclonal and has also been described in up to 1.5% CMML cases[2]. In conclusion, integrating all the clinical, pathological and genetic data the criteria for diagnosis of CMML were fulfilled.

1. Angona A, Fernández-Rodríguez C, Alvarez-Larrán A, et al. Molecular characterisation of triple negative essential thrombocythaemia patients by platelet analysis and targeted sequencing. Blood Cancer J. 2016;6(8):e463.

2. Parikh SA, Tefferi A. Chronic myelomonocytic leukemia: 2012 update on diagnosis, risk stratification, and management. Am. J. Hematol. 2012;87:610-619.

Panel Diagnosis

Chronic myelomonocytic leukemia type 1, myelodysplastic subtype (ICC 2022) / Myelodysplastic chronic myelomonocytic leukemia type 1 (WHO-5)

EA4HP24-BMWS-84

Myeloid Neoplasm with a rare *PDGFRB* rearrangement

Dr. Daniel Rivera, Dr. Hanadi El Achi, Dr. Jacob Armstrong, Dr. Andy Nguyen, Dr. Amer Wahed, Dr. Brenda Mai

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

Case Description

•The patient is a 36-year-old man without significant past medical history who presented with weakness, lower extremity edema, and pain. CT scan revealed splenomegaly of 23 centimeters (cm) and hepatomegaly of 20 cm without lymphadenopathy.

•Blood work revealed leukocytosis (61.5 x109/L), moderate anemia (hemoglobin level of 9.4 g/dL), and a normal platelet count (190 x109/L). Additionally, neutrophilia (29.4 x109/L), monocytosis (5.1 x109/L), and eosinophilia (7.2 x109/L). No nucleated red blood cells or blasts were identified on the peripheral blood smear.

Biopsy Fixation Details

•Formalin-fixed paraffin-embedded tissue.

Frozen Tissue Available

•Frozen Tissue Available: None.

Details of Microscopic Findings

Findings: Bone marrow was hypercellular (~100%) with trilineage hematopoiesis, granulocytic hyperplasia without dysplasia, and less than 5% blasts. The Myeloid to erythroid ratio was about 13:1. Immunohistochemical testing, CD34 highlighted scattered blasts comprising less than 5% of the total marrow population, including marrow sinusoids. CD61 highlighted megakaryocytes showing normal morphology and distribution. Reticulin stain showed focal areas of mild fibrosis (MF-1), while Trichrome stain did not reveal significant collagen fibrosis (MF-0).

Immunophenotype

No abnormal immunophenotype was found by flow cytometry.

Cytogenetics

Conventional cytogenetic studies showed t(5;14)(q33;q32) in 20 out of 20 metaphases. Interphase fluorescence in situ hybridization (FISH) showed a rearrangement of *PDGFRB* in 88% of nuclei.

Molecular Studies

BCR-ABL1 fusion transcript was not detected. No additional molecular abnormalities were detected.

Proposed Diagnosis

Myeloid Neoplasm (MN) with *PDGFRB* rearrangement.

Interesting Feature(s)

•MN with *PDGFRB* gene rearrangements could represent a diagnostic challenge that could result in misdiagnosis. The differential diagnosis includes chronic myelomonocytic leukemia with eosinophilia, myelodysplastic/myeloproliferative neoplasm with neutrophilia with eosinophilia, chronic eosinophilic leukemia, or other myeloproliferative neoplasms (MPN) with eosinophilia and therefore those must be excluded first. The most common cytogenetic alteration for MN with *PDGFRB* rearrangement is t(5;12)(q32;p13.2), resulting in *ETV6::PDGFRB* gene fusion. To date, more than 25 different *PDGFRB* fusion genes have been identified as a consequence of rearrangements of chromosome bands 5q in MN with *PDGFRB* rearrangement. Our case is unique in that a t(5;14)(q33;q32) was detected, which can result in a diagnostic challenge. Other reported entities associated with t(5;14) include B-cell acute lymphoblastic leukemia (B-ALL) and T-cell acute lymphoblastic leukemia. Cases of B-ALL with t(5;14) can also cause eosinophilia. According to the literature review, the t(5;14)(q33;q32) detected in this patient has been identified previously, although it is scarce. The t(5;14)(q33;q32) detected can result from a *PDGFRB::CCDC88C* gene fusion or *PDGFRB::TRIP11* gene fusion. Further investigation regarding these gene fusions is needed for prognostic evaluation and treatment optimization.

Panel Diagnosis

Myeloid/lymphoid neoplasms with eosinophilia and PDGFRB rearrangement (ICC 2022 / WHO-5)

EA4HP24-BMWS-98

The boundaries between primary myelofibrosis and MDS/MPN with SF3B1 mutation and thrombocytosis.

Dr. Arturo Bonometti

Humanitas University, Department of Biological Sciences, Pieve Emanuele, Italy

Case Description

An 86-year-old male with a recent history of recurrent headaches came to our attention for weight loss and a neutrophilic leukocytosis with thrombocytosis.
Laboratory examination showed the following results: Hb 8,4 g/dL, WBC 12.210/mm3, (N 10.620/mm3, M 580/mm3), PLT 738.000/mm3, LDH 826 U/L.

NGS analysis (Oncomine myeloid panel) revealed an isolated JAK2_V617F mutation with a 32,9% VAF. Bone marrow examination revealed bone marrow hypercellularity (70%), with granulocytic hyperplasia and left shifting, megakaryocytic hyperplasia with atypia and dense clusters formation, osteosclerosis grade 1, and a reticulin fibrosis grade 2. Erythropoiesis was highly reduced, and blasts were not increased.
Altogether a diagnosis of overt fibrotic stage of primary myelofibrosis was rendered and hydroxyurea therapy was started with reduction of the peripheral cytoses.
Sixteen months after the diagnosis, the treatment was interrupted for a severe anemization, and a novel bone marrow biopsy was performed together with novel cell count (Hb 8,6 g/dL, WBC 11.370/mm3, (N 8600/mm3, M 610/mm3), PLT 531.000/mm3).

Biopsy Fixation Details

FFPE, and EDTA decalcifyed.

Frozen Tissue Available

No

Details of Microscopic Findings

The novel bone marrow biopsy revealed a further increase in cellularity (90%), with findings on the granulocytic and megakaryocytic lineages similar to those observed in the previous biopsy. Even though was still reduced, the erythroid series was slightly more represented. Microvessel density was increased, with the presence of intrasinusoidal hematopoiesis, and stromal alteration included osteosclerosis grade 2, collagen fibrosis grade 1, and reticulin fibrosis grade 1. There was still no increase in blasts.
At the bone marrow smear examination, the erythroid series was hyperplastic (55% of the cells) with positivity at Perls reaction in more than half of the erythroid cells.

Immunophenotype

CD34+ cells: 1-2%
p53: 2%

Cytogenetics

46, XY[21]

Molecular Studies

NGS on the current sample revealed the presence of the JAK2V617F mutation at an highly reduced VAF (3%), and three additional pathogenic mutations in ASXL1, SF3B1, and TET2, all with >10% VAF.

Proposed Diagnosis

Primary myelofibrosis in overt fibrotic stage with molecular and immunocytochemical features of myelodysplastic/myeloproliferative neoplasms with SF3B1 mutation and thrombocytosis.

Interesting Feature(s)

Despite the late acquisition of SF3B1 mutation in MPNs being well known, this patient showed an impressive modification of the clonal architecture of its neoplasm, with marked reduction of the JAK2-mutation VAF and gain of additional mutation, including SF3B1, in the course of hydroxyurea.

Panel Diagnosis

(Overt) primary myelofibrosis (ICC 2022 / WHO-5) with MDS-type of progression

EA4HP24-BMWS-120

Erdheim-Chester disease with associated hematological neoplasm

Dr. Dragan Jevremovic, Dr. Karen Rech

Mayo Clinic, Division of Hematopathology, Rochester, USA

Case Description

65-year-old man presented with abdominal pain, nausea, diarrhea. Imaging showed retroperitoneal mass surrounding both kidneys, renal vessels, aorta, and sclerosing mesenteritis. There was a previous history of monocytosis (1.2x10(9)/L); bone marrow biopsy had been interpreted as normal. Radiologic findings of the retroperitoneum suggested IgG4-related disease, idiopathic retroperitoneal fibrosis, or possibly lymphoma. Retroperitoneal excision was performed and initially diagnosed as IgG4-related disease. The second review of the pathology diagnosed it as Erdheim-Chester disease.
PET/CT showed ECD bone lesions in bilateral tibia, femur, pubic bones, and clavicle. There was cardiac involvement by ECD (right atrial mass and pericardial effusion).
Patient started treatment with vemurafenib (BRAF inhibitor). After 3 months, there was a marked response on PET/CT, reduced atrial mass and resolved pericardial effusion. However, monocytosis increased and a repeat bone marrow assessment was done.
CBC: Hgb 12.2 g/dL, MCV 97, Platelets 134 x 109/L, WBC 11.5 (Monocytes 2.9x10(9)/L)).

Biopsy Fixation Details

Retroperitoneal mass:10% neutral buffered formalin.
Bone marrow core biopsy: B5/10% neutral buffered formalin, followed by decalcification if RDO Gold.

Frozen Tissue Available

No

Details of Microscopic Findings

Retroperitoneal mass: prominent fibrosis with scant inflammatory infiltrate containing lymphocytes, plasma cells, and increased bland histiocytes with amphophilic cytoplasm.
Bone marrow: Hypercellular marrow with panhyperplasia, atypia in all three lineages, and no increase in blasts. Peripheral blood and bone marrow monocytosis. Occasional clusters/nodules of plasmacytoid dendritic cells.

Immunophenotype

Retroperitoneal mass: Positive for CD163, factor 13A, BRAF V600E, and S100 (focal, 10-20% overall); negative for CD1a, langerin and OCT2.
Bone marrow: Increased CD68 staining. Occasional PDC nodules are positive for CD123 and CD303.

Cytogenetics

Retroperitoneal mass: not done.
Bone marrow:46,XY[20].

Molecular Studies

Retroperitoneal mass (VAF %):

TET2 p.R1261C (31.2)

NRAS p.G12V (9.2)

JAK2 p.V617F (8.2)

BRAF p.V600E (7.2)

TET2 p.V781fs (5.4)

KRAS p.A146V (4.5)

Bone marrow clot (VAF %):

TET2 p.R1261C (41.6)

TET2 pV781fs (16.0)

NRAS pG12V (9.2)

KRAS pA146V (8.3)

CBL pY371H (15.7)

FOXA1 copy number loss

ddPCR for *BRAF* V600E was negative

Proposed Diagnosis

Erdheim-Chester disease with associated hematological neoplasm (CMML).

Interesting Feature(s)

Shared genetic abnormalities between ECD and CMML suggest a common originating clone. Subclonal additive mutations in BRAF and JAK2 manifested as systemic histiocytosis diagnostic of ECD. Vemurafenib may have paradoxically activated MAPK signaling and selected for RAS mutant CMML clone.

Panel Diagnosis

Chronic myelomonocytic leukemia type 1 (ICC 2022) / Chronic myelomonocytic leukemia type 1 (WHO-5) accompanied by (clonally related) Erdheim-Chester disease

EA4HP24-BMWS-125

Pediatric Acute Leukemia with *NFIA*::*CBFA2T2* Fusion and Erythroid Differentiation

Dr. Cara Monroe[1,2], Dr. Sumire Kitahara[3], Dr. Hongyu Ni[3], Dr. Molly Shields[4], Dr. Alexandra Kovach[1,2], Dr. Brent Wood[1,2], Dr. Andrew Doan[4,2], Dr. Deepa Bhojwani[4,2], Dr. Ashley N. Gray[5,2], Dr. Gordana Raca[6,2], **Dr. Karin Miller**[1,2]

[1] Children's Hospital Los Angeles, Pathology and Laboratory Medicine, Los Angeles, USA; [2] University of Southern California, Keck School of Medicine, Los Angeles, USA; [3] Cedars Sinai Medical Center, Pathology and Laboratory Medicine, Los Angeles, USA; [4] Children's Hospital Los Angeles, Cancer and Blood Disease Institute, Los Angeles, USA; [5] Children's Hospital Los Angeles, Transplantation and Cellular Therapy, Los Angeles, USA; [6] Children's Hospital Los Angeles, Center for Personalized Medicine, Los Angeles, USA

Case Description

A 3-year-old previously healthy female presented with fatigue and leg pain. Complete blood count showed a normal white blood cell count (5.7K/uL), anemia (hemoglobin: 6.0 g/dL), and thrombocytopenia (platelets: 87 K/uL). MRI of the abdomen and pelvis demonstrated mild hepatosplenomegaly, extensive bone marrow edema, and no masses.

Biopsy Fixation Details

Peripheral blood (PB) was sent for flow cytometry (FC). Two bone marrow (BM) biopsies were performed, fixed in formalin, and decalcified.

Frozen Tissue Available

No

Details of Microscopic Findings

The peripheral smear had rare intermediate-sized blasts (5% of cellularity). The initial BM biopsy showed extensive necrosis. In viable areas, there were sheets of immature cells, which were medium to large in size with scant cytoplasm, open chromatin, and variably prominent nucleoli. A subsequent BM biopsy was more viable, hypercellular (>90%), and showed similar sheets of immature cells.

Immunophenotype

PB FC demonstrated a population of abnormal cells (9% of white cells) which expressed CD71 (bright), CD117, and CD4 (dim). They lacked CD3 (cytoplasmic), CD5, CD7, CD13, CD14, CD15, CD16, CD19, CD33, CD34, CD38, CD41, CD42b, CD45, CD56, CD61, CD64, CD79a (cytoplasmic), CD123, CD235a (glycophorin A), HLA-DR, and myeloperoxidase (MPO, cytoplasmic).

Immunohistochemistry (IHC) on the BM biopsy showed that the abnormal cells were positive for CD43, CD99, E-cadherin (partial, variable), and CD117. They were negative for CD45, TDT, CD34, hemoglobin, MPO, CD61, CD68, CD163, lysozyme, mast cell tryptase, CD1a, CD3, CD7, CD8, Pax5, CD10, CD138, and CD30 as well as stains for solid tumors including BCOR, chromogranin, cytokeratins, desmin, ERG, myoD1, myogenin, NKX2.2, PHOX2B, OCT3/4, synaptophysin, S100, and SALL4. INI-1 and BRG1 expression was intact. P53 was positive in 10-20% of the abnormal cells.

Cytogenetics
Karyotype (post induction-therapy sample) demonstrated: 46,XX,del(1)(p13p22),t(1;20)(p32;q11.2),del(11)(q13q24)[18]/46,XX[2]. Chromosome microarray showed copy number loss in 1p, 11q, and 17p (subclonal) and copy number gain in 17q.
Molecular Studies
Next generation sequencing (DNA) showed pathogenic variants in *ARID1a* (p.F1720fs, VAF 35%), *NRAS* (p.G12S, 6.5%), and *KRAS* (p.G13D, 4.8%). A targeted fusion panel (RNA) was negative. RNA-Sequencing demonstrated a novel *NFIA*::*CBFA2T2* fusion.
Proposed Diagnosis
Acute leukemia with *NFIA*::*CBFA2T2* fusion and erythroid differentiation most consistent with pure/acute erythroid leukemia (PEL)
Interesting Feature(s)
Immunophenotyping posed a challenge as definitive markers of hematopoietic origin, with the exception of CD43, were lacking. The remainder of the immunophenotypic workup suggested, but was not definitive for, erythroid differentiation. Further, the pattern of P53 by IHC and molecular studies was not consistent with biallelic TP53 alteration, typically seen in acute erythroid leukemia.
Detection of the *NFIA*::*CBFA2T2* fusion enabled classification of the acute leukemia as most consistent with PEL. *NFIA* is upregulated in the erythroid lineage, and *CBFA2T2* facilitates transcriptional repression. This particular fusion has not been previously reported in the literature. However, *NFIA* fusions with related genes, *CBFA2T1* (*RUNX1T1,* PMID: 33313700) and *CBFA2T3 (*PMID: 23032695, 3194901) have been reported in pediatric PEL. This case adds to the growing body of literature suggesting a distinct biology in a subset of pediatric patients with PEL.

Panel Diagnosis
Acute erythroid leukemia (WHO-5) / Acute myeloid leukemia, NOS (ICC 2022)

EA4HP24-BMWS-127

A vexxing diagnosis

Dr. Kaaren Reichard, Dr. Horatiu Olteanu

Mayo Clinic, Hematopathology, Rochester, USA

Case Description
61-year-old male who presented 4 years prior with relapsing polychondritis. Prior BM biopsies had no definitive diagnosis, no clonal abnormality; presumably autoimmune-related. He has been treated with steroids and rituximab. CBC: Hb 7.4, MCV 109, WBC 6.9, plt 67 with 85% PMN, 13% lymphs and 2% monocytes. Several years later VEXAS is described. Pt was then found to have **UBA1:** c.122T>C; **p.Met41Thr.**

Biopsy Fixation Details

Decal

Frozen Tissue Available

No

Details of Microscopic Findings

PB: Marked macrocytic anemia with mild polychromasia; WBC normal in number without dysplasia; no left shift or blasts; monocytopenia; platelets normal morphologyBM: BM aspirate is spicular and cellular; granulocytic hyperplasia (HP) with left-shift; erythroid hypoplasia with left-shift; vacuolization in pronormoblasts and promyelocytes; no increase in blasts; no granulocytic or erythroid dysplasia; multinucleated erythroid precursors; megakaryocyte HP with atypical cytologic features (eccentric localization of nuclear lobes, “wreath-like appearance", markedly condensed chromatin, prominent consolidation of cytoplasm imparting a bubble-gum type appearance, and forms with small nuclear remnants floating in the cytoplasm) No classic “dysplastic” forms. BM core biopsy; markedly hypercellular with granulocytic HP and left-shift, erythroid hypoplasia with left-shift and megakaryocytic HP with a spectrum of morphology as noted in aspirate. No increased blasts, mastocytosis or other neoplastic process.

Immunophenotype

CD61: megakaryocytic hyperplasia with atypia. CD71: decreased, left-shifted erythropoiesis.

Cytogenetics

Normal

Molecular Studies

NGS is negative. Panel: ANKRD26 ASXL1 BCOR CALR CBL CEBPA
CSF3R DDX41 DNMT3A ELANE ETNK1 ETV6 EZH2 FLT3 GATA1 GATA2 IDH1 IDH2
JAK2 KDM6A KIT KRAS MPL NPM1 NRAS PHF6 PTPN11 RAD21 RUNX1, SETBP1 SH2B3
SF3B1 SRP72 SMC3 SRSF2 STAG2 TERT TET2 TP53 U2AF1WT1
ZRSR2 .

Proposed Diagnosis

1) VEXAS syndrome characterized by a markedly hypercellular bone marrow (90%) with decreased, left-shifted erythropoiesis and occasional atypia, increased and left-shifted granulopoiesis, increased megakaryopoiesis with persistent and progressive significant atypia, vacuolization in early granulocytic and erythroid precursors and no increase in blasts (see comment).
2) Normal karyotype and NGS studies.

COMMENT

This is a challenging case due to the persistent spectrum of findings as has been previously well-documented in multiple bone marrow specimens. In the current specimen, as noted previously, the most striking feature is themorphologic atypia in the megakaryocytes. This feature, in comparison to the prior biopsies is now more significant and prominent. These features, in conjunction with the persistent unexplained cytopenias, are strongly worrisome for an underlying myeloid neoplasm. Unfortunately this interpretation is complicated by the patient's reported autoimmune disease, exposure to various treatment agents, and lack of a clonal marker.

Interesting Feature(s)

1) In florid cases of VEXAS, the PB findings often show macrocytic anemia, monocytopenia and thrombocytoenia (50%). While non-specific, the BM biopsy findings are reproducible and include (to varying degrees) megakaryocytic cytologic atypia (not the characteristic dysplastic appearance).

2) Most often normal karyotype except in cases with true associated MDS. Aside from *UBA1* mutation, next-generation sequencing studies for myeloid neoplasm-associated genetic mutations are typically absent or there may be a presence of a CHIP mutation, *DNMT3A, TET2*.

Panel Diagnosis

Vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic UBA1 mutations syndrome (VEXAS)

EA4HP24-BMWS-137

Acute myeloid leukemia (AML) with *BCR::ABL1*fusion in a patient with thrombocytopenia -absent radius (TAR) syndrome

Dr. Parnaz Daneshpajouhnejad[1], Dr. Alejandro Villar-Prados[2], Dr. Gabriel N. Mannis[2], Dr. Joshua R. Menke[3]

[1] Hospital of the University of Pennsylvania, Department of Pathology and Laboratory Medicine, Philadelphia, USA; [2] Stanford University School of Medicine, Department of Medicine, Division of Hematology, Palo Alto, USA; [3] Stanford University, Department of Pathology, Palo Alto, USA

Case Description

The patient is a 71-year-old woman with a history of TAR and baseline thrombocytopenia (~80). She has an extensive family history of TAR and has one sister with chronic myeloid leukemia (CML). She presented with respiratory distress, headache, and weakness, and CBC showed pancytopenia necessitating blood transfusions. A bone marrow biopsy and flow cytometry revealed AML and FISH and NGS showed BCR::ABL fusion. Treatment involved a modified induction regimen of decitabine, venetoclax and dasatinib, leading to a complete response with incomplete platelet recovery (CRi). Persistent positivity for t(9;22) prompted continued dasatinib monotherapy. The patient, discharged after 38 days, maintains CRi, with undetectable p210 BCR-ABL two months post-discharge, supported by dasatinib and platelet transfusions. Germline skin testing was performed to evaluate for *RBM8A* mutation.

Biopsy Fixation Details

Neutral buffered formalin

Frozen Tissue Available

N/A

Details of Microscopic Findings

The bone marrow biopsy revealed markedly hypercellular marrow (90%) for age with sheets of blasts comprising 50% of cellularity. Myeloid elements show left-shifted maturation with a myeloid-to-erythroid ratio of 10:1. Numerous megakaryocytes are noted with frequent small, hypolobated forms. A reticulin stain shows grade 2-3 myelofibrosis.

Immunophenotype

Flow cytometry: CD34+ blasts are positive for CD38, CD13, CD33, CD117 (dim to moderate bright), HLA-DR, CD45 (dim) and MPO (minor subset), while negative for CD3, CD19, among others.

Cytogenetics

FISH: t(9;22) (p210), 80.5% positivity

Karyotype (ISCN Nomenclature): 46,XX[20]

Molecular Studies

BCR-ABL p210 RT-PCR transcript levels (International Scale): expression level at >55.0%

Massively parallel sequencing studies (HemeSTAMP: 203 genes):*BCR::ABL1* fusion

STAT5B (Tyr392Asn, VAF 50%)

PPM1D (p.Ile496Val, VAF 50%)

CREBBP (p.Val238Leu, VAF 49%)

IKZF2 (p.Leu328Phe, VAF 46%)

Chromosomal microarray (peripheral blood): 105 kilobase (kb) deletion in chromosome 1, band 1q21.1, consistent with TAR

Proposed Diagnosis

AML (FAB classification M2), with moderate to severe myelofibrosis, grade 2-3, with *BCR::ABL1* fusion by WHO5 classification

Interesting Feature(s)

TAR, marked by absent radii and hypomegakaryocytic thrombocytopenia, is a rare autosomal recessive syndrome with a predisposition to AML. The pathogenesis of AML in TAR remains unclear with 6 cases of acute leukemia reported in the literature so far, including 4 AML, 1 T-ALL, and 1 acute leukemia lineage unspecified. Previous cases showed AML onset at a younger age, with one case of myelodysplastic syndrome with excess blasts, add(3p) and (12q) and a *CALR* mutation that progressed to AML and another case with de novo AML with t(8;21). Genetic analysis suggests that deletions in chromosome 1q21.1 result in the TAR phenotype, with the causative gene being *RBM8A*. Our patient with TAR syndrome was diagnosed with Ph+ AML, which accounts for about 1% of newly diagnosed AML cases and is the first documented case of a patient with TAR syndrome developing this already rare subtype of AML. The patient's family history of TAR and CML suggests a potential predisposition that may be driven by the cumulative effect of 1p21.1 deletion in combination with BCR::ABL fusion that "paved the way" for AML in this particular case. NGS showed multiple likely germline mutations with VAFs near 50%; none of these genes are present on chromosome 1 or are found to be co-mutated with *RBM8A* except *IKZF2*. This case underscores the need for further genetic exploration in understanding hematopoiesis and leukemogenesis in TAR syndrome.

Panel Diagnosis

Acute myeloid leukemia with BCR::ABL1 fusion (ICC 2022 / WHO-5)

EA4HP24-BMWS-139

Chronic myelomonocytic leukemia with ET type megakaryocytes - modulation of phenotype & morphology by genotype

Dr. Mufaddal Moonim[1], Dr. Amin Rahemtulla[2], Dr. Ketan Patel[2], Dr. Elisabet Nadal-Melsio[2], Dr. Andrew Innes[2]

[1] Imperial College Healthcare Trust, Histopathology, London, UK; [2] Imperial Colleage Healthcare Trust, Haematology, London, UK

Case Description

85/F presented with raised WCC and platelet count. Non-smoker, no allergies, no family history of leukaemia / lymphoma. Past history: 2011, macrocytosis, leucopenia & thrombocytopenia which did not satisfy criteria for MDS; 2019, Breast cancer treated with Sx+RT +Tamoxifen; Polymyalgia rheumatica treated with prednisolone.
Current FBC: Hb 130g/L, WBC 19.5, Plt 1015, Neut 15.1, Monos 1.9, Lymphs 2.1 (all x10^9/L), MCV 101fL. Ferritin 546 µg/L, iron saturation 17%. Reticulocytes 3.5%. Normal renal & liver function tests.
Film: Platelet clumps.Rare red cell fragment, stomatocyte, tear drop poikilocyte. Neutrophilia, occasional dysplastic neutrophil (hypogranular/dysplastic nucleus). Left shift. monocytosis - some atypical forms.(200 cell Diff; N 73%, L 13.5%, Mo 11.5%, Eo 1.5%, Ba 0.5%).One bare megakaryocyte nucleus seen.
Bone marrow aspirate: Hypercellular bone marrow aspirate which shows increased numbers of megakaryocytes, some of which are large and hyperlobated and rare micromegakaryocyte. Erythoid and granulocytic dysplasia noted with increased numbers of monocytes.

Biopsy Fixation Details

AZF

Frozen Tissue Available

Nil

Details of Microscopic Findings

Bone marrow cellularity: 80-90%, hypercellular for the age of the patient.

- The ME ratio is: 2:1
- Erythropoiesis is expanded and show megaloblastic changes.
- Myelopoiesis is significantly left shifted but shows maturation to neutrophils.
- CD42b highlights large numbers of megakaryocytes. Most are giant hyperlobated

forms. No micromegakaryocytes identified.
 Special stains show numerous iron laden macrophages and an increase in reticulin fibre pattern. No collagen or new bone formation identified. Fibrosis score: WHO grade 1.
 Immunohistochemistry for CD34, CD117, TdT, CD56, CD123 and WT-1 do not highlight any increase in immature precursors
 CD14 highlights a monocytosis (10-15% of TNC).
The features are those of a MDS/MPN overlap syndrome. The most likely possibility is Chronic myelomonocytic leukaemia (though the megakaryocyte morphology is unusual for this), and correlation with peripheral blood counts, aspirate, flow cytometry, cytogenetic and molecular tests is needed for accurate subtyping.

Immunophenotype

Bone marrow flow cytometry:
Myeloid blasts: 0.5%
Granulocytes 83.2% (Neut - 81.6% and Eos - 1.6%)
Monocytes: 8.3% (83% of monocytes express CD11b)
Lymphocytes: 8.5% (T-cells - 8%, B-cells - 0.1%, NK cells - 0.3%)

Cytogenetics

46, XX[20]

Molecular Studies

JAK2: c.1849G>T, p.(Val617Phe), VAF 46%, COSM12600, rs77375493, NM_004972.4
TET2: c.5707C>T, p.(Gln1903Ter), VAF 92%, COSM9635392, NM_001127208.3

Proposed Diagnosis

Chronic myelomonocytic leukaemia (CMML-0) [WHO 4th ed ICD-O 9945/3]
Chronic myelomonocytic leukaemia (CMML-1) [WHO 5th ed ICD-O 9945/3], [ICC Sep 2022]
Chronic myelomonocytic leukaemia with a JAK2 mutation.

Interesting Feature(s)

This case illustrates how the genotype changes both peripheral blood counts and bone marrow morphology due to the co-existence of two mutations which are driving different aspects of haemopoiesis - i.e. TET-2 driving the CMML-like phenotype (increased monocytes, dysplasia) and JAK2 driving the ET-like phenotype (elevated platelet counts and megakaryocyte proliferation with hyperlobated nuclei).
This case illustrates how one can predict what kind of mutations one would expect to find and the genotype also explains the phenotype (blood counts and marrow morphology) very well.
In the absence of molecular information, it would have been difficult to classify as it straddles two different disease categories (?CMML and ?progression in ET) and would have been labelled as MDS/MPN- unclassified.

Panel Diagnosis

Chronic myelomonocytic leukemia type 1, myeloproliferative subtype (ICC 2022) / Myeloproliferative chronic myelomonocytic leukemia type 1 (WHO-5)

EA4HP24-BMWS-151

Acute Promyelocytic Leukemia with a Variant Translocation

Dr. Cara Monroe[1,2], Dr. Paul Gaynon[3,2], Dr. Etan Orgel[3,2], Dr. Robert Lorsbach[4], Dr. Alexandra Kovach[1,2], Dr. Brent Wood[1,2], Dr. Gordana Raca[5,2], **Dr. Karin Miller**[1,2]

[1] Children's Hospital Los Angeles, Pathology and Laboratory Medicine, Los Angeles, USA; [2] University of Southern California, Keck School of Medicine, Los Angeles, USA; [3] Children's Hospital Los Angeles, Cancer and Blood Disease Institute, Los Angeles, USA; [4] Cincinnati Children's, Cincinnati, USA; [5] Children's Hospital Los Angeles, Center for Personalized Medicine, Los Angeles, USA

Case Description

A 12-month-old female with a history of cardiac defects presented with fever, listlessness, staring episodes, excessive drooling, decreased urine output, and left cheek swelling. Complete blood count showed leukocytosis (WBC 88 K/uL), anemia (hemoglobin 7.7 g/dL), and platelets of 183 K/uL with a WBC differential of 60% neutrophils, 18% lymphocytes, 3% monocytes, 6% metamyelocytes, 8% metamyelocytes, and 5% promyelocytes. PT (16.5s, range 8.8-12.5) and D-Dimer (20,960 ng/mL, range <570) were elevated. INR, PTT, and fibrinogen were within normal limits. Ultrasound showed prominent bilateral submandibular lymph nodes, a diffusely heterogenous left parotid gland, and a normal spleen.

Biopsy Fixation Details

The bone marrow (BM) biopsy was fixed in formalin and decalcified.

Frozen Tissue Available

No

Details of Microscopic Findings

The BM showed marked myeloid predominance (myeloid:erythroid ratio, 30:1) with a prominent population (30%) of bilobed cells with azurophilic cytoplasmic granules, consistent with promyelocytes. Auer rods were not identified. Blasts were not increased (1%). A subset of neutrophils showed dysplastic features, including nuclear hypolobation and hypogranular cytoplasm. Monocytes and promonocytes comprised 7% of the cellularity.

Immunophenotype

Peripheral blood and BM flow cytometry (FC) showed maturing myeloid cells (91% of white cells) with increased side-scatter light properties and autofluorescence, abnormal acquisition of CD13 and CD16, and diminished CD45. The promyelocytic component expressed myeloperoxidase (bright), CD33, and CD45 (dim) and lacked HLA-DR, CD34, and CD15; CD117 was dim to absent by FC and immunohistochemistry. A small, discontinuous population of phenotypically normal CD34+ stems cells (0.09%) was present by FC.

Cytogenetics

Karyotype showed trisomy 6: 47,XX,+6[20]. AML FISH panel was negative for *PML*::*RARA*, *RUNX1T1*::*RUNX1*, *CBFB*::*MYH11*, *BCR*::*ABL1*, rearrangement of *NUP98* and *KMT2A*, and monosomy 5 and 7. Chromosome microarray detected gain of chromosome 6 as the sole abnormality.

Molecular Studies

RT-PCR for *PML*::*RARA* was negative. A targeted fusion panel (RNA) including *RARA* variants was negative. NGS for DNA variants detected 3 variants of unknown significance: *ALK* (p.R1231Q, VAF 49%), *NOTCH1* (p.G300R, 50%), and *ERBB2* (p.R866H, 48%). *FLT3* TKD and ITD mutation analysis was negative. RNA-sequencing studies were then pursued and demonstrated a *TBL1XR1*::*RARB* fusion.

Proposed Diagnosis

Acute Promyelocytic Leukemia (APL, variant) with *TBL1XR1::RARB* fusion

Interesting Feature(s)

Multiple features of this case were suggestive of APL including increased, phenotypically aberrant promyelocytes. However, the abnormal myeloid maturation out of the promyelocyte population was unusual for APL, morphologically with dysplastic neutrophils and immunophenotypically with diminished CD45 and abnormal CD13 and CD16 acquisition. Due to concern for APL, the patient was initially treated with ATRA without response.

Molecular studies were negative for a *RARA* translocation. Identification of *TBL1XR1* fused with *RARB* by RNA-sequencing facilitated classification as an APL variant, non-responsive to ATRA, and prompted initiation of AML-directed chemotherapy. *RARB* fusions are a rare but recurrent abnormality in APL (PMID: 36465368 and 29921692), though the WHO5 and ICC currently recognize variant-APL with *RARA* translocations only. It is possible that variant fusion partners may account for the morphologic and immunophenotypic changes among maturing myeloid cells seen in this case.

Panel Diagnosis

Acute myeloid leukemia with maturation (WHO-5) / Acute myeloid leukemia, NOS (ICC 2022)

EA4HP24-BMWS-168

Isolated CNS Erythroblastic Sarcoma with *NFIA-RUNX1T1* fusion

Dr. Yukiko Kitagawa, Dr. Obaid Rehman, Dr. Kavita Umrau, Dr. Magdalena Czader

Indiana University, School of Medicine, Department of Pathology & Laboratory Medicine, Indianapolis, USA

Case Description

Patient is a 2-year-old female, with a 5 month-history of macrocephaly, and 4-day history of headache and gait disturbance. MRI showed heterogenous enhanced cortically based mass in the left parietal-occipital lobe with extension along the dura into posterior left frontal lobe.

The patient underwent debulking surgery twice followed by placement of VP shunt and intrathecal and intravenous chemotherapy including high dose methotrexate.

Currently patient is off therapy after intensification cycle.

Biopsy Fixation Details

Biopsy fixed in 10% buffered formalin.

Frozen Tissue Available

Not Applicable

Details of Microscopic Findings

Tumor is characterized by monotonous proliferation of intermediate to large cells with round to oval nuclei, fine chromatin, frequent nucleoli and scant basophilic cytoplasm morphologically consistent with erythroblasts. Numerous mitotic figures and delicate vascularization are seen. Focal necrosis is appreciated.

Immunophenotype

Neoplastic cells are positive for CD71, E-cadherin, CD43 and CD117, and partially positive for EMA. Hemoglobin is detected in rare neoplastic cells. P53 immunostain shows only weak partial staining.

Neoplastic cells are negative for: glycophorin A, CD33, CD45RB, CD3, CD20, GFAP, Synaptophysin, Neurofilament, Desmin, Myogenin, Vimentin, Keratin AE1/AE3, SALL4, Oct4, AFP, hCG, CD30, CD99.

Cytogenetics

Not applicable

Molecular Studies

DNA and RNA sequencing:

NFIA::RUNX1T1 fusion

ARID1A (M981fs) VAF: 44%

Proposed Diagnosis

Isolated CNS Erythroblastic Sarcoma with *NFIA-RUNX1T1* fusion

(WHO, ICC: Myeloid sarcoma)

Interesting Feature(s)

- This is the rare report of CNS based erythroblastic sarcoma with *NFIA-RUNX1T1* fusion with no evidence of bone marrow involvement (Tauziède-Espariat et al, Acta Neuropathologica Comm 2024).
- Myeloid sarcomas with *NFIA* and *RUNX1T1* or *CBFA2T3* rearrangement show similar clinicopathological features such as occurrence in infants/young children, erythroid differentiation and predilection to central nervous system involvement (King et al, Am J Clin Pathol 2021; Tauziède-Espariat et al, Acta Neuropathologica Comm 2024).
- Three of three cases studied by targeted sequencing showed mutations in *ARID1A* gene, a component of chromatin remodeling complex and postulated tumor suppressor gene. *ARID1A* mutations are known to occur in acute promyelocytic leukemia and select lymphoid malignancies (Madanet et al, Leukemia 2016; Burkhardt et al, Nat. Commun 2022). Interestingly, a loss of *ARID1A* function has been previously linked to increased *EPO* mRNA expression in primary human hepatocytes, and in mouse model leads to erythrocytosis and massive splenic erythropoiesis (Riou et al, eLife 2020).
- In one case *NFIA* fusion transcript was detected in placenta and therefore likely represented a congenital leukemia.

Panel Diagnosis

Myeloid sarcoma (ICC 2022 / WHO-5)

EA4HP24-BMWS-198

Myeloid/lymphoid neoplasm with *BCR::JAK2* rearrangement

Dr. Ling Zhang, Dr. Mohammad Hussaini, Dr. Hammad Tashkandi, Dr. Lynn Moscinski

Moffitt Cancer Center, Tampa, Tampa, USA

Case Description

69-year-old man was found to have leukocytosis (WBC of 44 x 10^9/L), mild anemia (HgB of 13.1 g/dL) without overt eosinophilia (0.65 x 10^9/L) on a routine blood study. A subsequent bone marrow (BM) biopsy showed myeloproliferative neoplasm, not other specified (MPN, NOS). The patient was treated on and off with Hydrea for 3 years. A follow-up CBCs showed WBCs of 86.9 x 10 ^{9}/uL, HgB of 9 g/dL and platelets of 16 x 10^9/L with 83.9% blasts. An BM biopsy showed hypercellularity (100%) and 84% blasts consistent with B-ALL with *BCR::JAK2* fusion. The patient was transferred care to MCC and started on modified DFCI, miniCVD + inotuzumab + Jakafi therapy, but showed refractory disease. He then underwent CAR-T (Tecartus) followed by an allogeneic HSC transplant with, however, a molecular

relapse occurred 6 months after, for which he received lymphodepletion followed by the 2ndCART (Tecartus) infusion and have achieved complete remission since then.

Biopsy Fixation Details

B Plus Fix fixative

Frozen Tissue Available

N/A

Details of Microscopic Findings

MPN, NOS: the peripheral blood smear revealed leukocytosis with mild left shifted granulocytosis without overt eosinophilia or circulating blasts. The BM biopsy demonstrated hypercellularity (100%) with slightly increased megakaryocytes, myeloid hyperplasia, and scattered eosinophils without reticulin fibrosis (MF1/3).

B-ALL: the BM smear showed increased lymphoblasts, occasional eosinophils and diminished myeloid and erythroid precursors. The BM core biopsy showed >90% cellularity with sheets of immature precursors/lymphoblasts and focally loose clusters of atypical megakaryocytes.

Immunophenotype

MPN: Occasional CD34 positive blasts identified by immunostains (IHC) and flow cytometry.

B-ALL: positive for CD34, PAX-5, CD79a, CD10, TdT and negative for CD3, CD117, MPO and lysozyme by IHC. The concurrent flow cytometry demonstrated a distinct population of B-lymphoblasts (Figure see PPT).

Cytogenetics

MPN: FISH: negative for *BCR::ABL1* fusion*, PDGFRA, PDGFRB, FGFR1 and CBFB* rearrangements, Cytogenetics reported complex cytogenetic aberrations: 46,XY,add(7)(p15),add(9)(p24),add(22)(q11.2)[18]/46,XY[2].

B-ALL: FISH study is negative for *BCR::ABL1* rearrangements. Karyotyping showed complex cytogenetic abnormalities: 46,XY,t(7;22;9)(p15;q11.2;p24)[2]/45,XY,-7,der(9)t(7;22;9)(p15;q11.2;p24),der(22)t(7;22;9)(p15;q11.2;p24)[19].

Molecular Studies

PCR study did not reveal *JAK2, MPL, CALR, FLT-3, or IDH1/2* mutations and NGS myeloid panel was negative at diagnosis of MPN.

FoundationOne Heme Panel including RNA-sequencing showed *BCR::JAK2* rearrangements and *MYC* T73A subclone at diagnosis of B-ALL as well as at diagnosis of MPN (retrospective study performed on BM unstained slides).

Proposed Diagnosis

/lymphoid neoplasm (MLN) with *BCR::JAK2* rearrangement.

Interesting Feature(s)

We present a rare scenario of MLN with *BCR::JAK2* rearrangement, whichis newly proposed and listed under MLN with *JAK2* rearrangement (MLN_*JAK2*R) by ICC and WHO classification. Given expansion of *JAK2* partners and potential targeted TKI therapy, an accurate diagnosis of MLN_J*AK2*R is demanded. As shown in our patient, eosinophilia is not prominent at phases of MPN and B-ALL, which could led to delay an accurate diagnosis if an RNA-sequencing was not applied. Presence of *JAK2* rearrangement, which can be identified by FISH, RT-PCR or RNA-sequencing, warrants further investigation of a

preexisting, concurrent, or subsequent myeloid or lymphoid neoplasm for appropriate treatment.

Panel Diagnosis

Myeloid/lymphoid neoplasms with eosinophilia and JAK2 rearrangement (ICC 2022 / WHO-5)

EA4HP24-BMWS-223

Acute myeloid leukemia with NPM1 mutation and associated myeloid sarcoma in a 1-year-old child

PhD/MD Marianne D.C. Goncalves[1], Dr. Humberto C. Carneiro[1], Dr. Rodrigo D.A. Natal[1], Dr. Luan B. Furtado[1], Dr. Luis H. Sakamoto[2], Dr. Yara Reina[2], Dr. Cristina Bortolheiro[2]

[1] Rede D'Or, Pathology, Sao Paulo, Brazil; [2] Rede D'Or, Hematology, Sao Paulo, Brazil

Case Description

A 22-month-old female patient presented with a two-month history of fever, recurrent otitis, bone pain, and neutropenia. Initial imaging studies revealed multiple bone lesions, predominantly in the upper and lower extremities. Pathological examination of these lesions showed focal chronic inflammatory infiltration. The patient was diagnosed with chronic aseptic multifocal osteomyelitis and treated with corticosteroids and multiple antibiotics. Bone marrow aspiration revealed hypercellular marrow with erythroid and megakaryocytic hypocellularity, replaced by an immature myeloid proliferation without abnormal immunophenotype and no PML-RARA detected by rtPCR. Bone marrow biopsy was uninformative due to inadequate sampling. Investigations for congenital bone marrow failure and congenital neutropenia syndromes were initiated. Exome analysis of peripheral blood did not identify any variant that could explain the clinical findings.
Two months later, the patient developed pancytopenia. A reassessment of the bone marrow showed hypocellular smear with relative granulocytic hypercellularity, promyelocyte maturation arrest, 9% blasts and erythrocytic and megakaryocytic series hypoplasia. Flow cytometry immunophenotyping of the blast cells gate revealed positivity to CD38, CD33, CD117, CD45, CD13, CD64 and MPO. The bone marrow biopsy was scarce, contained immature granulocytes and also showed abnormal megakaryocyte morphology and distribution. Karyotyping was normal. A diagnosis of myelodysplastic syndrome was hypothesized.
While waiting for molecular test results, generalized lymph node enlargement was detected as well as new bone lesions, and the pathological examination of both was compatible with myeloid sarcoma. Concurrently, next-generation sequencing of the bone marrow aspirate revealed an unexpected NPM1 gene mutation.

Biopsy Fixation Details

Lymph node, bone lesion and bone marrow biopsy fixed in buffered neutral formalin; trephine and bone lesion biopsy decalcified in EDTA.

Frozen Tissue Available

No.

Details of Microscopic Findings

PB: leukocytosis with increase in blasts and promyelocytes - blasts: 7%, promyelocytes: 17%, myelocytes: 8%, metamyelocytes: 2%, bands: 15%, segmented neutrophils: 15%, eosinophils: 0%, basophils: 0%, lymphocytes: 40%, monocytes: 5%. BM aspirate: hypercellular bone marrow, replaced by immature cells of the granulocytic lineage and 9% blasts. BM biopsy: hypercellular bone marrow at the expense of the granulocytic series, with maturation arrest and immature forms. Moderate dysmegakaryocytopoiesis. Lymph node and bone lesion biopsy: monotonous and immature cells with granular, pinkish cytoplasm and scattered megakaryocytes.

Immunophenotype

Flow cytometry: positive (CD38, CD33, MPO, CD45, CD117), weakly positive (CD64, CD13), negative (CD7, CD19, CD34, HLA-DR). Immunohistochemistry: positive (MPO), negative (CD34, CD117, TDT, CD10) no aberrant pattern of NPM1 immunoexpression was observed (clone NPM1/B23 – BSB-124 BioSB).

Cytogenetics

46,XX[20].

Molecular Studies

Bone marrow aspirate: PML-RARA (rtPCR) negative. NGS for myeloid malignancies: pathogenic variant in NPM1 W288Cfs*12, VAF: 14,2%. Exome and mitochondrial DNA sequencing: probably pathogenic variant in CEP164 Q1410*, VAF: 48.09% heterozygous; probably pathogenic variant in USH2A N443Mfs*18, VAF: 52,87%, heterozygous.

Proposed Diagnosis

Acute myeloid leukemia with NPM1 mutation and associated myeloid sarcoma.

Interesting Feature(s)

This case underscores the diagnostic challenges of NPM1 mutated AML and the importance of comprehensive genetic testing in pediatric patients with rare and overlapping hematological conditions.

Panel Diagnosis

Myeloid sarcoma (ICC 2022 / WHO-5) in NPM1 mutated AML

EA4HP24-BMWS-242

Myeloid Neoplasm in VEXAS Syndrome

Dr. Pooja Devi, Dr. Sam Sirotnikov, Dr. Dale M. Frank

University of Pennsylvania Perelman School of Medicine, Department of Pathology, Hematopathology, Philadelphia, USA

Case Description

A 78 year-old male presented with fever, weight loss, macrocytic anemia requiring transfusion, progressive thrombocytopenia, skin pruritis, diagnosed with neutrophilic dermatosis (Sweet syndrome) on skin biopsy, relapsing polychondritis, pneumonia and sinusitis.

June 2023

CBC: Hb 6.8 g/dl, MCV 111fl, Plt 194 thou/ul, WBC 13.8 thou/ul
High ESR, CRP, Ferritin
SPEP: faint IgG band
Kappa/Lambda:111.6/133.5
IgG/IgA/IgM: 2135/989/128
Autoantibodies negative
Infectious disease tests negative

Biopsy Fixation Details

ZBF

Frozen Tissue Available

NA

Details of Microscopic Findings

• Hypercellular marrow (40%) for age
• Erythroid precursors are decreased with cytologic atypia (multinucleation, nuclear irregularity, cytoplasmic vacuoles, megaloblastoid change) 10% of lineage
• Myeloid precursors are increased with full maturation; occasional Pelgeroid forms and cytoplasmic vacuoles are noted in precursors
• Megakaryocytes show cytologic atypia, prominent on core biopsy (small hypolobated, hyperchromatic forms highlighted by CD42b) >10% of lineage

Immunophenotype

Flow cytometry showed no abnormalities or increased blasts

Cytogenetics

Karyotype: 46,XY[20]
FISH: Negative for *BCR/ABL1/ASS1* t(9;22) translocation

Molecular Studies

NGS

Bone Marrow (July 2023*):

Variants of Potential Clinical Significance (% VAF)
DNMT3A 36.4%

*UBA1 not tested

Peripheral blood (December 2023):

Variants of Known Clinical Significance (% VAF)

UBA1 78.0%

DNMT3A 36.9%

TET2 3.6%

Proposed Diagnosis

Myelodysplastic syndrome (MDS), arising in VEXAS.

Note:

• Presence of vacuoles in myeloid and erythroid precursors raises a morphologic differential that includes copper deficiency, autoimmune disorders, MDS, and VEXAS.

• While macrocytic anemia is a feature of uncomplicated VEXAS, progression to transfusion dependent anemia and thrombocytopenia is frequently associated with development of MDS.

• Development of hematologic malignancies such as MDS and plasma cell neoplasms is common event (40% of cases) in VEXAS (Obiorah et al. Blood Adv. 2021 PMID: 34427584).

• The combined findings are compatible with MDS with low blasts (WHO 5th) / MDS, NOS, with multilineage dysplasia (ICC 2022), in a background of VEXAS.

Interesting Feature(s)

- We present an interesting case of VEXAS (Vacuoles, E1 enzyme, X-linked, Autoinflammatory, Somatic), a prototype of a hematoinflammatory syndrome manifesting with inflammatory and hematopathologic features.
- Somatic mutation of UBA1, the defining characteristic of VEXAS, was identified in our case.
- Rodrigues et al (Blood. 2023 PMID:37084382) described a spectrum of mutations and their outcomes in these patients. DNMT3 and TET2 are common mutations and presence of higher VAFs in the setting of VEXAS suggests a higher number of cells carrying the mutated gene leading to more severe manifestations like seen in our patient with a DNMT3 VAF of 36.4%. The significance of variants detected at lower levels is currently unclear.
- Current studies have hypothesized that the inflammatory environment caused by VEXAS favors emergence of clones and secondary MDS. DNMT3A mutation, which is closely related to MDS, may facilitate the amplification of VEXAS clones and even trigger severe manifestations (Manzoni et al. Clin. Hemat. In.2022 PMID:35950209).
- The WHO and ICC recently acknowledged VEXAS as part of the spectrum of pre-malignant clonal cytopenias alongside CCUS and MDS.
- Our patient received supportive treatment, but succumbed to sepsis six months after his initial presentation.
- VEXAS has only recently been described, effective treatments are still being investigated and no guidelines have been established.

Panel Diagnosis

Myelodysplastic syndrome, NOS with multilineage dysplasia (ICC 2022) / Myelodysplastic syndrome with low blasts (WHO-5) in the setting of VEXAS

EA4HP24-BMWS-244

Clonal and disease evolution of a myeloid neoplasm with germline *DDX41* mutation

Prof. Rong He, Prof. Horatiu Olteanu, Dr. Joanna Dalland, Dr. Jane Yuan, Prof. Min Shi, Prof. David Viswanatha

Mayo Clinic, Rochester, USA

Case Description

A 69-year-old man with a history of fluctuating cytopenias and excess alcohol intake presented with Hb 11.3g/dL, MCV 103.3fL, WBC 1.7x10^9/L, ANC 0.6x10^9/L, PLT 177x10^9/L. Folate, Vitamin B12, methylmalonic acid, and intrinsic factor antibody were normal. BM (case 1) showed normal cellularity without MDS features. Advised to reduce alcohol intake, he was observed. NGS 21 months later showed 3 VUSs. The patient remained well but experienced a drop in Hb to 8.4, prompting another BM (case 2) in 11 months, showing hypercellularity without diagnostic MDS features. NGS revealed higher variant allele fraction (VAF) in the 2 likely somatic variants and the likely germline *DDX41* R339H stayed at 49%. Hair follicles and saliva testing confirmed the germline nature of *DDX41* R339H. CBC showed Hb 7.9, MCV 110.4, WBC 1.8, ANC 0.5, PLT 159 18 months later, and BM (case 3) was hypercellular with multilineage dysplasia. NGS showed 2 additional variants* and continued increase of VAF in the 2 likely somatic variants. Karyotype was normal throughout. A diagnosis of MDS with multilineage dysplasia, highly suspicious for myeloid neoplasm with germline *DDX41* mutation was made. The patient was put on hypomethylating agent.

Biopsy Fixation Details

Decalcified and fixed in 10% neutral buffered formalin

Frozen Tissue Available

No

Details of Microscopic Findings

Case 1: normocellular with slight erythroid hyperplasia, slight granulocytic hypoplasia with left-shift, and unremarkable megakaryopoiesis. No diagnostic morphologic features of MDS.

case 2: hypercellular (60%) with erythroid hyperplasia, left-shifted granulopoiesis with slight increase of monocytic cells, and rare atypical megakaryocytes. No diagnostic morphologic features of MDS.

case 3: Significant anisopoikilocytosis in PB smear. BM was hypercellular (80%) with multilineage dysplasia: dyserythropoiesis with megaloblastic changes, nuclear budding and irregularity, and a subset of small hypolobated and osteoclast-like megakaryocytes. Slightly increased mononuclear myelomonocytic cells. No increase of blasts.

Immunophenotype

Case 3: No increase of blasts, monotypic B-cells, phenotypically abnormal T-cells or distinctly aberrant myeloid maturation.

Cytogenetics

Case 1-3: 46,XY[46]

Molecular Studies

BM Case 1: none

PB: *DDX41* R339H(49%) and L597E(22%), *CBL* G415R(17%)

BM case 2: *DDX41* R339H(49%) and L597E(39%), *CBL* G415R(34%)

BM case 3: *DDX41* R339H(49%) and L597E(42%), *CBL* G415 (41%), *EZH2* G630 (7%), *IDH1* L288Rfs*59(48%)*

Hair follicle/saliva: *DDX41* R339H germline

All were classified as VUS

*Unknown whether the *IDH1* variant was newly emerged as it was in a newly added NGS test region.

Proposed Diagnosis

Myeloid neoplasm with germline *DDX41* mutation (2017 WHO and 2022 ICC)

Interesting Feature(s)

This case highlights a myeloid neoplasm with germline *DDX41* mutation, showing a nice correlation between genetic, disease and morphologic evolution, which aids in diagnosis and guides therapy.

Classifying less well-documented missense germline (GL) *DDX41* variants poses challenges compared to the frameshift/start-loss loss-of-function variants per the ACMG guideline. Recent studies suggest gene-specific modifications, e.g., upgrading a GL *DDX41* VUS to pathogenic/likely pathogenic (P/LP) if co-existing with a somatic *DDX41* variant (PMID: 35671390, 37506341, 37874914). For R339H, despite independent VUS classification by 2 labs, the observed disease association, co-existence with somatic K597E, and increasing reported cases support its P/LP nature. This designation also qualifies the case for diagnosing myeloid neoplasm with germline *DDX41* P/LP variant by the 2022 WHO criteria.

Panel Diagnosis

Myelodysplastic neoplasm with low blasts (WHO-5) associated with germline DDX41 variant / Myelodysplastic syndrome, NOS with multilineage dysplasia (ICC 2022) and germline DDX41 mutation

EA4HP24-BMWS-259

A Case of Myeloid Neoplasm with Germline DDX41 Mutation and Normal Karyotype

Dr. Zubaidah Al-Jumaili, Dr. Nourhan Ibrahim, Dr. Hanadi El Achi, Dr. Jacob Armstrong, Dr. Brenda Mai

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

Case Description

A 68-year-old male with no significant past medical history presenting with pancytopenia

Biopsy Fixation Details

Formalin-fixed formic acid-decalcified paraffin-embedded tissue.

Frozen Tissue Available

N/A

Details of Microscopic Findings

- The peripheral blood showed pancytopenia, however dysplasia and circulating blasts were not identified.
- The bone marrow biopsy showed a variably cellular marrow (10-30%). There were increased blasts with scant to moderate agranular cytoplasm, round nuclei, fine chromatin, and distinct nucleoli; CD34 immunostaining on the core biopsy showed approximately 10-15% blasts.
- The erythroid elements were increased with dysplastic features including nuclear irregularity, budding, nuclear lobation, multinucleation, and basophilic stippling. Rare hypolobated and small megakaryocytes were noted on CD61 immunostaining.
- The plasma cells were increased, but kappa and lambda immunostaining were polytypic.

Immunophenotype

Flow cytometry on the bone marrow aspirate showed increased myeloblasts (~5-10% of total events). The myeloblasts were positive for: CD13, CD33, CD34 (bright), CD38 (dim), CD45 (dim), CD117, CD123 (dim), and HLA-DR and negative for CD2, cCD3, CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD11c, CD14, CD16, CD19, CD20, cCD22, CD56, CD64, cCD79a, CD138, nTdT, and cMPO

Cytogenetics

Chromosome analysis showed a normal male karyotype, 46,XY[20]

Molecular Studies

- Fluorescence in situ hybridization (FISH) for AML and MDS were negative.
- On next genome sequencing (NGS), a DDX41 p.M1 mutation was detected at a variant allele frequency of 48.5%; mutations in other genes were not detected. Although his bone marrow was consistently in remission on subsequent marrows, NGS studies detected persistence of his DDX41 mutation.

Proposed Diagnosis

Myeloid neoplasm with germline predisposition, myelodysplastic syndrome with increased blasts 2 with germline DDX41 pathogenic/likely pathogenic (P/LP) variant

Interesting Feature(s)

- Although the patient pancytopenia and has morphologic features of dysplasia with increased blasts (5-19%), he had a normal karyotype and lacked clonal cytogenetic and molecular abnormalities.
- The DDX41 mutation was found by expanded NGS testing, which emphasizes the importance of ancillary molecular testing in patients with myeloid neoplasms that lack clonal molecular and genetic abnormalities. There are no specific morphologic features that can suggest the presence of a DDX41 mutation, so routine NGS testing is vital for the detection of this entity.
- DDX41 germline mutations are one of the most common genetic alterations causing predisposition to myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML).
- Recent reports suggest that DDX41-related myeloid neoplasms could be considered as a distinct entity associated with better prognostic outcomes and better response to stem cell therapy.

Panel Diagnosis

Myelodysplastic neoplasm with increased blasts 2 (WHO-5) associated with germline DDX41 variant / Myelodysplastic syndrome/Acute myeloid leukemia (ICC 2022) with germline DDX41 mutation

EA4HP24-BMWS-263

Chronic myelomonocytic leukemia evolving into acute myeloid leukemia after acquisition of an NPM1 mutation

Dr. Jessie Xiong, Dr. Jingjing Zhang

University of Colorado, Pathology, Aurora, USA

Case Description

The patient is an older male (70s) with a reported history of Clonal cytopenia of undetermined significance (CCUS) since 2015. A bone marrow biopsy performed in 2015 was morphologically unremarkable. Next generation sequencing on the bone marrow aspirate showed pathologic mutations in SRSF2, TET2, and TP53. He was diagnosed with CCUS. However, evaluation of his monocyte count through the years reveals an absolute and relative monocytosis since 2015 (20% and 1.1x10^9/L), perhaps retrospectively warranting a diagnosis of Chronic myelomonocytic leukemia -1 (CMML1).

In 2023, he presented to our institute with acute, intractable headaches. MRIs and CT scans of the patient's head did not show a focal lesion. A bone marrow biopsy performed showed

a hypercellular marrow with monocytosis and multilineage dysplasia. A diagnosis of CMML-1 was rendered. The following pathogenic variants were reported on NGS a few weeks later: TET2 52.4% VAF, NPM1 4.1% VAF, TP53 47.6% VAF, SRSF2 49.5% VAF.
One month later, a PET/CT showed diffuse lymphadenopathy. A lymph node biopsy revealed a blast population. A diagnosis of myeloid sarcoma was rendered. A repeat bone marrow biopsy showed acute myeloid leukemia with 20-25% blast equivalents. While VAFS for TET2, TP53 and SRSF2 were steady, repeat biopsy showed that the VAF of NPM1 has now risen to 26.5%.
The patient ultimately received a bone marrow transplant and his 28 day marrow showed no evidence of disease and 100% donor chimerism.

Biopsy Fixation Details

10% formalin

Frozen Tissue Available

N/A

Details of Microscopic Findings

Initial Marrow : Markedly hypercellular (>90%). Multilineage dysplasia, monocytosis, and <5% blasts by aspirate differential.
One month later - Lymph Node: Diff-Quik cytospin slide shows sheets of large atypical mononuclear cells, with fine chromatin, prominent nucleoli, and scant to moderate cytoplasm with occasional pink granules (blasts), with a minor subset showing delicate nuclear folds (promonocytes/blast equivalents). The H&E biopsy shows similar features, with extensive nodal involvement by an atypical mononuclear cell population, with predominantly immature cytomorphologic features.
One month later - Marrow: The aspirate smears show increased blasts. Occasional erythrophagocytosis by blasts and abnormal monocytes is noted. Background hematopoiesis shows multilineage dysplasia. The core shows extensive necrosis, approximately 70% of the marrow space.

Immunophenotype

Positive: CD38, CD117 (minor subset), CD13 (spectrum), CD33 (bright), HLA-DR (minor subset), CD11b (variable), CD64
Negative: CD34, CD10, CD14 , CD7, CD56, CD19

Cytogenetics

46,XY[20]

Molecular Studies

2015: SRSF2, TET2, and TP53
2023:
Marrow 1:
- TET2 52.4%
- NPM1 4.1%
- TP53 47.6%
- SRSF2 49.5%

Marrow 2 (one month later)
- NPM1 26.5%
- SRSF2 48.7%

- TET2 51.5%
- TP53 46.4%

Proposed Diagnosis

2015: Chronic myelomonocytic leukemia (CMML1)
2023: Acute myeloid leukemia (AML)

Interesting Feature(s)

The patient's original CMML1 did not progress for almost a decade until the acquisition of NPM1. The first bone marrow biopsy in 2023 was a diagnostic dilemma, as it would not qualify for AML by ICC classifications. By ICC, an NPM1 mutation in CMML should be noted, but such a finding does not define de novo AML in the setting of known CMML.

By WHO 5th edition, while the presence of NPM1 mutation is normally considered AML defining regardless of blast count, rare cases with a low NPM1 VAF (<10%) with a low blast count (<5%) is currently a controversial area with recommendations to be cautious.

Panel Diagnosis

Myeloid sarcoma in acute myeloid leukemia with mutated NPM1 (ICC 2022 / WHO-5) evolving from CCMUS over CMML

EA4HP24-BMWS-264

Aggressive systemic mastocytosis associated with later-onset chronic myeloid leukemia

Dr. Carlo Pescia[1], Dr. Daniele Cattaneo[2,3], Dr. Cristina Bucelli[2], Dr. Valentina Bellani[2,4], Dr. Anna Chiara Migliorini[2], Dr. Federico S. Colombo[2], PhD/MD Alessandra Iurlo[2], Dr. Giorgio Alberto Croci[3,5]

[1] Università degli Studi di Milano, Milan, Italy; [2] Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Division of Hematology, Milan, Italy; [3] Università degli Studi di Milano, Department of Pathophysiology and Transplantation, Milan, Italy; [4] Università degli Studi di Milano, Department of Oncology and Hemato-Oncology, Milan, Italy; [5] Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Division of Pathology, Milan, Italy

Case Description

84-year-old male patient, with medical history of hypertension, chronic kidney disease, hypothyroidism, diverticulosis, myocardial infarction, osteoporosis.
Since 2012: persistent mild monocytosis (800/mmc).
September 2021: abdominal pain, dyspepsia, and significant weight loss (10 kg in 3 months); EGDS shows duodenitis with increased mast cells in the lamina propria, paired with an elevated tryptase level (85.2 mcg/L), splenomegaly (19 cm), hepatomegaly (17.5 cm) with ascites, and abdominal adenopathies. Laboratory results showed Hb 11.9 g/dL, WBC

5,080/mmc, PLT 325,000/mmc, and abnormal liver function tests. Bone marrow biopsy (BMB) revealed systemic mastocytosis (SM) with an associated hematological neoplasm, categorized as myeloproliferative neoplasm, unclassifiable.

August 2022: persistent diarrhea, laboratory findings: Hb 12.2 g/dl, WBC 4,700/mmc (ANC 1,000/mmc, Mo 1,100/mmc, AEC 1,200/mmc), PLT 368,000/mmc; a BMB at our Institution proved unsatisfactory due to poor quality. A transjugular biopsy performed due to hepatosplenomegaly with ascites and cholestasis showed mild portal fibrosis with admixed epithelioid and spindle mast cells, with a CD117+, tryptase+, CD25+, CD2+, CD30-/+ phenotype. Treatment with midostaurin for SM was started.

December 2022: peripheral edema; laboratory findings: Hb 11.7 g/dl, WBC 7,460/mmc (ANC 2,760/mmc, Mo 1,120/mmc, Ba 1,120/mmc), blasts 2% with myelocytes and metamyelocytes, PLT 1,123,000/mmc. Hydroxyurea was required for extreme thrombocytosis. Due to PLT count increase despite cytoreduction, a BMB+bone marrow aspirate (submitted case) was performed, documenting a blast phase of chronic myeloid leukemia. Consequently, tyrosine kinase inhibitor therapy with Imatinib was initiated.

Biopsy Fixation Details

10% buffered formalin

Frozen Tissue Available

No

Details of Microscopic Findings

BMB (Dec 2022) revealed an 80-90% cellularity with panmyelosis, dyserythropoiesis, and dysgranulopoiesis. Eosinophils were increased, and numerous small-sized, hypolobated megakaryocytes were observed, along with medium-sized blasts (10-15%). Spindle mast cells were present in fibrotic areas (<5%), and interstitial B and T lymphocytes comprised 10-15% of the cellular composition. Reticulin fibrosis was categorized as MF-1. Bone marrow aspirate revealed 27% blasts.

Immunophenotype

Mast cell aggregates were CD117+, tryptase+, CD25+, CD2-/+, and CD30-. Blasts exhibited a myeloid profile (CD34+, CD117+, MPO-/+, CD56-, TdT-, CD14-, tryptase-).

Cytogenetics

Karyotype: 46, XY (2021).

In August 2022, peripheral blood analysis showed positivity for *BCR::ABL1* p210 fusion transcript (7 copies/10,000), increased significantly to 2340.95 copies/10,000 in Dec 2022.

Molecular Studies

Molecular studies (NGS Oncomine® myeloid panel, 2021) identified *KIT D816V*, *KRAS*, *JAK2 V617F*, *MPL*, *NRAS*, *SRSF2*, and *ASXL1* mutations

Proposed Diagnosis

Systemic mastocytosis with associated hematological neoplasms (myeloproliferative neoplasm, unclassifiable, switching to blast phase of chronic myeloid leukemia).

Interesting Feature(s)

The later onset of a *BCR::ABL1* clone in SM-MPN progression represents a unicum and fewer than 10 cases of SM-AHN with CML as AHN have been reported. Intriguingly, mast cells were scarce in the bone marrow but abundant in extramedullary sites (liver and

gastrointestinal tract). Additional mutations in *ASXL1* and *SRSF2* further complicate the prognosis, portending inferior survival in ASM and CML.

Panel Diagnosis

SM-AMNs / AHNs (ICC 2022 / WHO-5): ASM and CMUS evolving to CMML, and CML (ICC 2022 / WHO-5)

EA4HP24-BMWS-272

"Paving the way" to Systemic Mastocytosis

Dr. Christian K. Hirt, Dr. Xiang Xu

Beth Israel Deaconess Medical Center, Harvard Medical School, Pathology, Brookline, USA

Case Description

79-year-old woman with history of hypertension who was referred to hematology for thrombocytopenia (platelets 41 K/uL) and mild anemia (Hgb 11g/dL). A bone marrow biopsy was performed which showed myelodysplastic neoplasm with multilineage dysplasia and a U2AF1 mutation. In addition, there was a small population of CD5-positive monoclonal B cells present. The patient was received no treatment and followed up by the clinician. After two years, she presented with skin rash, fatigue and weight loss. CBC showed worsening anemia and thrombocytopenia. A repeat bone marrow biopsy was performed.

Biopsy Fixation Details

The bone marrow biopsy was fixed in B Plus fixative and decalcified in RDO fixative prior to processing.

Frozen Tissue Available

n/a

Details of Microscopic Findings

On the initial bone marrow biopsy, histological sections revealed a hypercellular bone marrow with erythroid dysplasia, atypical megakaryocytic proliferation and small foci of lymphoid aggregates, but not increase in blasts or myelofibrosis.

On the 2nd bone marrow biopsy (2-years later), the bone marrow was hypercellular with sheets of atypical spindle/polygonal mast cells, with marked myelofibrosis. The background hematopoiesis showed erythroid and megakaryocytic dysplasia.

Immunophenotype

CD34 highlighted rare blasts on both biopsies comprising less than 5% of total cellularity. On the 1st biopsy a small focal B-cell aggregate was identified which co-expressed CD5 and CD20. In the 2nd biopsy, atypical mast cells are positive for mast cell tryptase, CD117, CD25 (80% of total cellularity).

Concurrent flow cytometric studies on both bone marrow biopsies showed a lambda light chain skewed B-cell population that co-expresses CD5 and CD23 (subset).

Cytogenetics

Cytogenetic study revealed normal karyotype. FISH study is negative for MDS or CLL panel.

Molecular Studies

Molecular studies (targeted next-generation sequencing) identified pathogenic mutation of *U2AF1* p.S34F on both bone marrow biopsies with similar allele frequency (39% vs 46%). On the 2nd biopsy, a new *KIT* D816V mutation was also detected with VAF of 29%.

Proposed Diagnosis

- Systemic mastocytosis with an associated haematological neoplasm (derived from underlying hematolymphoid neoplasms).
- CD5-positive B-lymphoproliferative disorder (monoclonal B-cell lymphocytosis)

Interesting Feature(s)

Systemic mastocytosis with an associated hematological neoplasm (SM-AHN) is a subgroup of systemic mastocytosis in current WHO classification. Over 90% of cases of systemic mastocytosis (SM) harbor pathogenic KIT mutations, particularly *KIT* D816V. U2AF1 is one of key components of spliceosome complex, which plays a critical role in RNA splicing. Mutations in U2AF1 have been discovered in varieties of myeloid neoplasms. However, U2AF1 mutation is rarely identified in SM-AHN. Herein, we present a case of systemic mastocytosis, which evolved from patient's underlying hematolymphoid neoplasms driven by newly acquiring a KIT D816V mutation on the base of a pathogenic mutation in splicing factor (U2AF1 p.S34F). Per current WHO classification, the AHN should usually be considered a secondary neoplasm with clinical and prognostic implications. In our case, the sequential steps of developing diseases (from MDS to SM) reveals a different clinical course and may implicate on further management.

Panel Diagnosis

SM-AMN / AHN (ICC 2022 / WHO-5): SM (at least SSM) and MDS, NOS with MLD (ICC 2022) / MDS with low blasts (WHO-5), and MBL of the CLL type

EA4HP24-BMWS-279

t(9,15)(q22;q24.1) *PML*::*SYK*: A new recurrent translocation in myeloid neoplasm with marked eosinophilia

Kara Tao[1], Prof. Xinyan Lu[2], Prof. Peng Ji[2], **Prof. Yi-Hua Chen**[2]

[1] Yale University, Research Intern, Pathology, Northwestern Memorial Hospital, Chicago, USA; [2] Northwestern Memorial Hospital, Pathology, Chicago, USA; [3] Northwestern Memorial Hospital, Pathology, Chicago, Germany

Case Description

A 67 year-old female presented for evaluation of persistent erythrocytosis and leukocytosis started 13 months ago. The peripheral blood (PB) showed erythrocytosis (HGB 17.4 g/dL; HCT 56.5%) and leukocytosis (WBC 36.8 k/uL) including absolute neutrophilia with a shift to

immaturity and marked eosinophilia (42%). Platelet count was normal (214 k/uL). Molecular analysis on PB was negative for *BCR::ABL1* fusion, *JAK2V617F, CALR* and *MPL* mutations. A bone marrow biopsy demonstrated a hypercellular marrow (>90% cellular) with granulocytic hyperplasia and marked eosinophilia (20-30%). Megakaryocytes were not increased and show small hypolobated forms. FISH analysis was negative for *BCR::ABL, PDGFA, PDGFB, FGFR1* rearrangements. NGS was positive for *ASXL1* mutation. A diagnosis of myeloproliferative neoplasm (MPN) was made without further subclassification. Subsequent chromosome studies demonstrated t(9;15)(q22;q24.1) involving *PML* gene and an unknown gene on chromosome 9. A comprehensive NGS revealed *PML::SYK* fusion. *SYK* rearrangements have been reported in only 3 cases of myeloid neoplasms, including t(9;12) *ETV6::SYK/TEL::SYK* (1 MDS and 1 MPN) and t(9;15)*PML::SYK* (MPN rapidly progressed to AML). To the best of our knowledge, our case is the second documented *PML::SYK* fusion in myeloid neoplasm. Interestingly, eosinophilia was noted in 3 of the 4 *SYK* rearranged myeloid neoplsms, 2 with marked eosinophilia Including our case.

Biopsy Fixation Details

Particle clot: Formalin

Frozen Tissue Available

No

Details of Microscopic Findings

1. Peripheral blood: Erythrocytosis with mild anisopoikilocytosis. Leukocytosis including neutrophilia and marked eosinophilia. Neutrophils show a shift to immaturity; no circulating blasts identified. Platelets normal in number with unremarkable morphology.

2. Bone marrow aspirate: Granulocytic predominance with relatively decreased erythroid precursors without over dysplasia. Marked eosinophilia (20 to 30%). Megakaryocytes are not increased with some small hypolobated forms.

3. Bone marrow core/particle clot : Markedly hypercellular (>90% cellular) with granulocytic hyperplasia and marked eosinophilia. No increased blasts by CD34 staining. Megakaryocytes were normal in number with small hypolobated forms. No reticulin fibrosis.

Immunophenotype

No increased blasts detected

Cytogenetics

Chromosome: t(9;15)(q22;q24.1)[20]

FISH: Negative for BCR::ABL1, PDGFA, PDGFB, FGFR1 rearrangements.

Molecular Studies

Comprehensive NGS: *PML::SYK* fusion and *ASXL1* mutation

Proposed Diagnosis

Myeloproliferative neoplasm with marked eosinophilia and t(9;15) *PML::SYK*

Interesting Feature(s)

1. SYK gene is located on chromosome 9q22 and encodes a non-receptor tyrosine kinase (TK). *SYK* rearrangements have been reported in 3 cases of myeloid neoplasms, including t(9;12) *ETV6::SYK/TEL::SYK* and t(9;15) *PML::SYK*. To the best of our knowledge, our case is the second documented *PML::SYK fusion* in myeloid neoplasm, suggesting that this is a recurrent cytogenetic abnormality in myeloid neoplasm.

2. Interestingly, of the four documented *SYK* rearranged myeloid neoplasms, 3 showed eosinophilia including 2 with marked eosinophilia, suggesting that *SYK* rearrangements may represent additional TK gene rearrangements that are not currently included in the WHO or ICC myeloid/lymphoid neoplasms with eosinophilia and TK gene rearrangements. IL-5 has been shown to activate *SYK* and lead to the proliferation of eosinophils. Constitutive activation of *SYK* may contribute to the eosinophilia.
3. Multiple *SYK* inhibitors are currently available and may have therapeutic potential for patients with *SYK* rearranged myeloid neoplasm.

Panel Diagnosis

Myeloid/lymphoid neoplasms with eosinophilia and other tyrosine kinase gene fusions (WHO-5) / Chronic eosinophilic leukemia (ICC 2022)

EA4HP24-BMWS-283

Leukocytosis in an infant associated with an NRAS mutation

Dr. Michele Paessler[1,2], **PhD/MD Gerald Wertheim**[1,2], Brian Lockhart[1]

[1] Children's Hospital of Philadelphia, Hematopathology, Philadelphia, USA; [2] Hospital of the University of Pennsylvania, Hematopathology, Philadelphia, USA

Case Description

1-day-old girl presented with petechiae, lymphocytosis (ALC 8,970), monocytosis (AMC 11,520) thrombocytopenia (PLT 38K). but no blasts. She was monitored in the NICU for a congenital infection and was discharged at DOL 5. Outpatient monitoring DOL 24, revealed splenomegaly and a CBC showing WBC of 72K with 10% CD34+ CD33+ blasts and PLT of 45K. Bone marrow aspirate at that time was trilinear with myeloid hyperplasia and M:E ratio of 10 and 5% myeloblasts. FISH was negative for +21, -7, KMT2Ar, BCR::ABL1. Next-generation sequencing panel was positive for NRAS c.35G>A (p.Gly12Asp) with VAF of 0.43. Sequencing of sorted B cells, T cells and myeloid cells showed NRAS mutation in all subsets. A skin sample also showed the NRAS mutation but VAF of 0.24, and cultured fibroblasts were NRAS WT. Findings were consistent with a diagnosis of juvenile myelomonocytic leukemia (JMML), though a mosaic germline RAS mutation could not be excluded.
Patient was started on azacitidine. At 15-months of age developed monocytosis (WBC 15.9, AMC 2,480), concerning for leukemic progression. Bone marrow study showed trilineage hematopoiesis without an increase in blasts or dysplasia. Genetic studies were negative for additional abnormalities. The patient continued on azacitidine therapy and was not considered for transplant at the time. Her WBC remains slightly elevated (most recent WBC 12.4), yet her platelets have normalized.

Biopsy Fixation Details

DOL 25: No biopsy obtained.

15 weeks biopsy: fixed in formalin.

Frozen Tissue Available

No frozen tissue. Aspirate cover slips available.

Details of Microscopic Findings

DOL 25: Trilineage hematopoiesis, myeloid hyperplasia (M:E = 10) and 5% myeloblasts.

15 months: Trilineage hematopoiesis and <5% myeloblasts.

Immunophenotype

Bone marrow aspirate (DOL 25): 10% of events were CD33+ CD34+ myeloblasts.

Bone marrow aspirate (15 months): 1.3% CD34+ myeloblasts without antigen abnormalities.

Cytogenetics

Normal at both time points.

Molecular Studies

NGS performed on bone marrow:

DOL 25:

NRAS (NM_002524.4), c.35G>A (p.Gly12Asp) (VAF: 0.43)

RELN (NM_005045.3), c.5904C>A (p.Asp1968Glu) (VAF: 0.44)

15-months:

NRAS (NM_002524.5), c.35G>A, (p.Gly12Asp) (VAF: .48)

RELN (NM_005045.4), c.5904C>A (p.Asp1968Glu) (VAF: .48)

Proposed Diagnosis

Leukocytosis with an activating NRAS mutation (JMML vs. Ras-associated autoimmune leukoproliferative disorder (RALD) vs. mosaic germline RASopathy)

Interesting Feature(s)

The case shows the clinical, morphologic and genetic overlap of an aggressive (JMML) and an indolent (RALD) disease. Although criteria for JMML were met, patients with activating NRAS or KRAS mutations should not be transplanted unless there are additional signs of aggressive disease (e.g. concomitant genetic mutations, increasing blasts, dysplasia).

Panel Diagnosis

Ras-associated autoimmune leukoproliferative disorder (with JMML-like presentation)

EA4HP24-BMWS-306

Myeloid/erythroid Sarcoma and Pure Erythroid Leukemia with *ZMYND8::RELA* Fusion

Prof. Daniel A. Arber, PhD/MD Jason Cheng, Prof. Sandeep Gurbuxani, Prof. Girish Venkataraman, PhD/MD Peng Wang, Dr. Angela Lager

University of Chicago, Pathology, Chicago, USA

Case Description

3 yo girl noted to have intraorbital swelling. MRI showed a mass (2.5 x 2.8 x 1.8 cm) filling the right maxillary sinus with infiltration into the right pterygopalatine fossa and oral cavity. Rhabdomyosarcoma was suspected clinically. CBC: WBC 11.5 K/uL, HGB 11.6 g/dL, MCV 79.0 fL, RDW 12.9%, PLT 101 K/uL. Differential count: 23% neutrophils, 6% bands, 57% lymphocytes, 9% monocytes. A biopsy of the maxillary sinus mass and a bone marrow biopsy were performed.

Biopsy Fixation Details

Tissue BX: 10% neural buffered formalin (NBF) fixative for overnight.

BMBX: B5 fixative and decalcified in 10% formic acid and 5% formaldehyde.

Frozen Tissue Available

None

Details of Microscopic Findings

The mass biopsy shows infiltrating sheets of neoplastic cells and large areas of necrosis.

BMBX shows a small portion of cellular bone marrow with predominantly atypical erythroblastic proliferation/infiltration and markedly reduced granulopoiesis and megakaryopoiesis. Aspirate smears show markedly increased erythroblasts with some dysplastic changes and no granulocytic dysplasia.

Immunophenotype

Right maxillary sinus mass lesion:

The neoplastic cells are weakly and partially positive for CD45, strongly positive for glycophorin C and GLUT-1, but negative for CD34, MPO, CD3 and PAX5. The neoplastic cells are negative other blast- and lineage-associated markers. A subset of cells is positive for TP53.

Bone marrow:

The atypical erythroblasts are negative for CD34, but strongly positive for glycophorin C. Additional IHCs and flow cytometry show that the atypical erythroblasts are positive for CD71 and CD36, but negative for all other blast- and lineage-associated markers.

Cytogenetics

BM aspirate:

48,XX,+6,del(7)(p22p21),der(10)t(10;13)(q22;q12),t(11;20)(q13;q13.1),+19[14]/96,idemx2[2]/46,XX[4]

Molecular Studies

RNA Fusion NGS FFPE of right maxillary sinus mass:

Detection of a *ZMYND8::RELA* fusion gene.

BM aspirate: No *TP53* mutation

Pathogenic Findings

RB1 loss-equivocal

NF1 rearrangement

Findings of Uncertain Clinical Significance

BCORL1 c.1607C>T, p.T536I (NM_021946.4) (VAF: 44%)

KDM6A c.274G>C, p.E92Q (NM_001291415.2) (VAF: 24%)

Proposed Diagnosis

Right maxillary sinus mass:

- **Myeloid/erythroid sarcoma with *ZMYND8::RELA* fusion.**

Bone marrow biopsy:

- **Pure erythroid leukemia**

Interesting Feature(s)

1. This case initially presented a maxillary sinus mass with clinical and radiological signs suggestive of rhabdomyosarcoma, but negative immunohistochemical studies for almost all blast- and lineage-associated markers.
2. Despite expression of TP53 by immunohistochemistry, the case did not harbor a mutation of *TP53*, highlighting the need for molecular confirmation of this mutation.
3. While most cases of pure erythroid leukemia are associated with *TP53* mutations and are classified as AML with mutated *TP53* in the International Consensus Classification, this mutation Is not present in rare cases occurring in infants and children. Cases of erythroid sarcoma and pure erythroid leukemia with *ZMYND8::RELA* (PLOS One 8:e63663, 2013) or other fusions (Blood Adv 4:6000-8, 2020; Pediatr Blood Cancer 70:e30333, 2023), including *RCC1::LCK, NFIA::CBFA2T3, NFIA::ETO2; NFIA::RUNX1T1,* and *MYB::GATA1,* and without *TP53* mutations have been reported. While this case has a complex karyotype, the recurring gene fusion (*ZMYND8::RELA)* seems to be a more consistent marker of this disorder, suggesting that these rare cases represent a distinct entity separate from AML with mutated *TP53* or AML with MDS-related cytogenetic abnormalities.

Panel Diagnosis

Myeloid sarcoma (ICC 2022 / WHO-5) in erythroid AML (WHO-5)

EA4HP24-BMWS-307

Myelodysplastic syndrome with multiple truncating *GATA2* mutations

Hovsep Ohan, Dr. Kidar Inamdar, Dr. Wei Liu, Dr. Juan Gomez-Gelvez, Dr. Yulei Shen, Dr. Sharmila Ghosh

Henry Ford Hospital, Pathology and Laboratory medicine, Detroit, USA

Case Description

53-year-old female presented with osteomyelitis of her left foot, macrocytic anemia and thrombocytopenia in April 2023. Past medical history was significant for acute promyelocytic leukemia (APL) at the age of 30 years, treated with AIDA induction therapy and 2 cycles of idarubicin and cytarabine followed by ATRA pulses every three months. She relapsed in 2001 and was treated with arsenic trioxide followed by SCT in 2002 and remained in remission by RT-PCR analysis. She developed slight thrombocytopenia in 2014. Several bone marrow biopsies performed were nondiagnostic.

Biopsy Fixation Details

1 hour of formalin fixation at room temperature, formalin fixation for 30 minutes at 50°C, followed by 4 hours of EDTA heated decalcification at 50°C.

Frozen Tissue Available

Not done

Details of Microscopic Findings

The bone marrow biopsy (4/2023) is hypercellular (60-70%) with trilineage hematopoiesis, 5% blasts and megakaryocytic dysplasia (small hypolobated forms) in a subset. No significant dysplasia was noted in erythroid and myeloid precursors. Grade 0-1/3 reticulin fibrosis was noted. Patient had normal white counts (7.1 x 10^3/uL), HB of 9.6 g/dl and platelet count 33K/uL.

Immunophenotype

Blasts CD34, CD117, HLA-DR, CD13, CD33 positive

Megakaryocytes Factor 8 positive

Cytogenetics

Normal female karyotype 46,XX[20]FISH: No abnormality detected

Molecular Studies

Myeloid Plus NGS: Variants of strong or potential clinical significance

DNMT3A p. R882P (VAF 41.9%)

RUNX1 p.H404Vfs* (VAF 42.7%)

GATA2 p. P254Afs* (VAF 7.9%)

GATA2 p. A198Rfs* (VAF 5.4%)

GATA2 p. S139Cfs* (VAF4.1%)

GATA2 p.V118Rfs* (VAF 6.6%)

GATA2 p. E6Gfs* (VAF 5%)

BCOR p. S822Qfs* (VAF4.5%)

Proposed Diagnosis

Myelodysplastic syndrome with increased blasts -1 (MDS-IB-1)

Interesting Feature(s)

The patient developed MDS more than 20 years after she had been diagnosed with APL. Therapy related myeloid neoplasms usually develop within 10 years of exposure to chemotherapy. Hence, this is more likely to be *de novo* MDS rather than therapy related. The patient had longstanding thrombocytopenia and several bone marrow biopsies performed remained non-diagnostic and no cause for the thrombocytopenia was identified. The diagnosis of MDS was challenging due to lack of significant dysplasia, < 5% blasts, normal karyotype and FISH, in the context of infection. The diagnosis was confirmed following NGS which revealed a complex mutational profile involving *DNMT3A, RUNX1, BCOR*, and 5 truncating mutations of the *GATA2* gene.

DNMT3A and *RUNX1* mutations were likely early events in clonal hematopoiesis followed by *GATA2* mutations that led to divergent clonal evolution. Five different mutations of *GATA2* gene is extremely unusual and has not been reported in MDS. These were frameshift mutations present at low allele frequencies of 4.1-7.9%. The combined mutations had a deleterious effect evidenced by the lack of response to therapy and progression to AML within 6 months. The *BCOR* frameshift mutation, was another subclone that further contributed to the adverse outcome.

GATA2 mutated MDS is known to be associated with cytopenias and subtle features of dysplasia as seen in our patient. They are also known to be associated with infections and one can speculate that the patient presenting with osteomyelitis may be related to *GATA2* mutations in the setting of MDS.

Panel Diagnosis

Myelodysplastic syndrome with excess blasts (ICC 2022) / increased blasts 1 (WHO-5) (with possible RUNX1 germline predisposition associated with a constitutional platelet disorder)

EA4HP24-BMWS-308

Acute myeloid leukemia with *DDX41* mutation

Dr. Sharmila Ghosh, Hovsep Ohan, Dr. Wei Liu, Dr. Juan Gomez-Gelvez, Dr. Yulei Shen, Dr. Kidar Inamdar

Henry Ford Hospital, Pathology and Laboratory Medicine, Detroit, USA

Case Description

70 years old male of European ancestry with PMH of multiple sclerosis, neurogenic bladder presented to ED for dysuria. There is no known family history of cancer or known Jewish ancestry or consanguinity.

His laboratory work-up was negative for PNH, acute EBV infection, acute hepatitis screen, HIV, ANA, Rheumatoid factor and monoclonal protein. CBC showed significant pancytopenia with macrocytic anemia, which appears to be present since 2015. A bone marrow was performed on 11/2022 for evaluation of pancytopenia.

Biopsy Fixation Details

Bone marrow cores are processed by formalin fixation for 1 hour at room temperature, 30 minutes of formalin fixation at 50°C, followed by 4 hours of EDTA heated decalcification at 50°C.

Frozen Tissue Available

Not done.

Details of Microscopic Findings

Nov 2022: Patient had pancytopenia - WBC 0.8 x 10^3/uL, HB 9.6 g/dl and platelet count of 59K/uL.

The bone marrow was hypocellular (20%) with decreased trilineage hematopoiesis, left shifted granulopoiesis and 24% blasts. Blasts were variable in size with high N:C ratio, round nuclei, fine chromatin, prominent nucleoli, basophilic cytoplasm, without Auer rods. No significant dysplasia was noted in any of the lineages.

Subsequent bone marrow biopsies done on 12/2022, 2/2023 and 12/2023 showed residual disease with blast count of 6%, 7% and 22% respectively.

Immunophenotype

Blasts positive for CD34, CD117, HLA-DR, CD13, CD33, MPO and TDT (minor subset)

Cytogenetics

Normal male karyotype 46,XY[20]

FISH: No abnormality detected

Molecular Studies

Myeloid Plus NGS: Variants of Strong or Potential Clinical Significance (Diagnostic, Prognostic & Therapeutic) TIER 1/2

(11/2022, blasts 24%)

DDX41 p. (D140Gfs*2) VAF 48.1%

DDX41 p. (R525H) VAF 3.2.0%

CUX1 p. (S1123C) VAF 48.2% (VUS)

(2/2023, blasts 6%)

DDX41 p. (D140Gfs*2) VAF 48.0%

DDX41 p. (R525H) VAF 5.0%

TET2 p. (Y1589*) VAF 4.7%

CUX1 p. (S1123C) VAF 45.8% (VUS)

Proposed Diagnosis

Acute myeloid leukemia *DDX41* mutation related

Interesting Feature(s)

We present a 71-year old male with *DDX41* related AML with some unusual findings. The patient has a biallelic *DDX41* gene mutation characterized by *DDX41* p. (D140Gfs*2) VAF 48.0% and a second missense mutation *DDX41* p. (R525H) VAF 5.0%, a rare mutation in myeloid neoplasms. *DDX41* p. (D140Gfs*2) has been described in germline sequencing studies and the persistently high VAF in consecutive bone marrow biopsies 49.2% (11/22),

48.1% (12/22) and 48.0% (8/23) suggests possible germline mutation in this patient. Germline studies were not performed for confirmation.

It can be presumed that the germline *DDX41* mutation predisposed to a somatic *DDX41* mutation as a secondary hit. The somatic mutation acted as driver mutation enhancing clonal advantage. It may be hypothesized that the VUS *CUX1* variant [CUX1 p. (S1123C) VAF 48.2%] present may have influenced the clonal process. Dermawan et al (AJCP 2022, 157: 586-594) have reported dysplasia in 19% of patients with VUS *CUX1* related myeloid neoplasm in their study Treatment was initiated with venetoclax and decitabine. The patient did not achieve remission and 4 months later acquired a new *TET2* nonsense mutation producing a cumulative leukemogenic effect.

The somatic *DDX41* mutation, as present in our patient, disrupts ATPase activity causing slow growth of tumor cells reflected in the late onset AML, prolonged cytopenias, hypocellular marrow and borderline blasts seen in our patient. Autoimmune disorders have been reported with germline *DDX41* mutation. The patient's history of multiple sclerosis may be of significance in the present context.

Panel Diagnosis

Acute myeloid leukemia with maturation (WHO-5) associated with germline DDX41 variant / Acute myeloid leukemia, NOS (ICC 2022) with germline DDX41 mutation

EA4HP24-BMWS-320

Divergent evolution from a founding clonal hematopoiesis clone to initial acute myeloid leukemia with *NPM1* mutation and later AML with mutated *TP53* after relapse

PhD/MD Jyoti Kumar[1,2], PhD/MD Mark D. Ewalt[1,2], PhD/MD Sanjay S. Patel[3], PhD/MD Ying Liu[1,2], PhD/MD Kseniya Petrova-Drus[1,2], PhD/MD Alexander Chan[1], PhD/MD Umut Aypar[4], PhD/MD Yanming Zhang[4], PhD/MD Maria E. Arcila[1,2], PhD/MD Mikhail Roshal[1], PhD/MD Wenbin Xiao[1]

[1] Memorial Sloan Kettering Cancer Center, Hematopathology Service, Department of Pathology and Laboratory Medicine, New York, USA; [2] Memorial Sloan Kettering Cancer Center, Diagnostic Molecular Pathology Service, Department of Pathology and Laboratory Medicine, New York, USA; [3] Weill Cornell Medicine/New York-Presbyterian Hospital, Division of Hematopathology, Department of Pathology and Laboratory Medicine, New York, USA; [4] Memorial Sloan Kettering Cancer Center, Cytogenetics Laboratory, Department of Pathology and Laboratory Medicine, New York, USA

Case Description

60-year-old male presented with worsening fatigue, anemia with circulating blasts. After diagnosis with AML (BM1-Day0), he received induction+modified HiDAC consolidation chemotherapy. Upon relapse, treatment with gliteritinib was initiated. 6 months later, patient received haplo-alloPBSCT followed by gilteritinib maintenance. 1 year later, patient relapsed (BM2-Day954) and received decitabine followed by gilteritinib due to progression of disease. He was started on azacitadine and venetoclax. 3 months later, persistent disease was noted (BM3-Day1382). The patient was admitted with neutropenic fever, complicated by hypoxemic respiratory failure and bacteremia. The patient's course worsened resulting in death 39 months after initial diagnosis.

Biopsy Fixation Details

BM core bx (formalin fixed); 2 H&E and 5 unstained slides provided from 3 time points.

Frozen Tissue Available

N/A

Details of Microscopic Findings

BM1-Day0: 90% blasts on aspirate, composed of medium to large sized blasts with oval to slightly irregular/folded nuclei, fine chromatin, occasional distinct nucleoli, many with perinuclear hof, and small amount of basophilic cytoplasm

BM2-Day954: no morphologic increase in blasts. Erythroid elements demonstrated dyserythropoeisis (occasional karyorrhexis, irregular nuclei). Frequent atypical megakaryocytes were noted (small, mono/hypolobated)

BM3-Day1382: showed persistent AML, 78% blasts on aspirate

Immunophenotype

BM1-Day0: abnormal myeloid blast population showed abnormal expression of CD11b(partial), CD25(dim), CD13(dim-bright), CD33(bright), CD34(major subset absent), CD38(intermediate-absent), CD64(partial), CD117(bright), CD123(uniform-bright), HLA-DR(dim-absent), MPO(subset)

BM2-Day954: abnormal myeloid blast population with abnormal expression of CD13(absent), CD123(absent), HLA-DR(absent)

BM3-Day1382: 2 abnormal myeloid blast populations. 1st population (52.2% of WBC) Immunophenotype: Abnormal: CD13(uniform-intermediate), CD33(negative-bright), CD34(negative), CD38(uniform-intermediate), CD117(uniform-intermediate), CD123(dim), HLA-DR(negative). 2nd population (20.1% of WBC) Immunophenotype: Abnormal: CD7(positive), CD11b, CD13(bright), CD33(bright), CD34(bright), CD38(bright), CD56(partial), CD117(dim-negative), HLA-DR(negative)

Cytogenetics

BM1-Day0: normal male karyotype 46,XY[20]

BM2-Day954: 46,XY,del(5)(q31q33),del(6)(q12q27),-7,+mar[1]/46,XY[20]

BM3-Day1382: complex karyotype

Molecular Studies

BM1-Day0: NPM1(38%), FLT3-ITD(34%), DNMT3A V716D(43%), IDH2 R140Q(41%), TP53 P190R(<1%) manual review

BM2-Day954: TP53 X125_Splice(28%), TP53 P190R(32%), DNMT3A V716D(28%), CUX1 A321Pfs(28%)

BM3-Day1382: TP53 X125_Splice(27%), TP53 P190R(28%), DNMT3A V716D(40%), NPM1(7%), FLT3-ITD(11%), IDH2 R140Q(9%), NRAS G12A(6%), NRAS A59D(8%), CUX1 A321Pfs(33%)

Proposed Diagnosis

Initial diagnosis: Acute myeloid leukemia with *NPM1* mutation

After relapse post transplant: Acute myeloid leukemia with mutated *TP53*, therapy-related

Interesting Feature(s)

NPM1 is a heterogeneous disease process that confers a more favorable prognosis, although 30-40% of patients relapse. AML with mutated *TP53*is another distinct entity associated with poor prognosis and *NPM1* mutations in this category are rare. Here, we report a unique case where the initial diagnosis was compatible with AML with *NPM1* mutation. Upon relapse after transplantation, there was clonal evolution of *TP53* variants, now consistent with AML with mutated *TP53*, which may represent a therapy-related process. This unique case confirms the presence of two different, yet related clones that are disease defining and demonstrating phenotypic shift by flow cytometry.

Panel Diagnosis

Acute myeloid leukemia with mutated NPM1 (ICC 2022 / WHO-5) and clonal evolution to acute myeloid leukemia with mutated TP53 (ICC 2022) / myelodysplasia-related (WHO-5)

EA4HP24-BMWS-335

An unusual myelodysplastic progression of essential thrombocytemia, with subsequent short-term blastic transformation

Dr. Edoardo Olmeda[1], Dr. Carlo Pescia[1], Dr. Daniele Cattaneo[3,4], M.Sc./M.A. Anna Chiara Migliorini[3], Dr. Alessandra Iurlo[3], Dr. Giorgio A. Croci[1,2]

[1] Foundation IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy, Division of Pathology, Milano, Italy; [2] University of Milan, Department of Pathophysiology and Transplantation, Milano, Italy; [3] Foundation IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy, Hematology Division, Milano, Italy; [4] University of Milan, Department of Oncology and Hemato-Oncology, Milano, Italy

Case Description

A 65-years-old woman with a past medical history of HCV infection (with entecavir therapy) and paroxysmal atrial fibrillation.

In 2019, following an acute myocardial infarction (inferior STEMI) blood tests revealed thrombocytosis (970'000/mmc), 12'380/mmc WBC and Hb 15 g/dL, with suppressed Epo.

In 2020 a bone marrow biopsy (BMB) was performed, favoring a diagnosis of essential thrombocythemia (ET) with JAK2 V617F mutation.

Due to thrombocytosis and recent AMI she started a dual antiplatelet therapy (DAPT) with cardioaspirin (100 mg/die), ticagrelor (180 mg/die) and hydroxyurea.

In mid 2022, she began with episodes of night sweats and blood tests revealed mild leukocytosis (15'320/mmc; ANC 10'940/mmc; Mo 1,700/mmc, 11.1%), Hb 12.9 g/dL and Plt 229,000/mmc, increased LDH (266 U/l (range 135-214)) and circulating CD34+ blasts (40/mmc). A second BMB and a bone-marrow aspirate (BMA) were performed, providing a picture consistent with a diagnosis of myelodysplastic (MDS)-like progression of essential thrombocythemia.

On January 2023 she progressed to acute myeloid leukemia (AML) confirmed with a BMB.

Biopsy Fixation Details

Tissue sample was fixed with 10% buffered formalin and decalcified with EDTA.

Frozen Tissue Available

No frozen tissue.

Details of Microscopic Findings

The 2020 BMB highlighted numerous and hyperlobated megakaryocytes, with giant forms and normal myeloid and erythroid series, with a cellularity of 40% and 2% of blasts.

In 2022 BMA showed hypercellularity, with left-shifting of myeloid lineage presenting cytoplasmic hypogranularity (69%) and megaloblastosis of erythroid series (19%).

A MDS-like picture was subsequently confirmed on bone-marrow biopsy, highlighting increased cellularity, mild dyserythropoiesis, and prevalence of immature forms in the myeloid series (M/E=6/1) with no fibrosis (MF-0). The blasts, both in the BMA and BMB, were 4%.

The 2023 BMB revealed a percentage of blasts of 40%, proving diagnostic of AML.

Immunophenotype

At AML progression, myeloid blast displayed a CD34+/CD117+/CD33+/MPO+/CD7+(60%)/sCD3-/CD19-/CD10-/TdT- phenotype.

Cytogenetics

Karyotype was normal - 46, XX [20] - at all time of evaluation.

Molecular Studies

In 2020 JAK2 V617F mutation was detected (PCR), with CALR wild-type.

In 2022 next generation sequencing was performed on peripheral blood (Oncomine® Panel).

JAK2 (VAF 42,92%), TET2 (VAF of 41,91% and 38,92%), and RUNX1 (VAF 9,30%) mutations were detected.

No BCR::ABL1 gene fusion was observed.

Proposed Diagnosis

Evolution of essential thrombocythemia into myelodysplastic syndrome/neoplasm, rapidly evolving into acute myeloid leukemia.

Interesting Feature(s)

The presented case offers an intriguing perspective on myelodysplastic evolutions of essential thrombocythemia, with «high-risk progression» mutations. The identified mutations (RUNX1) have demonstrated a clear association with a rapid progression towards acute myeloid leukemia, underscoring the importance of comprehensive genetic assessment in these patients. These findings highlight the diagnostic and therapeutic challenges associated with such evolution, emphasizing the importance of personalized and timely management strategies.

The case further confirms the heterogeneity of MPNs and underscores the complexity of its genetic interactions, providing significant insights for further studies on pathogenesis and therapeutic options.

Panel Diagnosis

Essential thrombocythemia (ICC 2022 / WHO-5) with MDS/MPN-type of progression and transformation to AML

EA4HP24-BMWS-343

Acute B-lymphoblastic leukemia with previous history of ET and follicular lymphoma

Caroline Hesselager, Maysaa Abdulla

Uppsala University Hospital, Uppsala, Sweden

Case Description

The patient is an 81-year-old woman who presented with thrombocytosis in 2012 and a diagnosis of MPN was made based on morphological findings of bone marrow biopsy and genetic findings. In 2022 the patient presented with lymphadenopathy and a diagnosis of low-grade follicular lymphoma was verified on biopsy from a supraclavicular lymph node. In 2023 she presented with anemia, thrombocytopenia, and high WBC. A diagnosis of B-ALL was made based on morphological and flow cytometry findings of bone marrow biopsy. In addition, prominent multilineage dysplasia was detected

Biopsy Fixation Details

4% buffered formalin.

Frozen Tissue Available

Bone marrow from 2023.

Details of Microscopic Findings

Bone marrow biopsy in 2012: 14 mm long with 40% cellularity and marked proliferation of megakaryocytes with domination of large megakaryocytes with abundant cytoplasm and hyper segmented nuclei. A few loose clusters of a few megakaryocytes were seen. No prominent increase of reticulin fibers. Findings were consistent with myeloproliferative disease; mainly Essential thrombocythemia.

Lymph node biopsy in 2022: morphological findings suggest a picture of low-grade follicular lymphoma.

Bone marrow biopsy in 2023: peripheral blood with 24% blasts, bone marrow smear with 78% blasts. In addition, there were features of dysplasia among erythropoiesis, granulopoiesis and megakaryocytes in this bone marrow biopsy and in the following bone marrow smears and biopsies taken as part of follow up.

Immunophenotype

Lymph node biopsy in 2022: immunohistochemical analysis showed positive expression of CD20, CD79a, CD10, BCL6 and BCL2.

Bone marrow biopsy in 2023: flow cytometry shows a leukemic cell population of 82% with positive expression of CD19, CD10, CD34, TDT, icCD79b and no expression of MPO, CD33 and CD117.

Cytogenetics

Bone marrow sample from 2023 displays two separate clones; 16 out of 27 analyzed cells shows a complex karyotype including del(5q) and monosomy 7 and 10 separate cells shows near-triploidy karyotype.

Molecular Studies

Allele specific PCR in peripheral blood 2012 showed a JAK2.pV617F mutation.
In the bone marrow sample from 2023 FISH-analysis shows no BCR::ABL1 rearrangement and no KMT2A (MLL)/11q23 rearrangement. A myeloid NGS panel was performed on CD15-positive and CD19-positive cell fractions respectively. Pathogenic variants were found commonly for DNMT3A, JAK2p.V617F, TET2 and TP53. The CD15-positive cell fraction also harbored pathogenic/probable pathogenic variants in KDM6A and MPL and the CD19-positive cell fraction showed a pathogenic variant in the PTPN11 gene.

Proposed Diagnosis

MPN in 2012, follicular lymphoma in 2022, B-ALL and multilineage dysplasia in 2023.

Interesting Feature(s)

The patient developed different hematological neoplasms in the past 11 years. It would be interesting to investigate clonal development and possible clonal relation between these diseases and evaluate the possibility of transformation of myeloproliferative disease or follicular lymphoma to acute lymphoblastic leukemia.

Panel Diagnosis

Essential thrombocythemia (ICC 2022 / WHO-5) and (probable transformation of a follicular lymphoma to) acute lymphoblastic B-cell leukemia

EA4HP24-BMWS-382

Exploring the Significance of Positive JAK2 V617F in a 5q Deletion Myeloid Neoplasm with Myelodysplastic and Myeloproliferative Overlap Features: A Case Report"

PhD/MD Samah Kohla[1,2], Dr. Imran N. Ahmad[1], Dr. Jassim A. Khan[3], Dr. Amna Gameil[3], Dr. Suzanna El Akiki[4,2], Dr. Aliaa Amer[1], Dr. Sarah Elkourashy[3,2], Dr. Feryal Ibrahim[1], Dr. Samah Kohla plays the role of the principal author, taking responsibility for diagnosing the case, composing the abstract, and submitting the report. Dr. Imran Ahmad played a contributory role in the diagnosis, while Dr. Jassim Khan participated in writing the manuscript. Dr. Amana Gamil contributed to the clinical aspects, and Dr. Susanna El Akiki conducted the molecular analysis. The manuscript underwent review by Dr. Feryal Ibrahim, Dr. Aliaa Amer, and Dr. Sara ElKourashy.

[1] Hamad Medical Corporation, Department of Lab Medicine and Pathology, Hematology Division, NCCCR, Doha, Qatar; [2] Weill Cornell Medicine, Qatar, Doha, Qatar; [3] Hamad Medical Corporation, Department of Hematology, NCCCR, Doha, Qatar; [4] Hamad Medical Corporation, Department of Lab Medicine and Pathology, Cytogenetic and Molecular Division, Doha, Qatar

Case Description

A 62-year-old man, who underwent a total thyroidectomy in 2004 due to papillary thyroid cancer, is currently taking Levothyroxine. He was referred to the hematology department due to persistent and significantly elevated platelet levels. His complete blood count (CBC) revealed a hemoglobin (HgB) level of 9.2 g/dL, a white blood cell count (WBC) of 8.4×10^3/uL, and a platelet count of 1,244×10^3/uL.

Biopsy Fixation Details

Paraffin-embedded biopsy fixed in AZF

Frozen Tissue Available

NA

Details of Microscopic Findings

The Peripheral smear showed moderate normochromic normocytic anemia. Leukocytes were left shifted with few dysplastic neutrophils, basophilia, and <1 blasts. Platelets were markedly increased with many giant forms, few large bare nuclei and megakaryocytic fragments were also seen. Differential count revealed (Neutrophils 53%, Myelocytes 1%, Monocytes 1%, Basophils 6%, Eosinophils 4%, Lymphocytes 34% and Blasts <1%). Bone marrow (BM) aspirate and touch were significantly hemodilute showed < 2% blasts. BM biopsy was hypercellular (90%) with increased trilineage hematopoiesis. Megakaryocytes are markedly increased with anisocytosis, clustering, atypia, and pleomorphism, displaying a combination of small to medium forms with hypolobated/monolobated nuclei and large forms with hyperlobated nuclei. BM sinusoids were dilated with intrasinusodial hematopoesis. Minimal to mild reticulin fibrosis was noted (MF1/3) while trichrome was negative Iron Stain showed 29% ring sideroblasts.

Immunophenotype

NA

Cytogenetics

FISH analysis for BCR/ABL1 is normal.

Karyotype: 46,XY,del(5)(q22q35),del(11)(q23)[25].G- banding chromosome analysis revealed an abnormal clone in all metaphases with an interstitial deletion of chromosome 5 at cytogenetic bands 5q22q35, along with deletion of chromosome 11 at 11q23.

Molecular Studies

Positive for the JAK2 V617F missense mutation, consistent with a diagnosis of MPN. NGS diagnostic workup of 76 genes associated with myeloid neoplasms was evaluated and the following were the significant single nucleotide missense variants identified showed *SF3B1 (31%), JAK2(30%), and TP53 (33%).*

Proposed Diagnosis

Myelodysplastic neoplasm with low blasts and isolated 5q deletion (MDS-5q), positive for JAK2 V617F mutation.

Interesting Feature(s)

The overall findings were consistent with the WHO diagnostic criteria of MDS with isolated 5q deletion. Our panel has reached this diagnosis with reference to the WHO's recent classification of haematological tumors where a small fraction of MDS with isolated 5q deletion are associated with ***JAK2 V617F*** positivity having proliferative features in the bone marrow and higher median platelet counts. It is unclear whether their clinical presentation or prognosis differs from that of the MDS with isolated del(5q) and wild-type JAK2. Owing to the paucity of data in this regard, the differential diagnosis would include the pre-fibrotic stage of primary myelofibrosis and MDS/MPN NOS. Until more evidence is published, it is recommended by WHO that these cases with combined del(5q) and JAK2 p.V617F mutation be classified as MDS with isolated del(5q) rather than included in the MDS/MPN-NOS category.

Panel Diagnosis

Myelodysplastic/myeloproliferative neoplasm with thrombocytosis and SF3B1 mutation [and del(5q), JAK2 and TP53 mutation]

EA4HP24-BMWS-391

Ras-associated autoimmune leukoproliferative disorder (RALD): A patient with large cell transformation of mycosis fungoides, plasma cell neoplasm and persistent monocytosis

Dr. C. Cameron Yin, Dr. Paul Young, Dr. M. James You, Dr. Jie Xu, Dr. L. Jeffrey Medeiros, Dr. Shaoying Li, Dr. Sofia Garces

MD Anderson Cancer Center, Hermatopathology, Houston, USA

Case Description

The patient is a 32-year-old woman with a history of ITP requiring splenectomy at age 16 and mycosis fungoides (MF). She also had persistent monocytosis for nine years. Two years ago, she presented with worsening skin lesions and lymphadenopathy. PET scan revealed increased metabolic activity in the skin, multiple lymph nodes and bone marrow (BM). A complete blood cell count showed leukocytes 9.2 K/uL, hemoglobin 13.2 g/dL and platelets 488 K/uL, with 31% monocytes. A left inguinal lymph node biopsy revealed MF with large cell transformation. BM aspiration and biopsy showed a plasma cell neoplasm and monocytosis.

Biopsy Fixation Details

The BM core biopsy was decalcified and the biopsy and aspirate clot specimens were fixed in formalin.

Frozen Tissue Available

No

Details of Microscopic Findings

The BM core biopsy and aspirate smear from two years ago showed normocellular (50%) BM with trilineage hematopoiesis, increased plasma cells (12%) and increased monocytes (15%). A more recent follow-up BM sample showed persistent monocytosis, mild dysgranulopoiesis and dyserythropoiesis, and 3% blasts.

Immunophenotype

Immunohistochemical analysis showed increased CD138+ plasma cells (10-15%), with kappa light chain restriction. There were a few scattered CD3+, CD7+ T-cells and CD20+ B-cells. T-cells showed no loss of CD7 expression.

Flow cytometry immunophenotypic analysis revealed a population of aberrant plasma cells; positive for CD38, CD138, CD56 and monotypic cytoplasmic immunoglobulin kappa light chain. No aberrant T-cells were detected. The CD34+ blasts and monocytes were immunophenotypically unremarkable.

Cytogenetics

46,XX[20]

Molecular Studies

Next generation sequencing (NGS) using a panel of 81 genes showed:

DNMT3A c.2512A>G p.N838D (variant allele frequency, VAF, 40.3%)
KRAS c.35G>A p.G12D (VAF, 40.9%)

Proposed Diagnosis

Ras-associated autoimmune leukoproliferative disorder (RALD) in a patient with large cell transformation of mycosis fungoides, plasma cell neoplasm and persistent monocytosis

Interesting Feature(s)

We present a case of RALD in a young patient with concurrent mycosis fungoides with large cell transformation, plasma cell neoplasm and persistent monocytosis. RALD is an autoimmune lymphoproliferative syndrome (ALPS)-related disorder characterized by leukocytosis with absolute monocytosis, BM abnormalities, splenomegaly, lymphadenopathy, autoimmune phenomena and activating somatic mutations in *KRAS* or *NRAS*. Rare patients with RALD develop juvenile myelomonocytic leukemia (JMML) or chronic myelomonocytic leukemia (CMML). While patients with RALD should be carefully monitored, diagnosis of progression to malignancy should be established with caution because the diagnosis of progression may lead to aggressive therapy such as stem cell transplantation. In particular, current diagnostic criteria for CMML are unable to distinguish CMML with a normal karyotype from RALD. Helpful features for diagnosis of an overtly malignant phenotype include acquisition of cytogenetic or molecular abnormalities (e.g. monosomy 7). In the current case, although mild multilineage dysplasia was noted in a follow-up BM specimen, a diagnosis of CMML/JMML was not rendered because of a lack of cytogenetic abnormalities, overt dysplastic features, cytopenias and immunophenotypic aberrancy. The patient remains under close monitoring. Other interesting features in this patient are the co-occurrence of other hematolymphoid malignancies generally known to carry *KRAS/NRAS* mutations as pathogenic drivers including plasma cell neoplasms and mycosis fungoides. Moreover, NGS analysis showed *KRAS* G12D and *DNMT3A* N838D.

Panel Diagnosis

Ras-associated autoimmune leukoproliferative disorder with mycosis fungoides, smoldering myeloma-level clonal plasmacytosis and clonal monocytosis suspect of evolving CMML type 1

EA4HP24-BMWS-395

A rare case of simultaneous coexistence of chronic lymphocytic (CLL) and chronic myeloid leukemia (CML)

Dr. Ibrahim Ganwo[1], Dr. Shehab Mohamed[2], Dr. Dina Soliman[1], Dr. Aliaa Amer[1], Dr. Feryal Ibrahim[1]

[1] HMC, Department of Laboratory Medicine & Pathology, Hamad Medical Corporation, Doha, Qatar; [2] HMC, Department of Hematology-Oncology, Hamad Medical Corporation, Doha, Qatar

Case Description

Our patient is a 44-year-old male patient presented with history lower back pain and loss of weight and found to have moderate leukocytosis. Clinical physical examination yield submandibular lymphadenopathy, otherwise is unremarkable

PAN CT scan showed generalized lymphadenopathy and mild splenomegaly

Biopsy Fixation Details

Parafine embedded bone marrow biopsy fixed in AZF

Frozen Tissue Available

N/A

Details of Microscopic Findings

Peripheral blood smear shows moderate leukocytosis with neutrophilia with shift to the left, basophilia and lymphocytosis with occasional smudge cells. No increase in prolymphocytes.

Bone marrow aspirate is hypercellular with increased granulopoiesis, decreased erythropoiesis, 3% blasts and increased lymphoid cells (33%).

Bone marrow biopsy is markedly hypercellular (~100%) showing granulocytic hyperplasia and decreased erythropoiesis. Megakaryocytes are increased, the majority are dwarf forms. By immunostains CD34 positive cells are estimated 1-2%. The bone marrow is also infiltrated with abnormal lymphoid cells, mostly showing nodular pattern and few marrow areas show interstitial lymphoid infiltration, the lymphoid cells composed of small and mature B-cells positive for CD20, CD79a, PAX-5, CD5, CD23, BCL2, and CD43 by immunostians. The lymphoid infiltrate is roughly estimated at approximately 40% of core cellularity. There is mild increase in the reticulin fibers (MF 1).

Immunophenotype

Flow cytometry on peripheral blood shows a monotypic B-cell population comprising approximately 44% (of total cells) and expressing CD19, CD20 (dim), CD5 (majority), CD23, CD43, CD200, IgD and IgM (majority) with lambda light chain restriction. There is partial expression of CD79b and expression of FMC7 on the minority of monotypic cells (~23%). There is no significant expression of CD10, CD49d and the majority is negative for CD38.

Cytogenetics

Chromosomal analysis on peripheral blood showed abnormal karyotype: 46,XY,t(9;22)(q34;q11.2)[16]/46,XY[25].nuc ish(D13S319x1,13q34x2)[96/200].

FISH analysis for CLL panel on peripheral blood:
The iFISH evaluation was performed on interphase nuclei cells from leukemic blood sample using above mentioned probes from Vysis. The analysis revealed an ABNORMAL hybridization signal pattern with one signal for D13S319 probe indicating deletion at 13q14.3 in 48% of the cells analyzed

Molecular Studies

Positive for an e13a2 BCR::ABL1 gene fusion by single step RT-PCR.

Proposed Diagnosis

Overall findings consistent with simultaneous bone marrow involvement by chromic myeloid leukemia (CML), morphologically in chronic phase and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL)

Interesting Feature(s)

This case was suspected as CLL based on peripheral blood lymphocytosis with some smudge cells on peripheral blood smear, however, the presence of neutrophilia with shift to the left and basophilia raised the susceptibility of co-existence of CML. Chromosomal analysis on peripheral blood confirmed the co-existence of CML.
The presence of simultaneous coexistence of chronic lymphocytic (CLL) and chronic myeloid leukemia (CML) in the same patient is rare and reporting this case emphasize the vital role of a multiparametric approach combining clinical, morphologic, immunophenotypic, and cytogenetic/molecular data to make an accurate diagnosis and initiation of proper management on the right time if needed as in this reported case. This case was suspected as CLL based on peripheral blood lymphocytosis with some smudge cells on peripheral blood smear, hoever, the presnece of neutrophilia with shift to the left and basophilia raised the sus

Panel Diagnosis

Chronic myeloid leukemia and chronic lymphocytic leukemia (ICC 2022 / WHO-5)

EA4HP24-BMWS-401

An unusual orbital granulation tissue

PhD/MD Fanny Drieux[1], M.Sc./M.A. student Noémie Roberto[1], PhD/MD student Sydney Dubois[2], Dr. Lise-Marie Roussel[3], Dr. Pascaline Etancelin[4], Dr. Dominique Penther[4], PhD/MD Victor Bobée[5], Dr. Alexandru Serban[1], PhD/MD Marick Laé[1], Dr. Liana Veresezan[1]

[1] Centre Henri Becquerel, Department of Pathology, Rouen, France; [2] Centre Henri Becquerel, Department of Hematology, Rouen, France; [3] Centre Henri Becquerel, Department of Head and Neck Surgery, Rouen, France; [4] Centre Henri Becquerel, Department of Genetic Oncology, Rouen, France; [5] Rouen University Hospital, Department of Biological Hematology, Rouen, France

Case Description

A 71-year old male with a history of sinonasal adenocarcinoma treated by ethmoidectomy and left orbital exenteration in combination with radiotherapy in 2004, presented recurrent bleeding of the left orbital cavity during the follow up, requiring iterative cauterization. Due to the persistence and majoration of the orbital bleeding in 2023, four biopsies of the orbital cavity revealed an ulcerative granulation tissue, with expansion of vessels. A large resection of the granulation tissue was finally performed, with a clinical suspicion of lymphoma or a relapse of adenocarcinoma.

Biopsy Fixation Details

We received a dozen of fresh fragments, fixed in formalin 4% and embedded in ten paraffin blocks.

Frozen Tissue Available

Two fragments of frozen tissue are available.

Details of Microscopic Findings

Microscopic analysis showed ulcerative granulation tissue among an hemorrhagic background. Vessels were surrounded by a proliferation of medium and undifferentiated atypical cells, showing irregular nuclei and prominent nucleoli, among sheets of polynuclear cells. There were also scarce and large atypical cells, initially interpreted as dystrophic fibroblasts in the post-radiotherapy context.

Immunophenotype

By immunohistochemistry, the medium cells were positive for ERG, MPO and showed a high KI67 proliferative index. They were negative for vascular (CD34, CD31), lymphoïd (CD20, PAX5, CD79a, CD3, CD5, CD2, CD7) or epithelial (AE1/AE3, MNF116, EMA) markers, and no expression of EBER transcripts. Further exhaustive immunohistochemistry study revealed expression of CD15 and lysozyme by the medium cells but no expression of CD117, CD68, TdT or CD99, while the scarce and large atypical cells were CD42B positive corresponding to polymorphic megakaryocytes. There were also clusters of erythroblastic cells, also highlighted by glycophorin and E-cadherin.

Regarding the presence of the orbital localization of hematopoietic cells, a retrospective verification of clinical parameters revealed hyperleukocytosis (45G/L), increased

neutrophils (31G/L), anemia (11g/dl), thrombopenia (120G/L) and persistent monocytosis (9.1G/L, 20%). LDH were elevated.
In this context, a diagnosis of myeloid neoplasm was suggested, probably « chronic myelomonocytic leukemia ».

Cytogenetics

The karyotype performed from the fresh materiel after 16 hours of culture, was normal (46, XY[20]).

Molecular Studies

This hypothesis was further supported by the results of the targeted sequencing performed on the FFPE biopsy (Qiaseq technology, NextSeq), detecting mutations of *SRSF2* (p.P95H 46%), *TET2* (2 mutations, 41 and 46%) and *NRAS* (p.Y46D 42%).

Proposed Diagnosis

The final proposed diagnosis was "chronic myelomonocytic leukemia ».

Interesting Feature(s)

This case illustrates the necessity of genetic studies to refine a specific myeloid neoplasm diagnosis, and emphasizes the importance of clinical parameters in the interpretation of a well known but unusual localization of myeloid cells. Moreover, it also illustrates a potential pitfall of ERG expression by immature granular cells, which may be misinterpreted in a routine workflow by general pathologists.

Panel Diagnosis

Myeloid sarcoma (ICC 2022 / WHO-5) in CMML-MP, radiotherapy-related (ICC 2022) / MP-CMML, post radiotherapy (WHO-5)

EA4HP24-BMWS-405

Myelodysplastic/myeloproliferative neoplasm with *NPM1*, *DNMT3A* and *NOTCH3* mutations evolving into acute myeloid leukemia in an adolescent patient

Dr. Robert Lorsbach[1], Dr. David Grier[1], Dr. Somak Roy[1], Dr. Teresa Smolarek[2], Dr. Benjamin Mizukawa[3]

[1] Cincinnati Children's Hospital Medical Center, Pathology & Laboratory Medicine, Cincinnati, USA; [2] Cincinnati Children's Hospital Medical Center, Genetics, Cincinnati, USA; [3] Cincinnati Children's Hospital Medical Center, Hematology/Oncology, Cincinnati, USA

Case Description

A 16-year-old female presented to our ED with a 1 month history of nausea/vomiting, headache, light-headedness, and dizziness. Physical examination revealed only pallor with no evidence of lymphadenopathy or hepatosplenomegaly.

CBC showed leukocytosis (WBC 36.6 K/uL), anemia (hemoglobin 2.7 g/dL/hematocrit 8.1%), and platelets of 65 K/uL. Peripheral blood (PB) differential (500 cell count): 75% neutrophils, 5% immature granulocytes, 1% eosinophils, 8% monocytes, 2% blasts, and 9% lymphocytes.

Biopsy Fixation Details

PB was sent for flow cytometry (FC). Fresh bone marrow (BM) aspirate at diagnosis and 3 weeks later was sent for FC and ancillary studies. The BM biopsy was fixed in formalin and decalcified.

Frozen Tissue Available

None

Details of Microscopic Findings

The PB at diagnosis showed neutrophilic leukocytosis with profound anemia and thrombocytopenia. Many of the neutrophils were dysplastic and included forms with nuclear hyperlobation and fewer hypogranulated forms; immature myeloid cells comprised 5% of leukocytes, and blasts accounted for 2% of cells. The BM was 100% cellular with myeloid hyperplasia and left-shifted maturation (myeloid:erythroid ratio, 11:1). Dysplastic megakaryocytes were present. Blasts were not increased in H&E sections. A second BM obtained 3 weeks later revealed 25% blasts.

Immunophenotype

FC identified a CD117+/CD34- myeloid blast population in PB and BM accounting for 3.2% and 5.5% of analyzed cells, respectively. These blasts expressed CD13, CD33, HLA-DR, moderate CD45 and had low-moderate side scatter; they were negative for CD11b, CD14, CD64, and all other antigens evaluated. The CD117+/CD34- immunophenotype was confirmed by immunohistochemistry. FC performed on a subsequent BM identified 29% blasts.

Cytogenetics

Metaphase cytogenetics yielded a normal female karyotype: 46,XX[20]. FISH studies were negative for numeric or structural abnormalities of chromosomes 5/5q, 7/7q, 8, 12p13.2 (*ETV6*), 17p13.1 (*TP53*) or 20q; no evidence of *BCR::ABL1*, *KMT2A*, *RUNX1::RUNX1T1*, *CBFB::MYH11*, *DEK::NUP214*, *NUP98*, *ETV6*, *FIPL1::PDGFRA*, *PDGFRB*, or *FGFR1* fusions or rearrangements. Chromosome microarray detected no clinically significant acquired copy number changes or loss of heterozygosity identified

Molecular Studies

DNA NGS identified several abnormalities (VAF in parentheses): *NOTCH3*, 2565delC, N856fs*4 (50.63%); *NPM1*, 863_864insTCTG, W288fs*12 (38.51); *DNMT3A*, 2645G>A, R882H (47.36%); *NRAS*, 37G>C, G13R (25.42%); *NRAS*, 35G>C, G12A (2.4%), *GATA2*, 913_914insGG, L305fs*22 (1.74%). multiple *FLT3* mutations (each < 3.5%). Subsequent whole exome sequencing performed on skin fibroblast DNA confirmed that the *NPM1*, *DNMT3A* and *NOTCH3* mutations were somatic.

Proposed Diagnosis

Myelodysplastic/myeloproliferative neoplasm NOS with *NPM1*, *DNMT3A*, and *NOTCH3* mutations, evolving into acute myeloid leukemia.

Interesting Feature(s)

The peripheral blood and bone marrow findings at diagnosis raised a broad differential diagnosis that included CML, atypical CML, chronic neutrophilic leukemia and other myeloproliferative neoplasms. Myelodysplastic/myeloproliferative neoplasms (MDS/MPN) are very rare in the pediatric population, and a prodromal phase of MDS/MPN is unusual for AML with *NPM1* mutations.

Panel Diagnosis

Acute myeloid leukemia with mutated NPM1 (ICC 2022 / WHO-5) evolving from (aCML-like) MDS/MPN, NOS (ICC 2022)

EA4HP24-BMWS-411

MDS/MPN - using mutation analysis

Dr. Lakshmi Venkatraman[1], Dr. Michelle Moore[2], Dr. Nicholas Cunningham[3], Dr. Graeme Greenfield[3], Dr. Amy Logan[5], Dr. Kathryn Clarke[3], Dr. Mark Catherwood[4,5], Dr. Lakshmi Venkatraman[1]

[1] Belfast Health and Social Care Trust, Cellular Pathology, BELFAST, UK; [2] Belfast Health and Social Care Trust, Cellular Pathology, BELFAST, UK; [3] Belfast Health and Social Care Trust, Haematology, BELFAST, UK; [4] Belfast Health and Social Care Trust, Haematology, BELFAST, UK; [5] Belfast Health and Social Care Trust, Regional Molecular Diagnostic Service, BELFAST, UK

Case Description

A 63 y old male presented in October 2023 with a history of shortness of breath and dry cough. His blood count showed anaemia, leucocytosis and thrombocytopenia. Blood film showed 58% neutrophils with immature myeloid cells, absolute but not relative monocytosis. He had no organomegaly or adenopathy.

Biopsy Fixation Details

FFPE, EDTA decalcification. 10% buffered formalin

Frozen Tissue Available

No

Details of Microscopic Findings

Markedly hypercellular (more than 90%)marrow with myeloid hyperplasia and left shift, relative decrease in erythroid cells and few normal as well as dysplastic megakaryocytes. Full range of maturation.

Immunophenotype

No increase in CD34 positive blasts. CD15 highlights the maturing myeloid cells. CD61 demonstrates variation in megakaryocyte size.

Cytogenetics

47, XY +8[6]/ 46XY[14]

Three copies of FGFR1. No rearrangements of BCR::ABL1, FGFR1, PDGFRA, PDGFRB, ETV6, JAK2.

Molecular Studies

JAK2 negative, BCR::ABL negative

EZH2: VAF 93%

IDH2: VAF 43%

RUNX1: VAF 41%

No SETBP1, CSF3R, SETD2 or ETNK1 mutations.

Proposed Diagnosis

Atypical CML- ICC 2022

MDS / MPN with neutrophilia who 2022

Interesting Feature(s)

•Morphology suggestive of Atypical CML/ MDS MPN with neutrophilia

•Molecular findings: absence of SETBP1, ETNK1

•- the mutations identified are not specific for any myeloid neoplasm

•- EZH2, IDH2, RUNX1 all more common in aCML/MDS-MPN with neutrophilia than CNL

•- EZH2 mutations also usually exclusive of CSF3R OR SETBP2

•- high risk of transformation to AML and poor prognostic mutation group

•- EZH2 93% implies LOH, is the primary event; other two mutations are secondary events

•- NGS did not identify the +8 seen on cytogenetics

Panel Diagnosis

Atypical chronic myeloid leukemia (ICC 2022) / Myelodysplastic/myeloproliferative neoplasm with neutrophilia (WHO-5)

EA4HP24-BMWS-428

The concurrent presence of plasma cell myeloma and a JAK2 V617F-positive hypoplastic fibrotic myeloid neoplasm with dysmegakaryopoiesis presents a diagnostic challenge.

PhD/MD Samah Kohla[1,2], Dr. Deena Mudawi[3], Dr. Sarah Elkourasy[3,2], Dr. Amna Gameil[3,2], Dr. Jassim Khan[3], Dr. Dina Soliman[1,2], Dr. Aliaa Amer[1], Dr. Shehab Fareed[3], Dr. Ferayl Ibrahim[1], Dr. Samah Kohla took on the role of the main author, overseeing the case diagnosis and abstract drafting. Dr. Deena Mudawi, Dr. Sarah Elkurashy, Dr. Amna Gameil, and Dr. Shehab Fareed contributed to the clinical aspects and reviewed the manuscript. Furthermore, Dr. Jassim Khan, Dr. Dina Soliman, Dr. Aliaa Amer, and Dr. Feryal Ibrahim were involved in reviewing the manuscript.

[1] Hamad Medical Corporation, Lab Medicine and Pathology Department, Hematology Division, NCCCR, Doha, Qatar; [2] Weill Cornell Medicine Qatar, Doha, Qatar; [3] Hamad Medical corporation, Clinical Hematology Department, NCCCR, Doha, Qatar

Case Description

A 74-year-old male patient known case of multiple comorbidities, including, DM, dyslipidemia, CKD, and compensated heart failure on medications, has a history of successfully treated HCV infection in 2018. In 2015, he was diagnosed with IgG Lambda MGUS. He was admitted due to symptomatic anemia and thrombocytopenia In April 2023. The CBC revealed marked anemia with hemoglobin of 5.6 gm/dl, moderate thrombocytopenia with platelets of 55 x 10^3, and normal WBCs and ANC at (4.4 and 202 x10^3/ul respectively). SPE and immunofixation showed IgG lambda monoclonal band (8 g/L) with increased KaFLC and LaFLC and normal K/R ratio. DAT was positive attributed to medications and/or underlying disease. Bone marrow (BM) examination was performed.

Biopsy Fixation Details

BM biopsy fixed with AZF

Frozen Tissue Available

NA

Details of Microscopic Findings

Peripheral blood showed marked anemia, mild reticulocytosis, increased rouleaux, many NRBCs, few with dysplastic features (megaloblastic), leukoerythroblastic picture, dysgranulopoietic features (hypo/non-segmentation and hypogranulation), 2% blasts, and moderate thrombocytopenia. BM aspirate was dry tap. Touch was non-informative. BM biopsy was markedly hypocellular (5-10%) with markedly decreased trilineage hematopoiesis and some morphologically atypical megakaryopoiesis with a significant subset of small forms with hypolobular nuclear features with no significant clusters. There was increased fibrosis with diffuse loose thin bundles of collagen

deposition (MF2-3). There was a relative increase in background plasma cells, monocytes/histiocytes, and T-lymphocytes.

Immunophenotype

Immunohistochemistry showed no increase in CD34-positive cells. CD138 highlighted increased plasma cells comprising (~10-20% of total cellularity) with lambda restriction and aberrant CD117, CD56, and positive for Cyclin-D1. CD61 & VWF highlighted decreased megakaryocytes with atypical small and hyoplobated forms. CD68 &CD163 highlighted scattered interstitial histiocytes with occasional phagocytic activity. CD3 highlighted scattered interstitial small lymphocytes as well as focal small apparent prevascular aggregates of small T lymphocytes. MPO highlighted the marked depression of granulopoiesis. E-cadherin & GPA showed residual collections of erythroid cells. Cytokeratin was negative.

Cytogenetics

Not done

Molecular Studies

Positive for JAK2 V617F gene mutation.

Proposed Diagnosis

-Markedly hypocellular BM with panhypoplasia and atypical megakaryocytes. No increase in blasts. Grade 2 fibrosis (MF-2).

-Plasma cell myeloma, lambda light chain restricted (10-20%) of BM cellularity.

Interesting Feature(s)

This is a case of plasma cell neoplasm for correlation with radiologic studies and SPE, the plasma cells are Cyclin D1 positive. The BM is atypically hypocellular and showed features of dysmegakaryopoiesis and fibrosis. The patient has a positive JAK2 V617F gene mutation. Further evaluation of a primary myeloid neoplastic process is difficult given the lack of BM aspirate material and the marked hypocellularity of BM biopsy. The presence of reticulin fibrosis and apparent megakaryocytic dysplasia raises the possibility of myeloid neoplasm, which is supported by the positive JAK2 gene mutation, however further subclassification is not possible in this specimen. Possibilities could include an MPN with end-stage hypoplastic and fibrotic features or a hypocellular MDS or atypical MDS/MPN. Given the inability to perform cytogenetic analysis, NGS for myeloid neoplasm should be requested. Exclusion of secondary causes for marrow failure such as drugs/toxins or autoimmune disease is also suggested.

Panel Diagnosis

(Overt) primary myelofibrosis ((ICC 2022 / WHO-5), spent phase and multiple myeloma

EA4HP24-BMWS-429

Bone marrow findings of chronic myeloid leukemia with concurrent *BCR-ABL* fussion and *JAK2* V617F mutation at diagnosis

Dr. Francisco Javier Diaz de la Pinta[1], Dr. Rebeca Manso[1], Dr. Carlos Soto[2], Dr. Daniel Naya Errea[3], Dr. Alvaro Arriero Garcia[2], Dr. Sara Perlado Marina[2], Dr. Mireia Atance[2], Dr. Rocio Nieves Salgado[2], Dr. Juan Manuel Alonso Dominguez[2], Dr. Carlos Blas Lopez[2], Dr. Socorro Maria Rodriguez-Pinilla[1]

[1] FUNDACION JIMENEZ DIAZ UNIVERSITY HOSPITAL, PATHOLOGY, MADRID, Spain; [2] FUNDACION JIMENEZ DIAZ UNIVERSITY HOSPITAL, HEMATOLOGY, MADRID, Spain; [3] INFANTA ELENA HOSPITAL, HEMATOLOGY, VALDEMORO, Spain

Case Description

A 48-year-old-woman came to our hospital due to thrombocytosis during last two years and alternating leukocytosis. At first visit, thrombocytosis was detected at blood test (564 x 10^3/µl) with mild leukocytosis (14 x 10^3/µl), and subsequent bone marrow biopsy was performed. Hemoglobin level was not altered. Patient did not have any previous clinical history of interest

Biopsy Fixation Details

Paraffin embeded tissue. Formol 10%

Frozen Tissue Available

Peripheral blood available at hematology department

Details of Microscopic Findings

Bone marrow smears showed slightly increased cellularity with an increase of megakaryocytic lineage with abnormal morphology (most hypolobated nuclei but also presence of hyperlobated-shaped typical of essential thrombocythemia). Bone marrow biopsy was also slightly hypercellular with markedly increased megakaryocytes at centromedullar location. Dwarf hypolobated megakaryocytes were predominant but there were also large hyperlobated megakaryocytes with loose clustering and occasional staghorn-like nuclei. Myeloid and erythroid cells did not present dysplastic morphology, myeloid:erythroid (M:E) ratio was preserved (3:1) and there were no increase of CD34+ blasts. Reticulin fibrosis was not increased either (MF0).

Immunophenotype

Flow cytometry study did not detect abnormal phenotype of hematopoietic cells. By immunohistochemistry, CD61 highlighted hyperplastic and diverse morphology of megakaryocytic lineage, while CD71 and MNDA confirmed preserved M:E ratio

Cytogenetics

Deletion of 5q and was not detected by FISH study (Metasystem)

Conventional cytogenetic study on bone-marrow showed a karyotype with the following formula: 46,XX,t(9;22;17)(q34;q11.2;q21)[9]/46,XX[2], with presence of triple translocation

involving chromosomes 9, 22 and 17. This included the reciprocal translocation t(9;22)(q34;q11.2)

Molecular Studies

Initial molecular studies performed in peripheral blood detected *JAK2* V617F mutation by PCR (VAF: 0.37%).

t(9;22)(q34;q11.2) was subsequently confirmed by FISH study (Abbot Molecular).

Proposed Diagnosis

At hematology department a differential diagnosis between chronic myeloid leukemia (CML) and myelodysplastic neoplasm with 5q deletion was initially suggested, based on predominant hypolobate megakaryocytic morphology.

At pathology department a diagnosis of chronic myeloproliferative neoplasm (MPN) was made and it was recommended to correlate with molecular and cytogenetic findings. Following these, case was finally discussed at mutlidisciplinary committee and a definitive diagnosis of CML with concurrent *BCR-ABL* fusion and *JAK2* V617F mutation was made

Interesting Feature(s)

MPNs with concurrent *JAK2* V617F and *BCR-ABL* fusion are estimated to have a prevalence rate of 0,4% among all MPNs tested for both genomic alterations.

Our case highlights how in these cases clinical and morphological features tend to correlate with clonal composition with leukocytosis and presence of small hypolobate megakaryocytes corresponding to *BCR-ABL* fusion and onset of thrombocytosis and hyperlobated megakaryocytes with preserved M:E ratio due to *JAK2* V617F clone. When *BCR-ABL*+ CML is simultaneously present in conjunction with *JAK2* V617F, it seems to play a dominant role with the latter. Interestingly, previous reports suggest that clearance of the dominant *BCR-ABL* clone with tyrosine kinase inhibitors permits *JAK2* clone to exhibit its clinicopathological phenotype with increase of platelet count and bone marrow histomorphology of essential thrombocythemia or myelofibrosis, so it is important to be aware of this genetic combination to avoid misinterpretations.

Panel Diagnosis

Chronic myeloid leukemia and JAK2 V617F+ CHIP (ICC 2022 / WHO-5)

EA4HP24-BMWS-432

CALR mutated myeloid neoplasm with features of myeloproliferative neoplasm and myelodysplastic syndrome (neoplasm)

Dr. Rashmi Kanagal-Shamanna, Dr. Tariq Muzzafar, Dr. Sa A. Wang, Dr. Carlos Bueso-Ramos

MD Anderson Cancer Center, Hematopathology, Houston, USA

Case Description

A 76 y.o. male with a history of DLBCL with testicular involvement s/p R-CHOP and radiation (2012), MDS with 9% blasts, and diploid cytogenetics (diagnosed outside 3/2018), post treatment with azacitidine with no response.

Biopsy Fixation Details

Decalcified biopsy; formalin fixation

Frozen Tissue Available

Yes

Details of Microscopic Findings

RBCs are decreased in number with anisopoikilocytosis; neutrophils show hypogranular cytoplasm. Circulating blasts are noted (~6%). Platelets are decreased and include large hypogranular forms.

Bone marrow biopsy shows small sub-cortical fragments with variably cellular (~50%) marrow. Megakaryocytes are markedly increased in number (seen in diffuse sheets) and are dysplastic (small hypolobated forms). Immature cells are increased in the background. There is increased fibrosis. Bone marrow smears are aparticulate. There is dysgranulopoiesis. Erythroid precursors are decreased. Megakaryocytes are dysplastic (small hypolobated forms). Blasts represent ~13% of the cells.

Iron stain: Absent ring sideroblasts.

Reticulin: Diffuse increase, coarse fibers; Trichrome: Multifocal interstitial collagen deposits

Grade: MF-2

Immunophenotype

CD34: Highlights increased blasts (up to 19% of total cells)

CD61: Sheets of megakaryocytes

Flow cytometry: Increased myeloblasts

Cytogenetics

45,XY,der(6;7)(p10;q10)[18]/46,XY[2]This results in a loss of 7p and 6q.

Molecular Studies

NGS: Positive for mutations in CALR (type 3, described in MPNs in the literature; VAF 35%), U2AF1 (27%), ASXL1 (19%), and SETBP1 (x2, 15%, <2%)

FLT3: Negative for internal tandem duplication and D835 mutation
Leukemia translocation (RNA-based screen for selected fusions) panel: Negative

Proposed Diagnosis

The findings are diagnostic of an advanced phase myeloid neoplasm showing myelofibrosis, dysgranulopoiesis, dysmegakaryopoiesis (myelodysplasia). In the presence of a dominant MPN driver (frame-shift CALR mutation), the findings are best regarded as an advanced phase myeloproliferative neoplasm (MF-2) progressing with myelodysplastic features. CALR K374fs (type 3) mutation has been reported in MPNs in multiple publications. CALR mutations in MDS and MDS/MPN (other than RARS-T) are rare.
In the setting of treated MDS, post cytotoxic therapy/ therapy-related and to enable clinical trial enrollment, the final diagnosis was issued as below:
MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASM, UNCLASSIFIABLE WITH MYELOFIBROSIS (MF-2), *CALR* MUTATED (POST-CYTOTOXIC THERAPY/ THERAPY RELATED)

Interesting Feature(s)

Categorizing an advanced-phase myeloid neoplasm based on molecular features can be extremely challenging.
In this case, the diagnosis was between MDS (morphology, and especially in the setting of a previously treated MDS) and MPN (CALR mutation at a high frequency).
Points in favor of MPN:

- Myelofibrosis (MF2)
- *CALR* mutated [CALR mutations in MDS and MDS/MPN (other than RARS-T) are extremely rare]. This type 3 *CALR* mutation, K374fs has been reported in MPNs.
- Karyotype der(6;7) results in loss of 7p (not 7q or monosomy 7 typical of MDS)
- Other gene mutations (*U2AF1, ASXL1, SETBP1*) are frequent in progressing MPN and don't signify much in the presence of an MPN driver mutation.
- The absence of *TP53* mutation is unusual in t-MDS or MDS with fibrosis (this case).

Points in favor of MDS

- Anemia (however, upon detailed review, pt. has a documented history of GI bleeding explainign severity).
- Absence of cytosis
- History of cytotoxic therapy, sheets of dysplastic megakaryocytes
- Presence of "dysgranulopoiesis" and "dysmegakaryopoiesis

Panel Diagnosis

Myelodysplastic neoplasm with fibrosis post radio-chemotherapy (WHO-5), MDS/AML, radio-chemotherapy-related (ICC 2022)

EA4HP24-BMWS-442

Progression of MPL-mutated myeloproliferative neoplasm with bi-allelic *TP53* mutations

Dr. Shikha Malhotra, Dr. Sarah Ondrejka, **Dr. Megan O. Nakashima**

Cleveland Clinic, Laboratory Mediciine, Cleveland, USA

Case Description

The patient is an 83-year-old woman who was reportedly diagnosed with essential thrombocythemia in 2005. In 2014 a bone marrow biopsy was reported with megakaryocytic atypia including large size with hyperlobated nuclei. FISH was negative for *BCR::ABL1* and molecular testing was negative for *JAK2* V617F and exon 12-15 mutations and *CALR* mutation, but positive for *MPL* W515L mutation (VAF 5.3%). Karyotype was 46,XX[20]. The patient was started on hydroxyurea, with which she maintained a platelet count between 300-500 x 10^9/L. In December of 2023, she was found to have new pancytopenia (HGB 7.8 g/dL, WBC 2.78 x 10^9/L, PLT 37 x 10^9/L). A bone marrow biopsy was performed.

Biopsy Fixation Details

Fixed in zinc-formalin. Trephine EDTA-decalcified.

Frozen Tissue Available

None

Details of Microscopic Findings

2023: Aspirate smears showed erythroid hyperplasia and left-shift with 17% blasts and significant dyserythropoiesis including multinucleation, nuclear budding/fragmentation, and megaloblastoid or giant forms. Ring sideroblasts were also present (15%). Blasts were increased (17% on differential count. There was also some dysgranulopoiesis (nuclear hypersegmentation or cytoplasmic hypogranularity) and small, hypolobated megakaryocytes. The core biopsy was hypercellular (80-90%) due to panhyperplaisa. With erythroid hyperplasia and left-shift. There were also increased micromegakaryocytes, without tight clustering. 2014 marrow has been requested for comparison.

Immunophenotype

Erythroid precursors were positive for CD71 and subset-positive for e-cadherin. Blasts showed subset CD34 positivity. Megakaryocytes were highlighted with CD61.

Cytogenetics

43~45,XX,add(1)(p32)[5],add(2)(p11.2)[4],add(3)(q21)[3],add (5)(q11.2),-8[5],-17,-19[3],add(19)(q13.3)[4],-20[3],-21[5],-22[4],+1- 6mar[cp8]/46,XX[13]

Molecular Studies

MPL p.W515L, NM_005373.2, c.1544G>T VAF: 31.9%.

TP53 p.R213*, NM_000546.5, c.637C>T VAF: 62.6%

Proposed Diagnosis

High grade myeloid neoplasm, favor accelerated phase myeloproliferative neoplasm

Interesting Feature(s)

This is a high grade myeloid neoplasm with trilineage dysplasia, and especially prominent erythroid dysplasia. While the megakaryocyte morphology is not suggestive of a de novo MPN, hydroxyurea can mask typical MPN megakaryocytes morphology. Given the persistence of the *MPL* mutation, this is favored to represent progression of the previously reported process, which was morphologically compatible with an essential thrombocythemia. However, there has been significant molecular genetic evolution, including the acquisition of a complex karyotype and TP53 mutation, which is functionally biallelic due to the loss of one chromosome 17. The severe dyserythropoiesis is reminiscent of cases previously called pure erythroid leukemia, most of which are now thought to harbor bi-allelic *TP53* mutation.

Panel Diagnosis

Essential thrombocythemia (ICC 2022 / WHO-5) (pre-PMF?) with MDS-type progression in accelerated phase

EA4HP24-BMWS-444

A patient with Rosai-Dorfman disease (RDD), Ras-associated autoimmune leukoproliferative disorder (RALD) progressing to chronic myelomonocytic leukemia (CMML) and a clonal plasma cell proliferation

Dr. Sofia Garces, Dr. Hong Fang, Dr. Vasiliki Leventaki, Dr. Wei Wang, Dr. L. Jeffrey Medeiros, Dr. C. Cameron Yin

MD Anderson Cancer Center, Hematopathology, Houston, USA

Case Description

39-year-old woman with pancytopenia since age 2y and splenectomy for massive splenomegaly at age 5y was diagnosed with autoimmune lymphoproliferative syndrome (ALPS). The patient also had nodal RDD at age 18y. She presented to our hospital at age 34y with multicompartmental lymphadenopathy and hypermetabolic bone marrow (BM) on PET-CT. Complete blood cell count showed leukocytosis (21.4 K/uL) with monocytosis (12%) and BM evaluation showed RALD. A concurrent submandibular lymph node (LN) biopsy showed RDD. The patient was treated with clofarabine and cobimetinib but progressed to CMML one year later. She received a matched unrelated donor stem cell transplant (SCT) and remains in complete remission.

Biopsy Fixation Details

BM core biopsy was decalcified. Both BM and submandibular LN sample were fixed in formalin.

Frozen Tissue Available

No

Details of Microscopic Findings

BM (2019) showed 90% cellularity, myeloid predominance, monocytosis and multilineage dyspoiesis. Submandibular LN biopsy (2019) showed abnormal histiocytes undergoing emperipolesis. BM (2020) showed 80% cellularity, monocytosis and worsening dysplasia.

Immunophenotype

Flow cytometry (2019 and 2020 BM) showed <1% CD34+ myeloblasts with decreased HLA-DR and CD38; neutrophils with abnormal CD13/CD16 and monocytes with increased CD16. There was also a minute aberrant population of plasma cells: CD56+, CD117+, kappa+. Submandibular LN biopsy showed RDD with S100+, CD68+, CD1a- histiocytes.

Cytogenetics

2019 BM: 46,XX[20]

2020 BM: 46,XX,r(7)(p11.2)[15]/46,XX[5]; FISH positive for del(7)(q31) (81.5%) and monosomy 7 (12.5%)

Molecular Studies

Next generation sequencing (NGS) 81-gene panel:

2019 and 2020 BM: *KRAS* c.35G>T p.G13C, VAF 36.5% and 52%, respectively.

2019 submandibular LN: *KRAS* c.35G>T p.G13C, VAF 30%

Proposed Diagnosis

2019 BM: RALD and a small population of clonal plasma cells

2019 submandibular LN: RDD

2020 BM: CMML evolving from RALD. Persistent clonal plasma cells

Interesting Feature(s)

We present a patient with RALD who progressed to CMML. The patient also had concurrent nodal RDD and a clonal plasma cell proliferation.

RALD is a chronic, generally indolent ALPS-related disorder. Patients present with persistent monocytosis, lymphoproliferation, and autoimmune phenomena. RALD has clinicopathologic features that overlap with JMML and CMML, including somatic mutations in *KRAS/NRAS*. Most patients with RALD have an indolent clinical course, but rare cases evolve into CMML/JMML. Distinguishing RALD from CMML/JMML is challenging due to lack of clear-cut pathologic criteria for malignant transformation. Acquisition of genetic abnormalities support overtly malignant disease. In the submitted case, the clinicopathologic features were not significantly different in the 2019 and 2020 BM, but acquisition of monosomy 7 in the latter prompted a malignant diagnosis. The patient was then treated with SCT and remains in remission for over 3 years. Additional interesting features in this case include the co-occurrence of nodal RDD and coexistence of clonal plasma cells in the BM. Both RDD and clonal plasma cell proliferations are known to carry *KRAS/NRAS* mutations as pathogenic drivers in case subsets. Moreover, in the current case NGS showed *KRAS* G13C in the BM samples with RALD and CMML, and in tissue involved by RDD.

Panel Diagnosis

Essential thrombocythemia (ICC 2022 / WHO-5) (pre-PMF?) with MDS-type progression in accelerated phase

EA4HP24-BMWS-457

Transformation of a JAK2 positive Myeloproliferative neoplasm with 2 early/undifferentiated cell populations

Dr. Penny Wright[1], Dr. Catherine Neal[2], PhD/MD Geraldine Duncan[2]

[1] Canterbury Health Laboratories, Anatomic Pathology, Christchurch, New Zealand; [2] Canterbury Health Laboratories, Haematology, Christchurch, New Zealand

Case Description

67-year-old man diagnosed as JAK2 positive ET on bone marrow January 2017. Treated with Hydroxyurea. Persistent episodes of palmar rash of uncertain aetiology although erythromelalgia considered. May 2017 Coeliac disease diagnosed on duodenal biopsy.

May 2022 Hydroxyurea stopped due to neutropenia. Became leucoerythroblastic, and developed generalised lymphadenopathy and hepatosplenomegaly. Bone marrow and inguinal lymph node biopsy showed infiltration by 2 immature cell populations consistent with blast transformation. Treated with Ruxolitinib and then Doxorubicin and Vincristine. Developed skin lesions on the face and biopsy showed infiltration by immature cells. Peripheral blood findings deteriorated and he died in February 2023.

Biopsy Fixation Details

Formalin. EDTA

Frozen Tissue Available

No

Details of Microscopic Findings

BM trephine Jan 2017: Cellularity 70%. Increased, hyperlobulated megakaryocytes with clusters. Reticulin MF-1.

BM trephine July 2022: Cellularity 70%. CD34 positive cells approximately 10%. Megakaryocytes increased with clusters and abnormal lobation. Nodular aggregates of immature cells positive for CD117, CD5, CD2, CD7 and partial CD3. Reticulin MF-2.

Inguinal LN July 2022: Diffuse infiltrate positive for CD3, TdT, CD34, ERG, CD99, CD7, CD43, CD5,CD10, CD45 and Ki67. Immature cells positive for CD7, CD117, CD45, CD1a and CD2 more dispersed. Extramedullary haematopoiesis.

Skin, biopsies, face Sept 2022: Dermal infiltrate positive for CD45, CD43, CD7, CD68 and CD99 with variable CD2, CD3, CD4, CD5 and CD1a. Scattered cells positive for CD117 and TdT.

Immunophenotype

Flow cytometry analysis demonstrated the presence of 2 immature populations in the lymph node:

1) Approximately 5% of mononuclear cells (5% of CD45 positive cells) were immature cells positive for CD7 (brighter than the normal T cells in this sample), CD34, CD45 (weak), CD13 (weak), CD5 (weak), CD10 (partial), TdT (weak), CD99 (weak), HLA-DR and intracellular CD3 (partial, approx. 1/3 positive). These cells were mostly negative for CD2, CD1a and CD117, all were negative for surface CD3, CD4, CD8, CD56, CD57, CD16, CD25, myeloperoxidase, CD33, CD64, CD14, CD61, CD138, CD19 and CD20.
2) Approximately 25% of mononuclear cells (25% of CD45 positive cells) were immature cells positive for CD7 (also brighter than the normal T cells in this sample), CD117 (variable, approximately 1/2 positive), CD45 (weak to moderate), CD2 (weak/partial) and HLA-DR (weak). These cells were mostly negative for CD56, all were negative for surface and intracellular CD3, CD5, CD4, CD8, CD10, CD57, CD16, CD25, CD1a, CD99, CD34, TdT, myeloperoxidase, CD13, CD33, CD64, CD14, CD61, CD138, CD19 and CD20.
The two immature populations described in the lymph node were also present at low levels in the bone marrow:
CD34+/CD7+(moderate)/CD5+/CD13+(weak) = 1.4% of CD45+,
CD117+/CD7+(moderate)/CD34-/CD13- = 1.5% of CD45+.

Cytogenetics

Bone marrow July 2022 46,XY[20]

Molecular Studies

BCR-ABL1 gene fusion not detected on peripheral blood.
Lymph node FISH showed no evidence of FGFR1 rearrangement.
DNA from bone marrow analysed using the Illumina TruSight Myeloid 54 NGS panel showed clinically significant variants in the ASXL1, IDH1, NRAS, SRSF2, STAG2 and TET2 genes. Also confirmed the JAK2 V617F variant detected in 2017.

Proposed Diagnosis

Blast transformation of a JAK2 positive MPN with 2 immature cell populations.

Interesting Feature(s)

Mutation profile consistent with progression of a JAK2 positive MPN. Two immature populations - one phenotype similar to near-ETP-ALL and the other undifferentiated.
Original diagnosis probably pre-fibrotic PMF

Panel Diagnosis

Prefibrotic primary myelofibrosis (ICC 2022 / WHO-5) with early T-precursor lymphoblastic/undifferentiated blast phase

EA4HP24-BMWS-472

Myelodysplastic syndrome/myeloproliferative neoplasm with concurrent JAK2 V617K mutation and del(5q)

Dr. Gonçalo Pereira, Dr. Tiago Maia, Dr. José Cabeçadas

Instituto Português de Oncologia de Lisboa, Francisco Gentil, Anatomic Pathology, Lisbon, Portugal

Case Description

A 68-year-old male presented with severe (7.4g/dL) macrocytic anemia and thrombocytosis (> 1,500,000/µL). A bone marrow trephine biopsy and molecular studies revealed a myeloid neoplasm with del(5q) and *JAK2* V617F mutation. The patient was treated with hydroxyurea (initiated before bone marrow biopsy) and lenalidomide (initiated after bone marrow biopsy), which resulted in normalization of hemoglobin and platelet count. After 11 months of therapy, the patient is in clinical/hematologic remission. So far, he did not undergo a new bone marrow biopsy nor genetic/cytogenetic reevaluation

Biopsy Fixation Details

Hammersmith protocol

Frozen Tissue Available

No

Details of Microscopic Findings

Bone marrow with areas of hypercellularity with trilinear hematopoiesis and preserved maturation of all series. A megakaryocytic proliferation consisting in pleomorphic hypolobated forms with aggregates was conspicuous. Hypoplastic erythroid lineage with features suggestive of dyserythropoiesis. Blasts within normal limits and distribution. A slight proliferation of mast cells and myelofibrosis (MF-2) were present. Bone marrow aspirate was not adequate for cytomorphological evaluation.

Immunophenotype

Aberrant expression of CD25 in the mast cells, without co-expression of CD30 or other criteria for mastocytosis

Cytogenetics

Interstitial 5q deletion in 9 of 10 metaphases [46,XY,del(5)(q13q33)]

Molecular Studies

Presence of*JAK2* V617Fmutation (Sanger). No mutations were found in CALR and MPL genes. No BCR-ABL1 gene fusion was found (RT-PCR multiplex). NGS study was not performed and *KIT* gene status was not assessed

Proposed Diagnosis

Myelodysplastic syndrome/myeloproliferative neoplasm with concurrent JAK2 V617K mutation and del(5q)

Interesting Feature(s)

- Myeloid neoplasms with concurrent del(5q) and *JAK2* V617F mutation have been described, either with both alterations present at diagnosis or one emerging/being

selected during disease evolution. Our case has typical clinical and morphological features of the myelodysplastic syndrome/myeloproliferative neoplasm, with good clinical behaviour so far, despite the presence of myelofibrosis.

- The presence of mast cell proliferations with aberrant immunophenotype has not been described so far within these neoplasms.

Panel Diagnosis

Myelodysplastic syndrome with del (5q) (ICC 2022) / with low blasts and isolated 5q deletion (WHO-5), and JAK2 mutation

EA4HP24-BMWS-475

A case of triple-negative MPN with alternative mutations affecting JAK-STAT signaling and epigenetic regulation

Dr. Francisco J. Llamas Gutierrez[1], Dr. Louis Escuer[1], Dr. Faustine Lhomme[3], Dr. Cecile Le Naoures Mear[2], PhD/MD Cedric Pastoret[5]

[1] CHU de Rennes, Pathology, Rennes, France; [2] CHU de Rennes, Pathology, Rennes, France; [3] CHU de Rennes, Hematology, Rennes, France; [4] CHU de Rennes, Pathology, Rennes, France; [5] CHU de Rennes, Molecular Biology, Rennes, France

Case Description

A 41 year-old woman consulting for right thoracic paraesthesia. Ultrasonography revealed a 14 cm splenomegly. Peripheral blood showed isolated thrombocytosis: PLT : 471 G/L, Hb : 13.8 g/dL, WBC 7.3 G/L.

Biopsy Fixation Details

formalin-fixed, EDTA decalcification

Frozen Tissue Available

No

Details of Microscopic Findings

Slightly hypercellular bone marrow. Increase in number and size of megakaryocytes; some of them present hyperlobulated nuclei or form loose clusters. Myeloid to erythroid ratio is normal. Blasts are not increased. Reticulin network is normal.

Immunophenotype

CD31 highlights the morphology and distribution of megakaryocytes.

Cytogenetics

No

Molecular Studies

NGS myeloid panel:
-DNMT3A (NM_022552.2) Mut : Ex13 c.1541G>A p.(Cys514Tyr) VAF=5.4%
-SH2B3 (NM_005475.2) Mut : Ex6 c.1174C>T p.(Arg392Trp) VAF=63.9%
No Jak2, Calreticuline or MPL mutations

Proposed Diagnosis

Triple négative MPN, essential thrombocythemia

Interesting Feature(s)

Mutations in other genes that affect JAK-STAT signalling (SH2B3) or epigenetic regulation (DNMT3A) providing évidence of a clonal haematopoietic disorder. The high VAF for SH2B3 rises de question of a germline variant.

Panel Diagnosis

Essential thrombocythemia (ICC 2022 / WHO-5) (pre-PMF?)

BONE MARROW WORKSHOP PART III: Myeloid and lymphoid early/undifferentiated precursor cell neoplasms and mixed phenotype acute leukemias

Oral Presentations

EA4HP24-BMWS-33	All mixed up: Acute leukemia with mixed phenotype, myelodysplasia-related cytogenetics, and TP53 mutation
EA4HP24-BMWS-102	Acute undifferentiated leukemia, an increasingly rare diagnosis
EA4HP24-BMWS-152	Mixed phenotype acute leukemia, T/myeloid, BCL11B-activated
EA4HP24-BMWS-104	Early-T precursor lymphoblastic leukemia with PICALM::MLLT10 fusion: a challenging diagnosis)
EA4HP24-BMWS-415	Distinct B/myeloid and T-lymphoblast populations at separate anatomic sites in mixed-phenotype acute leukemia with BCR::ABL1 fusion

EA4HP24-BMWS-33

All mixed up: Acute leukemia with mixed phenotype, myelodysplasia-related cytogenetics, and *TP53* mutation

Dr. Nicholas C. Taylor[1,2], Dr. Juli-Anne Gardner[1,2], Dr. Joanna L. Conant[1,2], Dr. Ashley K. Volaric[1,2], PhD/MD Debra G.B. Leonard[1,2], **Dr. Katherine A. Devitt**[1,2]

[1] University of Vermont Medical Center, Pathology & Laboratory Medicine, Burlington, USA; [2] Larner College of Medicine, University of Vermont, Burlington, USA

Case Description

A 90-year-old female with a past medical history of hypothyroidism and recent transient ischemic attack was referred to hematology/oncology for new onset pancytopenia with severe neutropenia and symptomatic anemia. She reported feeling extremely weak for 6 months. Work-up for secondary causes was unrevealing.

Laboratory findings included macrocytic anemia (Hgb 7.4 gm/dL, RR 11.6-15.2; MCV 109 fL, RR 81-98) and leukopenia (WBC 2,050/cmm, RR 4,000-12,400) with neutropenia (ANC 610/cmm, RR 2,200-8,850) and 19% circulating blasts. Platelet level was unreportable due to clumping.

Biopsy Fixation Details

Fixation in neutral buffered formalin

Frozen Tissue Available

No

Details of Microscopic Findings

The peripheral blood showed abnormal neutrophil granulation and lobation. The bone marrow was hypercellular with 60% blasts in a background of trilineage dyspoiesis, and 8% ring sideroblasts. Immunohistochemistry showed the blasts to be CD34+ with distinct myeloid (MPO, lysozyme) and B-lymphoid (TDT, PAX5, CD79a) subsets.

Immunophenotype

A population of CD45dim, CD34+, HLA-DR+ blasts accounted for 60% of events by flow cytometric analysis, with two distinct populations with differing lineage-defining phenotypes. The first population expressed CD117, cMPO (at 50% intensity threshold of mature neutrophils), CD13, CD33dim, CD4dim, comprising ~60-70% of the blasts. The second population expressed CD19, cCD79a, nTDT, CD38, CD58, CD9dim, comprising ~20-30% of the blasts.

Cytogenetics

Complex karyotype including loss of chromosomes 5 and 7. Negative for *KMT2A* gene rearrangement and *BCR::ABL1* gene fusion.

41,XX,add(3)(q12),-4,-5,-6,-7,-8,-9,+11,-12,-16,-17,-18,-19,+4~6mar[cp16]/ 46,XX[4].nuc ish(ABL1,BCR)x2[200],(MLLx3)[143/200]

Molecular Studies

Next generation sequencing of the bone marrow for a panel of genes identified a pathogenic variant in *TP53* (p.H179Q, c.537T>G, 63.7% variant allele frequency).

Proposed Diagnosis

Acute leukemia, myelodysplasia-related, with mixed phenotype, B/Myeloid (WHO 5th ed)
Mixed phenotype acute leukemia, B/myeloid, with mutated *TP53* (ICC)

Interesting Feature(s)

- Interesting rare case of acute leukemia that challenges existing diagnostic frameworks
- Overlaps with myelodysplasia-related AML categories, ALAL (MPAL B/myeloid), and AML with mutated *TP53*, depending on the classification system used
- Not purely myeloid, not fitting into the established acute myeloid leukemia categories, but current MPAL categories are not encompassing
- An MPAL diagnosis is prohibited by the WHO when a case fits another well-defined entity including AML-MR. For ICC, entities defined by single gene abnormality take precedence in the myeloid realm, but in MPAL only myelodysplasia-related cytogenetic abnormalities or mutations are mentioned hierarchically.
- Guidance is lacking on terminology of mixed phenotype acute leukemias that may have some features of a defined single-lineage leukemia

Panel Diagnosis

AML-MR (WHO)/ AML with TP53 mutation /ICC) and MPAL (B/myeloid) immunophenotype

EA4HP24-BMWS-102

Acute undifferentiated leukemia, an increasingly rare diagnosis

Alexandra E. Kovach[1,2], Sara-Jane Onyeama[3], Ashley Gray[2,3], Neena Kapoor[2,3], Deepa Bhojwani[2,3], Gordana Raca[1,2]

[1] Children's Hospital Los Angeles, Pathology and Laboratory Medicine, Los Angeles, USA; [2] University of Southern California, Keck School of Medicine, Los Angeles, USA; [3] Children's Hospital Los Angeles, Pediatrics, Hematology/Oncology, Los Angeles, USA

Case Description

A 17-year-old previously healthy female presented with hyperleukocytosis (white blood cell count 106 K/uL).

Biopsy Fixation Details

Formalin with decalcification

Frozen Tissue Available

Yes

Details of Microscopic Findings

The peripheral blood and bone marrow contain abundant large uniform blasts with scant pale agranular basophilic cytoplasm and occasional nucleoli.

Immunophenotype

Flow cytometry (peripheral blood): Positive CD4 (dim), CD5 (dim), CD7, CD33, CD34, CD38, CD45 (dim), CD58 (dim), CD71 (dim) and TdT; cCD3, CD10, CD13, CD14, CD15, CD19, CD22, CD24, CD41, CD42, CD56, CD61, CD64, cCD79a, CD117, CD123, CD235, HLA-DR and cMPO

Cytochemistry (bone marrow aspirate): negative for MPO and NSE

Immunohistochemistry (bone marrow core biopsy): positive for CD79a (weak); negative for lysozyme, MPO and PAX5

Cytogenetics

Karyotype: 48,X,-X,r(7)(p15q36),+10,+10,+19[3]/46,XX[17]

Fluorescence in situ hybridization (FISH) panel for acute myeloid leukemia (AML): Four copies of the centromere signal for chromosome 10 (63%), consistent with the tetrasomy 10 observed by karyotype analysis in the abnormal clone. In addition, FISH showed three RUNX1 (21q22) signals in 8.5%-9.5% of analyzed cells, suggesting the presence of a sub-clone with a gain of the RUNX1 locus or chromosome 21.

Chromosome microarray: Gain of two extra copies of chromosome 10, gain of chromosome 19, loss of the X chromosome, copy number losses (deletions) in 7p and 7q (consistent with a ring chromosome), CN-LOH in 12p and a low-level gain of chromosome 21 were observed.

Molecular Studies

Variants of strong significance were observed in the *PTEN* and *IKZF1* genes and a variant of potential significance was detected in the *RUNX1* gene.

Proposed Diagnosis

Acute undifferentiated leukemia

Interesting Feature(s)

With increasing clinical application of sophisticated molecular technologies to subclassify leukemia and other malignancies, the proportion of cases without an identified genetic driver is decreasing. Similarly, comprehensive immunophenotyping of leukemia – including antigens defining rare forms of differentiation such as basophilic, erythroid and megakaryoblastic – can typically identify a leukemic cell lineage. As such, true acute undifferentiated leukemia (AUL) is increasingly rare. Here we present comprehensive features of a bona fide AUL. The patient had a slow response to acute myeloid leukemia (AML)-directed chemotherapy with minimal residual disease detected after the first two cycles of chemotherapy. She underwent hematopoietic stem cell transplantation and remains in remission 3 years later. *PTEN*, *IKZF1* and *RUNX1* mutations are observed in different hematologic neoplasms, but only *RUNX1* mutations have been reported in association with undifferentiated leukemias. Gains of chromosomes 19 and 21 and 7q deletions are recurrent in myeloid malignancies, but the significance of these abnormalities in undifferentiated leukemia is uncertain.

Panel Diagnosis

AUL (WHO/ICC)

EA4HP24-BMWS-152

Mixed phenotype acute leukemia, T/myeloid, BCL11B-activated

Dr. Yiannis Petros Dimopoulos, Dr. Wei Wang, Dr. Shaoying Li, Dr. Guilin Tang, Dr. Sanam Loghavi, **Dr. Sa A. Wang**

University of Texas, MD Anderson Cancer Center, Hematopathology, Houston, USA

Case Description

A 66-year-old male presented worsening fatigue, diaphoresis, and was found to have leukocytosis, (WBC 28.4 x10^9/L, 81% circulating blasts), anemia (Hgb 7.4 g/dl), and thrombocytopenia (platelets 54x10^9/L). He presented to our hospital for treatment after initially receiving hydroxyurea.

Biopsy Fixation Details

Formalin-fixed, paraffin-embedded.

Frozen Tissue Available

No.

Details of Microscopic Findings

On aspirate smears, the blasts were intermediate in size with irregular nuclear contours, prominent nucleoli, and scant cytoplasm without Auer rods (figure 1A). The bone marrow biopsy was hypercellular for age (80%) with an extensive immature mononuclear cell infiltrate (figure 1B).

Immunophenotype

A subset of the blasts was positive for MPO with cytochemistry (figure 2A). Immunohistochemistry (IHC) performed on the biopsy showed the blasts were diffusely positive for BCL11B (figure 2B).

Flow cytometric immunophenotyping (figure 3) showed a distinct blast population, positive for CD2, cytoplasmic CD3, CD7, CD13, CD33 (partial), CD34, CD36 (partial), CD38, CD45 (dim), CD54 (dim), CD117, CD123, CD133, HLA-DR, cytoplasmic MPO (subset, ~13%), and TDT (dim). The blasts were negative for surface CD3, CD4, CD5, CD14, CD15, CD19, CD25, CD56, CD64, CD1a, CD8, and CD10.

Cytogenetics

Conventional karyotype revealed 47,XY,+mar[1]/46,XY[19]. Fluorescence *in situ* hybridization (FISH) was negative for *BCR::ABL1* or *KMT2A* rearrangement. Optical genome mapping (OGM) revealed a t(8;14)(q24.21;q32.2), with putative *CCDC26::SETD3* (figure 4).

Molecular Studies

FLT3-internal tandem duplication was detected by PCR, followed by capillary electrophoresis. Next generation sequencing revealed the presence of mutations involving *RAD21* (c.578dupT p.L193fs*4, variant allelic frequency (VAF): 20%) and *TET2* (c.4393C>T p.V10I, VAF: 43%).

Proposed Diagnosis

Acute leukemia of ambiguous lineage (T/myeloid) with *BCL11B* rearrangement (WHO 5th)

Mixed phenotype acute leukemia (T/myeloid) with *BCL11B* activation (ICC)

Interesting Feature(s)

BCL11B activated (BCL11B-a) acute leukemia may manifest as T-lymphoblastic leukemia with an early T-phenotype (ETP), mixed phenotype acute leukemia (T/myeloid), or AML with minimal differentiation. These leukemias may be considered as a molecularly defined subset of acute leukemia. BCL11B-a can result from 14q32 rearrangements (juxtaposing *BCL11B* to superenhancers), amplifications generating superenhancers from noncoding elements distal to *BCL11B*, but can also be due to other BCL11B-activating genetic alterations. Rearrangements that do not result in activation of BCL11B (particularly *BCL11B::TLX3*) need to be excluded. These cases frequently co-occur with *FLT3*, WT1, and epigenetic mutations, and are important to recognize as they may be sensitive to FLT3 or JAK/STAT inhibition. BCL11B IHC (usually negative in ETP-ALL or AML) is strongly positive in BCL11B-a acute leukemia.

By conventional karyotyping, T-ALL blasts usually do not actively divide under conventional culture, often producing a normal karyotype. Translocations can be cryptic. Thus, FISH is required for confirmation, with probes designed to target certain breakpoints and avoid the breakpoints that do not activate BCL11B. The rearrangements additionally often do not produce fusion products that can be detected by RNA sequencing. As such, OGM is superior to other technologies in identifying these rearrangements.

This case represents a classic case of BCL11B-a acute leukemia, confirmed by OGM and BCL11B-IHC and showing strong CD2 expression and a mixed T/myeloid phenotype. Of note, the T cell component of this patient's disease had an ETP-like phenotype.

Panel Diagnosis

MPAL (T/myeloid), BCL11b activated (WHO/ICC)

EA4HP24-BMWS-104

Early-T precursor lymphoblastic leukemia with PICALM::MLLT10 fusion: a challenging diagnosis

Dr. Tianyu Yang, Dr. Judith Brody

Northwell Health, Pathology, Greenvale, USA

Case Description

A 29-year-old female with past medical history of chronic low back pain, celiac disease, and covid-19 infection two weeks before, presented with headache for 2 days. CBC showed normal WBC count with neutropenia and 78% blasts, normocytic anemia, and normal platelet count. She also had elevated LDH (339 u/l). CT scan reported no lymphadenopathy or organomegaly. Peripheral blood smear showed undifferentiated blasts with agranular cytoplasm and high nucleus-to-cytoplasmic ratio. Flow cytometry was performed on

peripheral blood and bone marrow specimen, blasts were positive for HLA-DR, CD34, CD117, CD123, CD33, CD13, CD15 (partial), CD7, CD11b, cytoplasmic CD3 (dimmer than mature T cells); negative for CD2, surface CD3, CD4, CD5, CD8, CD10, CD14, CD19, CD41, CD61, CD56; myeloperoxidase was negative by cytochemistry stain and by immunohistochemical (IHC) stain on core biopsy; about 30% of cells were TdT positive by indirect immunofluoresence staining; CD1a was negative by IHC stain. A diagnosis of early-T precursor lymphoblastic leukemia was rendered based on the phenotype of blasts. Cytogenetic studies revealed abnormal karyotype of 46,XX,t(10;11)(p?12;q?21)[7]/92,idemx2[6]/47,idem,+4[1]/46,XX[6]. FoundationOne Heme studies were performed, PICALM::MLLT10 fusion was identified, and multiple genetic mutations were detected, including NOTCH1 S2341fs*13, TP53 V225I, CDKN1B loss, ETV6 V322fs*6 and A67fs*9, IK3R2 R574*, PTPN11 E76G, SUZ12 R654*, TLL2 Y954*, and MYCN P44L (sub-clonal). Patient was treated with chemotherapy (daunorubicin, vincristine, PEG asparaginase, and dexamethasone). Day 29 bone marrow achieved morphologic remission, however, small population of T-lymphoblasts (0.3%) was demonstrated by flow cytometry. One year after presentation, patient received allogeneic stem cell transplantation (SCT) from a matched unrelated donor. She is now in remission 23 months from diagnosis.

Biopsy Fixation Details

Bouin's fixative

Frozen Tissue Available

Not available

Details of Microscopic Findings

Peripheral blood smear: undifferentiated blasts, variable cell size, agranular cytoplasm, and high N/C ratio.

Bone marrow core biopsy: increased cellularity of 95-100% with diffuse proliferation of immature cells/blasts; erythroid and myeloid cells are markedly decreased (minimal), megakaryocytes are present in adequate number and mostly normal morphology.

Immunophenotype

Blasts positive for HLA-DR, CD34, CD117, CD123, CD33, CD13, CD15 (partial), CD7, CD11b, cytoplasmic CD3 (dimmer than mature T cells); negative for CD2, CD3 (surface), CD4, CD5, CD8, CD10, CD14, CD19, CD41, CD61, CD56; myeloperoxidase negative by cytochemistry stain and by immunohistochemical stain on core biopsy; about 30% of cells TdT positive by indirect immunofluoresence staining; dim partial CD3 stain and negative CD1a by IHC stain on core biopsy.

Cytogenetics

Abnormal female karyotype

Karyotype: 46,XX,t(10;11)(p?12;q?21)[7]/92,idemx2[6]/47,idem,+4[1]/46,XX[6]

Molecular Studies

PICALM-MLLT10 fusion

NOTCH1 S2341fs*13

TP53 V225I

CDKN1B loss

ETV6 V322fs*6, A67fs*9

MYCN P44L - subclonal†

PIK3R2 R574*
PTPN11 E76G
SUZ12 R654*
TLL2 Y954*

Proposed Diagnosis

Early-T precursor lymphoblastic leukemia

Interesting Feature(s)

Blasts show expression of multiple myeloid and stem cell markers; whereas expression of T cell antigens are limited (dim cytoplasmic CD3 and surface CD7). Differential diagnosis of AML with myelodysplasia related changes was considered at the time; mixed phenotype acute leukemia was excluded given lack of MPO expression.

Panel Diagnosis

ETP-ALL. *DD* AML with minimal differentiation (WHO)/AML, NOS (ICC)

EA4HP24-BMWS-415

Distinct B/myeloid and T-lymphoblast populations at separate anatomic sites in mixed-phenotype acute leukemia with *BCR::ABL1* fusion

Dr. Gina Sotolongo[1], Luis Carrillo[1], Ken Young[1,2], Dr. Eric Carlsen[1,2]

[1] Duke University Hospital, Pathology, Durham, USA; [2] Duke Cancer Institute, Durham, USA

Case Description

The patient is a 60-year-old man with a history of renal transplant (on tacrolimus, mycophenolate mofetil, and prednisone) that presented with dysphagia, 50-pound weight loss, and bilateral tonsillar enlargement. Complete blood counts showed WBC 16.2*10^9 (mostly neutrophils), Hgb 10.3 g/dL, PLT 152*10^9. He underwent bilateral tonsillectomy. He subsequently underwent bone marrow biopsy to further characterize his disease process. After final diagnosis, he received dasatinib + prednisone with evidence of complete remission, followed by ongoing blinatumomab + ponatinib.

Biopsy Fixation Details

Tonsils: formalin. Bone marrow core biopsy: acetic zinc formalin.

Frozen Tissue Available

No.

Details of Microscopic Findings

Tonsils: The tonsils are enlarged and show a marked interfollicular expansion by intermediate-sized monotonous lymphoid cells with fine chromatin and variably prominent nucleoli.

Bone marrow: Aspirates show an increase in blasts (29%). Blasts are large with regular nuclear contours, dispersed chromatin, prominent nucleoli, and scant granular cytoplasm. An MPO cytochemical stain is weakly positive in a subset of blasts. Background trilineage hematopoiesis is progressive with no significant dyspoietic changes. The core biopsy shows hypercellular marrow (80%) with increased blasts.

Immunophenotype

Tonsils (IHC + flow cytometry): CD2 negative, surface CD3 negative, **cytoplasmic CD3 positive** (based on IHC positivity), CD4 negative, **CD5 dim**, **CD7 bright**, CD8 negative, CD10 negative, CD19 negative, CD20 negative, CD22 negative, CD25 negative, CD33 partial dim positive, CD34 partial positive, CD38 variable, CD45 dim, CD56 negative, CD117 negative, CD123 mostly negative, MPO negative, TdT positive. *Phenotype compatible with T-lymphoblasts.*

Bone marrow: (IHC + flow cytometry): CD2 negative, surface CD3 negative, cytoplasmic CD3 negative, CD4 negative, CD5 negative, CD7 negative, CD8 negative, **CD10 partial positive**, CD11c negative, CD13 mostly positive, CD14 negative, **PAX5 partial positive**, **CD19 partial positive**, CD20 negative, **CD22 partial positive**, CD25 mostly positive, CD33 partial positive, CD34 positive, CD38 bright, CD45 dim, CD56 negative, CD64 negative, CD79a partial positive, CD117 negative, CD123 dim, HLA-DR positive, lysozyme negative, **MPO partial positive**, TdT positive. *Phenotype compatible with B-lymphoblasts and myeloblasts. A discrete T-lymphoblast population is not detected.*

Cytogenetics

Tonsils: FISH: Send-out FISH for *BCR::ABL1* on FFPE is positive in 78% of cells.

Bone marrow: Karyotype: 45,XY,-7,t(9;22)(q34;q11.2)[12]/46,XY[12]. FISH: FISH for *BCR::ABL1* is positive in 36% of cells. No evidence of deletion at *CDKN2A* or rearrangements involving *KMT2A* or TCR alpha/delta.

Molecular Studies

Tonsils: NGS: A lymphoid NGS panel is negative for pathogenic variants. T-cell clonality: A dominant rearrangement in TCRG is detected (Vg5:JgP1).

Bone marrow: NGS: A myeloid NGS panel detected pathogenic variants in *BCORL1* (p.Gly1682ArgfsTer4, VAF 14%) and *RUNX1* (p.Tyr380LeufsTer220, VAF 25%).

Proposed Diagnosis

Mixed-phenotype acute leukemia with *BCR::ABL1* fusion (International Consensus Classification 2022 and WHO 5th edition online beta version).

Interesting Feature(s)

Cases of MPAL with combined B-lymphoblast, T-lymphoblast, and myeloblast phenotypes are rare. Molecular abnormalities associated with these neoplasms are incompletely characterized. It is unclear whether monosomy 7 and mutations in *BCORL1* and *RUNX1* are present in all 3 blast populations. To our knowledge, cases of MPAL with segregation of different blast lineages by anatomical site have not been previously reported.

Panel Diagnosis

AML-MR (WHO/ICC) with MPAL immunophenotype and BCR-ABL1 fusion, post transplant

Cases Discussed by the Panel

EA4HP24-BMWS-16	Any space for acute leukemia of mixed or ambiguous lineage in the current time of leukemia-defining genetic aberrations? A case of *TP53* mutated MPAL.
EA4HP24-BMWS-19	Myeloid/Lymphoid neoplasm with FGFR1 rearrangement presenting in blast phase with MPAL phenotype
EA4HP24-BMWS-27	Acute undifferentiated leukemia with cryptic *NUP98::BPTF* fusion identified by NGS
EA4HP24-BMWS-51	Acute Undifferentiated Leukemia with *NPM1* mutation and extramedullary involvement, arising from prior myelodysplastic syndrome
EA4HP24-BMWS-54	Acute leukemia with *PICALM::MLL10* fusion
EA4HP24-BMWS-59	Early T-cell precursor subtype T-lymphoblastic leukemia (ETP-ALL) initially presenting with mixed/ambiguous phenotype
EA4HP24-BMWS-65	Acute Undifferentiated Leukemia with PHF6 mutation and clonal T-cell receptor and immunoglobulin gene rearrangement
EA4HP24-BMWS-76	Hematopoietic neoplasm of ambiguous lineage
EA4HP24-BMWS-109	Early T-cell Precursor (ETP) Acute Lymphoblastic Leukemia/ Lymphoma (ETP-ALL)
EA4HP24-BMWS-126	Mixed phenotype acute leukemia (MPAL) with BCR::ABL1 fusion
EA4HP24-BMWS-131	Mixed Phenotype Acute Leukemia with Monocytic Differentiation
EA4HP24-BMWS-132	Mixed Phenotype Acute Leukemia with Complex Karyotype
EA4HP24-BMWS-134	AUL with KMT2A rearrangement
EA4HP24-BMWS-135	Myeloid/lymphoid neoplasm with FLT3 rearrangement with simultaneous presentation of CML-like marrow proliferation and T-LBL/L of lymph nodes
EA4HP24-BMWS-145	CD56+ Early T Precursor Acute Lymphoblastic Leukemia with RUNX1 rearrangement.
EA4HP24-BMWS-154	Post cytotoxic chemotherapy-associated B/myeloid mixed phenotype acute leukemia
EA4HP24-BMWS-158	Acute Undifferentiated Leukemia
EA4HP24-BMWS-161	Mixed-phenotype acute leukemia, T/myeloid (MPAL-TM)
EA4HP24-BMWS-164	Mixed phenotype acute leukemia, B/T/Myeloid
EA4HP24-BMWS-194	Acute myeloid leukemia, myelodysplasia related, with B/monocytic-dendritic phenotype.
EA4HP24-BMWS-216	Acute undifferentiated leukaemia with FLT3-ITD shows molecular signatures of AML
EA4HP24-BMWS-219	Mixed Phenotype Acute Leukemia (T/Myeloid) masquerading as Acute Myeloid Leukemia
EA4HP24-BMWS-227	Chronic Myeloid Leukemia Presenting with Mixed Phenotype B/Myeloid Blast Phase: A Diagnostic Challenge
EA4HP24-BMWS-228	Mixed-phenotype acute leukemia (MPAL), B/T
EA4HP24-BMWS-232	A challenging case of *PICALM::MLLT10* rearranged acute leukemia with unusual immunophenotype and genotype

EA4HP24-BMWS-233	Acute leukemia with trilineage differentiation, likely unrelated to reported prior MPN
EA4HP24-BMWS-236	Acute myeloid leukemia without lineage-defining antigen expression
EA4HP24-BMWS-237	Extramedullary Mixed Phenotype Acute Leukemia Associated with Warthin's Tumor and Bone Marrow Involvement by Acute Myeloid Leukemia
EA4HP24-BMWS-238	B-lymphoblastic leukemia with aberrant MPO positivity
EA4HP24-BMWS-239	Myelodysplastic syndrome with biallelic TP53 inactivation exhibiting a T/Myeloid immunophenotype
EA4HP24-BMWS-240	Myeloid/B Mixed-Phenotype Acute Leukemia with MACROD1-RUNX1 Fusion
EA4HP24-BMWS-247	Myeloid/lymphoid neoplasm with FGFR1 rearrangement: Mixed-phenotype acute leukemia (Acute leukemias of ambiguous lineage).
EA4HP24-BMWS-270	Blast Phase of Chronic Myeloid Leukemia, *BCR::ABL1* Positive, Presenting as B-Lymphoid/Myeloid Extramedullary Tumor
EA4HP24-BMWS-274	Mixed Phenotype Acute Leukemia
EA4HP24-BMWS-278	Diagnostic Challenges in a Patient with Acute Leukemias: Myeloid or Lymphoid?
EA4HP24-BMWS-346	Acute leukemia with unusual phenotype and differentiation aspects
EA4HP24-BMWS-350	Acute Leukaemia of Ambiguous Lineage or Acute Myeloid Leukaemia?
EA4HP24-BMWS-366	Acute leukemia with PAX5 p.P80R and recurrence as a genetically-related histiocytic proliferation
EA4HP24-BMWS-368	Mediastinal Mass with Myeloid Blasts: what's in a name?
EA4HP24-BMWS-394	A Diagnostic Dilemma for a Patient with Skin Lesions and Marked Leukocytosis: Mixed Phenotype Acute Leukemia or BPDCN with Aberrant CD3 Expression?
EA4HP24-BMWS-399	Mediastinal germ cell tumor with associated haematological malignancy (B-and myeloid lineage leukemia carrying complex karyotype and sharing identical *TP53* mutation with extragonadal germ cell tumor)
EA4HP24-BMWS-400	An interesting case of Mixed Phenotype Acute Leukaemia B/myeloid
EA4HP24-BMWS-406	Acute undifferentiated leukaemia with low level FLT3 ITD mutation and Trisomy 13 and discrepant immunophenotypes on flow cytometry and immunohistochemistry.
EA4HP24-BMWS-421	B-lymphoblastic leukemia with BCR::ABL1-like features (predicted *IGH*::*CRLF2* rearrangement) and partial monocytic differentiation
EA4HP24-BMWS-424	A difficult case of acute leukaemia
EA4HP24-BMWS-445	MDS with Multilinear Dysplasia, Excess blasts and RUNX1-mutation with Mixed Myeloid and early T-cell Precursor phenotype
EA4HP24-BMWS-458	Patient with blast phase multilineage (B and myeloid) neoplasm with *FGFR1* rearrangement and *RUNX1* mutation
EA4HP24-BMWS-459	Acute leukemia with minimal (or ambiguous?) differentiation

EA4HP24-BMWS-464	Trilineal mixed-phenotype acute leukemia
EA4HP24-BMWS-467	Early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) with high mutation burden
EA4HP24-BMWS-476	Acute leukemia, mixed myeloid/plasmacytoid dendritic cell phenotype involving bone marrow and lymph node.

EA4HP24-BMWS-16

Any space for acute leukemia of mixed or ambiguous lineage in the current time of leumemia-defining genetic aberrations? A case of *TP53* mutated MPAL.

Prof. Alexandar Tzankov

University Hospital Basel, Pathology, Basel, Switzerland

Case Description

F, 84 without hematologically significant previous medical history presented to her general practitioner and thereafter to the hematology department because of fatigue. At that time here CBC were:

- RBC 2.78 x 10^{12}L
- Hb 72 g/L
- PLT 48 x 10^{9}/L
- WBC 0.49 x 10^{9}/L
- Neut 19.3%
- Lymph 54%
- Mono 8.5%
- Eos 1.2%
- Baso 5.6%
- Blasts 11.4%

Biopsy Fixation Details

FFPE, EDTA decalcified

Frozen Tissue Available

No

Details of Microscopic Findings

Hypercellular bone marrow with diffuse infiltration by small monotnous blasts with single small nucleoli and with amphophilic cytoplasms.

Immunophenotype

FCM/FACS

- Myeloid blasts (18 %): CD34+, CD117+, HLA-DR+, CD13+, CD10-, CD11b-, CD16-, CD64-, CD14-, CD35-, CD300e-, CD105-, CD71-, CD36-, CD33± (35%),CD56-, CD19± (47%), CD7+ (30%), cTdT± (40%), cyCD3-, sCD3-, cyCD79a-, cyMPO+ (18%), CD38+ (79%), CD22+ (37%), CD15dim (23%), CD203c-, CD4-, CD123dim (79%), CD42a/CD61-, CD25-, CD9-, CD42b-, CD41-
- B-lymphoblasts (14 %): CD34+, CD117-,HLA-DR+, CD13dim(45%), CD10-, CD11b-, CD16-, CD64-, CD14-, CD35-, CD300e-, CD105-, CD71-, CD36-, CD33-, CD56-, CD19+ (80%), CD7-, cTdT+ (80%), cyCD3-, sCD3-, cyCD79a±(dim), cyMPO-, CD38+ (84%), CD22+ (80%), CD15dim (20%), CD203c-, CD4-, CD123+ (79%), CD42a/CD61-, CD25-, CD9-, CD42b-, CD41-

IHC

- CD34+, dimTdT, MPO±, dimPAX5, CD79a+,
- negative for: CD2, CD3, CD11c, CD20, CD56, CD123, IRF8, NPM1mut
- pathological overexpression of p53 as in biallelic *TP53* missense mutation or monoallelic missense mutation and deletion of the wild-type allele

Cytogenetics

Not assessed since management strategy was active surveillance.

Molecular Studies

TP53 R273H (VAF 86%)

No mutations in hotspots of: *ABL1, BRAF, CBL, CSF3R, DNMT3A, FLT3, GATA2, HRAS, IDH1, IDH2, JAK2, KIT, KRAS, MPL, MYD88, NPM1, NRAS, PTPN11, SETBP1, SF3B1, SRSF2, U2AF1, WT1**

No mutations in all exons of: A*SXL1, BCOR,CALR, CEBPA, ETV6, EZH2, IKZF1, NF1, PHF6, PRPF8, RB1, RUNX1, SH2B3, STAG2, TET2, ZRSR2*

No fusions among 687 fusion transcripts analyzed by the Oncomine Myeloid RNA Panel*

*the absence of other mutations is rather typic for AML with *TP53* mutations

Proposed Diagnosis

Acute MPO-positive (mixed phenotype) leukemia with mutated *TP53*

Interesting Feature(s)

The case raises the question of whether *TP53* mutated mixed phenotype acute leukemias with myeloid differentiation and especially with expression of MPO should not be classified as AML with mutated *TP53*.

Evidence of the rationale to reduce the "waste basket" of mixed phenotype acute leukemias for selected entities already exists:

- Lymphoblastic blast-phase CML and lymphoblastic M/LN-Eo are not considered MPAL
- AML with *RUNX1::RUNX1T1* fusion frequently express CD19, PAX5 and CD79a* and are not considered MPAL
- AML with *RUNX1*-mutations can aberrantly express CD79a and PAX5[#] and should not be considered MPAL
- Vice versa in ALL:
  - dim MPO expression in B-ALL is acceptable (not considered MPAL)[&]
  - one or more myeloid markers (CD11b, CD13, CD33, CD65, CD117) even define ETP-ALL.

References:

- *Cancer Res 2004;64:7399-404;
- [#]Pathobiology 2019;86:162;
- [&]Blood 2016;127:2391.

Panel Diagnosis

AML (WHO) /AML with TP53 mutation (ICC), with MPAL immunophenotype

EA4HP24-BMWS-19

Myeloid/Lymphoid neoplasm with FGFR1 rearrangement presenting in blast phase with MPAL phenotype

Dr. Anna Ruskova

Auckland City Hospital, Department of Pathology and Laboratory Medicine (LabPLUS), Auckland, New Zealand

Case Description

14-year-old girl, presented with 2 week's history of abdominal pain. Previously well. FBC showed pancytopenia and circulating blasts. Bone marrow aspirate 56% blasts with a phenotype of MPAL. Complex karyotype including t(2;8). FISH: signal pattern consistent with 8p11.23 (*FGFR1*) gene rearrangement in 84% of the nuclei.

Biopsy Fixation Details

Fixation formalin for 24 hrs. Decalcification 10% formic acid for 6 hrs

Frozen Tissue Available

none

Details of Microscopic Findings

FBC Hb 72g/L, MCV 107 fL, WCC 8.44 x 10^9/L, Blasts 1.9 Neu 0.4, Ly 5, Eos 0.54, Mon 0.08, Plt 108

BM aspirate: hypercellular

NRBCs 8%, Blasts 56%, Myelocytes 1%, Metamyelocytes 7%, Band+Seg Neu 17%, Eos 6%, Mon 2%, Ly 6%

Erythropoiesis: Moderately reduced. Normoblastic. No dysplasia.

Granulopoiesis: Mildly reduced with dysplastic changes - abnormal segmentation and hypogranulation

Megakaryocytes: Mildly reduced with normal morphology

Lymphoid Cells: normal

Other: Blasts medium to large with high N:C ratio and basophilic cytoplasm. Chromatin is immature with prominent nucleoli. No Auer rods and rare granulation is observed.

MPX: Blasts predominantly negative, 8% MPX positive blasts

BM trephine biopsy

Increased cellularity at 100% of the intertrabecular space.

Intertrabecular architecture abnormal with heavy infiltration by blasts.

Erythropoiesis: Markedly reduced cellularity

Granulopoiesis: Moderately reduced cellularity, band and segmented forms are present. Eosinophils relatively prominent

Megakaryocytes: Moderately reduced, normal morphology.

Other: large population of blast cells at 70% of all cells

Immunohistochemistry and other stains:

CD34 and TdT: CD34+ and TdT+ blasts account for 70% of the all cells

Immunophenotype

Blasts are identified by their side scatter and CD45 characteristics. There are two subsets of blasts which have similar CD19 and CD45 expression. The larger subset with low side scatter have a clear B-lymphoblastic phenotype with positive TdT, CD38, CD34, CD10, CD19, along with dim CD79a and very dim cCD22 expression. The second subset of blasts, showing a higher side scatter also express TdT and bright CD19 along with HLA-DR, CD117 (partial), CD34, CD38 (bright), CD13, CD33 and dim cyto CD79a expression. Myeloperoxidase is present in a small percentage of these blasts. There are no monocytoid or T-cell markers seen on either of the subpopulations.

Additional markers: CRLF2: negative

Cytogenetics

45,XX,t(2;8)(q37;p11.2),-7,t(7;14)(q32;q32)[19]/46,XX[1]

FISH:

Normal enumeration of chromosome 4, 10 and 17 centromeres

No *BCR::ABL1, KMT2A* or *ETV6::RUNX1* gene rearrangements

No *RUNX1T1::RUNX1* or *CBFB::MYH11* gene rearrangement

***FGFR1* gene rearrangement -** Nuclear FISH studies revealed a signal pattern consistent with a 8p11.23 (*FGFR1*) gene rearrangement in 84.3% of nuclei examined.

Molecular Studies

Myeloid targeted gene panel: 111 genes frequently found mutated in myeloid malignancies were analysed for mutations. The sample has a missense mutation in *PIK3CG* (V994L; VAF 18%) – variant of unknown significance

Bone marrow failure syndrome panel (performed @ Blueprint Genetics) –

Sequence and Del/Dup (CNV) analysis using the Blueprint Genetics Bone marrow failure syndrome panel did not detect any known disease-causing or rare variants that could explain the patient's phenotype.

Proposed Diagnosis

Myeloid/Lymphoid neoplasm with *FGFR1* rearrangement, presenting in blast phase with MPAL phenotype

Interesting Feature(s)

M/LN-Eo presenting in blast phase

Borderline BM eosinophilia, No PB eosinophilia

FGFR1 rearrangement can occur in de novo Ph-like ALL but the presence of blasts of both lymphoid and myeloid lineage favours M/LN-Eo

Panel Diagnosis

Myeloid/lymphoid neoplasm with FGFR1 rearrangement (WHO/ICC), presenting as MPAL (B/myeloid)

EA4HP24-BMWS-27

Acute undifferentiated leukemia with cryptic *NUP98::BPTF* fusion identified by NGS

Dr. Joanna L. Conant[1,2], Dr. Katherine A. Devitt[1,2], Dr. Patricia T. Greipp[3], Dr. Juli-Anne Gardner[1,2]

[1] University of Vermont Medical Center, Pathology and Laboratory Medicine, Burlington, USA; [2] Larner College of Medicine at the University of Vermont, Burlington, USA; [3] Mayo Clinic, Department of Laboratory Medicine and Pathology, Rochester, USA

Case Description

A previously healthy 35-year-old male presented with generalized abdominal tenderness, chest pain, and shortness of breath. CT scan showed liver masses and multiple enlarged lymph nodes. Complete blood count (CBC) showed anemia (Hgb 2.8 g/dL, RR 13.3-17.0 g/dL), leukocytosis (WBC 80.6K/cmm, RR 4.3K-10.6K/cmm), and thrombocytopenia (80K/cmm, RR 132K-337K/cmm). A diagnosis of T-cell acute lymphoblastic leukemia was made at an outside hospital. He was treated with hyperCVAD part A.

Two weeks later, he was seen at our institution for continuation of care and to proceed with hyperCVAD part B. CBC with differential showed mild anemia, normal WBC count, 33% blasts. Repeat bone marrow was consistent with acute undifferentiated leukemia. He was treated with CALGB 10403 before he developed multi-organ failure and died 10 weeks after initial diagnosis.

Biopsy Fixation Details

2nd marrow: Formalin fixation

Frozen Tissue Available

No

Details of Microscopic Findings

Peripheral blood revealed numerous blasts. Bone marrow approached 100% cellularity, and blasts accounted for 85% of nucleated cells.

Immunophenotype

1st marrow (by report): Blasts negative for myeloperoxidase (MPO) stain. Flow cytometry: Positive for CD34, CD38, HLA-DR, TdT, CD2 (dim), CD7, CD22, CD33, CD71 (dim), and CD56; negative for CD4, CD8, and other myeloid, monocytic, and B-cell markers. Presence/absence of CD3 expression was not documented.

2nd marrow: Immunohistochemical (IHC) staining: Positive for CD34 and TdT; Negative for CD3, CD79a, and MPO. Flow cytometry: Positive for CD34, CD38, HLA-DR, dim TdT, CD7 and CD33; Negative for CD2, CD3, cCD3, CD5, CD4, CD8, CD1a, CD19, CD22, cCD22, CD79a, CD10, cMPO, CD13, CCD117, CD14, CD64, and CD56.

Cytogenetics

Karyotype from both marrows showed a normal male karyotype (46,XY[20]). Second marrow demonstrated a cryptic *NUP98* rearrangement using fluorescence *in-situ*

hybridization (FISH) break-apart probe in interphase nuclei, following identification of fusion product by NGS.

Molecular Studies

1st marrow (by report): Next generation sequencing (NGS) DNA panel detected pathogenic variants in *APC* (p.Y96*, c.288T>G, 26.4% variant allele frequency, VAF), *ASXL1 (*p.G646Wfs*12, c.1934dupG, 41.2% VAF), *ETV6* (p.R359*, c.1075C>T, 86.9% VAF), *KDM6A* (p.V1113Sfs*41, c.3337_3339delins13, 68.5% VAF).

2nd marrow: NGS DNA assay detected previously identified variants in *ASXL1* (25.5% VAF), *ETV6* (60.2% VAF), *KDM6A* (71.4% VAF), and a pathogenic variant in *NOTCH1* (p.S2467*, c.7400C>A, 37.4% VAF). *APC* gene was not evaluated. RNA assay identified a cryptic *NUP98::BPTF* fusion. *NUP98* gene (11p15) and *BPTF* (17q24) gene were fused in-frame with *NUP98* exon 12 spliced to *BPTF* exon 21. T-cell receptor gene rearrangement analysis was negative.

Proposed Diagnosis

Acute undifferentiated leukemia (AUL) (ICC and WHO 5th ed).

Interesting Feature(s)

- Highlights critical need to classify disease based on lineage-specific markers vs. lineage-associated markers using flow cytometry and IHC staining. While there was dim CD2 & CD7 expression on the first bone marrow, no cytoplasmic or surface CD3 was reported for flow cytometry; CD3 IHC was not performed. Given lack of expression of any lineage-specific markers, a diagnosis of AUL is more appropriate.
- A cryptic *NUP98* rearrangement was identified by NGS and interphase FISH analysis, highlighting the importance of a multimodal approach to genetic evaluation.
- There are only 4 published reports of *NUP98::BPTF* fusions in hematologic malignancies: 3 AML and 1 T-ALL. All had similar breakpoints with fusions between *NUP98* exons 11-12, and *BPTF* exons 21-24. This is the first reported *NUP98::BPTF* fusion in AUL.

Panel Diagnosis

AUL (WHO/ICC)

EA4HP24-BMWS-51

Acute Undifferentiated Leukemia with *NPM1* mutation and extramedullary involvement, arising from prior myelodysplastic syndrome

Dr. Katelynn M. Wilton[1,2], Dr. Meredith Nichols[1,2], Dr. Jingwei Li[1,2], Mary Gerard[3], Dr. Eric Winer[3], Dr. Scott Lovitch[1], Dr. Ruben Carrasco[1], Dr. Geraldine Pinkus[1], Dr. Sam Sadigh[1]

[1] Brigham and Women's Hospital, Pathology, Boston, USA; [2] Massachusetts General Hospital, Pathology, Boston, USA; [3] Dana Farber Cancer Institute, Hematology / Oncology, Boston, USA

Case Description

A 64-year-old woman with macrocytic anemia was diagnosed with myelodysplastic syndrome with multilineage dysplasia and ring sideroblasts, with stable disease 4 years later **(B1).** She developed eye discomfort, monoclonal IgA kappa paraprotein (M-spike 0.04g/dL) and an ethmoid/orbital mass on imaging. Biopsy showed plasmacytoma. She underwent radiation therapy. Six months later, she had progressive fatigue with worsening anemia, thrombocytopenia and leukocytosis with circulating blasts. Bone marrow biopsy was performed **(B2)**. She received Vyxeos, with chemoablation, followed by decitabine/venetoclax, but had progression**.** Biopsy of an FDG-avid sinonasal mass was also performed **(B3).** She achieved remission and is undergoing evaluation for transplant.

Biopsy Fixation Details

Bone marrow biopsies: Bouins fixative, RapidCal Immuno decalcificationSinonasal biopsy: Formalin

Frozen Tissue Available

No

Details of Microscopic Findings

Initial bone marrow biopsy **(B1)** showed hypercellular myeloid predominant marrow with multi-lineage dysplasia and no increase in blasts. At the time of leukemia progression, bone marrow biopsy **(B2)** showed sheets of large-sized blasts (68%) with round to irregular nuclei, finely dispersed chromatin, variably distinct nucleoli and abundant basophilic cytoplasm with vacuolation. Sinonasal biopsy **(B3)** showed sheets of large-sized blasts with irregular or folded nuclei, smooth chromatin, distinct nucleoli and abundant amounts of eosinophilic cytoplasm.

Immunophenotype

At leukemia diagnosis **(B2)** blasts were:

Flow cytometry : $CD45^{dim}$ $CD56^{+}$ CD33+ HLA-DR^{var} $CD13^{dim}$ CD64+ CD34- CD117- CD14-

Immunohistochemistry: CD56+ CD31+ $CD117^{weak}$ $CD123^{weak}$ mutant-$NPM1^{diffuse/strong}$ CD34- lysozyme- CD45- MPO- CD3- CD20- CD4- CD68-CD163- CD43- CD15- CD14-

After treatment, flow cytometry showed immunophenotypically similar blasts.

In the sinus biopsy (**B3**), blasts were CD33+ lysozyme^{weak} CD117^{weak} mutant-NPM1+ CD138- MPO-

Cytogenetics

Before treatment **(B1-2)**: 46XX[20]

After treatment: 48,XX,+8,+8,add(19)(p13)[cp16]/46,XX[4]

Molecular Studies

An 88 gene myeloid NGS panel showed:

At MDS diagnosis (**B1**): ***CBL***(c.1151G>A p.C384Y, 3.4% VAF), ***SF3B1***(c.2098A>G p.K700E, 27.4% VAF)

At leukemia diagnosis (**B2)**: ***CBL*** (c.1151G>A p.C384Y, 10.8% VAF), ***SF3B1*** (c.2098A>G p.K700E, 20.3% VAF), ***NPM1***(c.859_860insTCTG, p.W288Cfs*12, 13.3% VAF), ***RUNX1***(c.326A>T, p.N109I, 1.5% VAF)

Post therapy: ***CBL***(c.1151G>A p.C384Y, VAF 26.2%), ***NPM1*** (c.859_860insTCTG, p.W288Cfs*12, 32.8% VAF), two new variants in ***FLT3*** (c.2503G>C, p.D835H, 26.2% VAF; c.2503G>T, p.D835Y, 5% VAF)

Chemoablated marrow: ***SF3B1*** (c.2098A>G p.K700E, 4% VAF).

Proposed Diagnosis

Acute undifferentiated leukemia with mutated *NPM1*, arising from prior MDS, with extramedullary involvement of prior plasmacytoma site.

Interesting Feature(s)

Despite a myeloid mutational profile, *NPM1* mutation and previous clonally-related MDS, this acute leukemia lacked lineage defining markers and therefore cannot currently be classified as acute myeloid leukemia. This raises the question of whether *NPM1* mutation or progression from clonally-related MDS should be considered myeloid lineage defining. This acute leukemia also colonized an irradiated prior plasmacytoma site suggesting that tumor and radiation-related changes may have supported leukemia colonization. While polysomy 8, *NPM1*, *RUNX1* and *FLT3* mutations accumulated during disease progression, *SF3B1* mutation disappeared in leukemia and reappeared post-chemoablation, reflecting possible exclusion from leukemia cells.

Panel Diagnosis

AML-MR (WHO/ICC)

EA4HP24-BMWS-54

Acute leukemia with *PICALM::MLL10* fusion

Alexandra E. Kovach[1,2], Karin P. Miller[1,2], Jamie Stokke[2,3], Gordana Raca[1,2]

[1] Children's Hospital Los Angeles, Pathology and Laboratory Medicine, Los Angeles, USA; [2] University of Southern California, Keck School of Medicine, Los Angeles, USA; [3] Children's Hospital Los Angeles, Pediatrics, Hematology/Oncology, Los Angeles, USA

Case Description

A 17-year-old previously healthy male presented with leukocytosis, lymphadenopathy and an anterior mediastinal mass. Peripheral blood flow cytometry was performed and chemotherapy initiated due to airway compromise. Following initial treatment, a bone marrow biopsy was performed two weeks after diagnosis. Eleven months after diagnosis, a recurrent mediastinal mass was identified.

Biopsy Fixation Details

Bone marrow (two weeks after chemotherapy initiation): Formalin and EDTA decalcification.

Recurrent mediastinal mass (eleven months after diagnosis): Formalin.

Frozen Tissue Available

No

Details of Microscopic Findings

Peripheral blood from diagnosis: Large blasts with scant agranular basophilic cytoplasm and ovoid nuclei.

Bone marrow after chemotherapy initiation: Approximately 25% leukemia.

Recurrent mediastinal mass 11 months after diagnosis: Sheets of intermediate-sized mononuclear cells with irregular nuclear contours, finely dispersed chromatin and moderately abundant eosinophilic cytoplasm.

Immunophenotype

Peripheral blood flow cytometry from diagnosis: Positive for CD7, CD13, CD33 (dim), CD34, CD38, CD45, CD56 (partial), CD71, CD123 and HLA-DR (partial); negative for cCD3, CD5, CD19, CD41, CD42, CD61, cCD79a, CD117, and cMPO.

Bone marrow after chemotherapy initiation: Identical flow cytometry results to those of prior peripheral blood; CD61 negative by immunohistochemistry.

Recurrent mediastinal mass: Positive for CD4, CD7 (weak, partial), CD13, CD33, CD34, CD45, CD71 (variable), CD117 (partial), HLA-DR, cMPO (partial) and TP53; negative for c/sCD3, CD19 and CD56 (immunohistochemistry and/or flow cytometry).

Cytogenetics

Peripheral blood: An abnormal rearrangement between the *PICALM* gene (11q14.2-21) and the *MLL10* gene (10p12-13) was detected by conventional karyotype: 46,XY,t(10;11)(p12;q21)[5]/46,XY[15]. No abnormalities were detected on a standard acute myeloid leukemia (AML) fluorescence *in situ* hybridization (FISH) panel. No copy number

abnormalities of established clinical significance were detected by chromosome microarray (CMA).
Recurrent mediastinal mass: Copy number losses in the 8q, 9q and 17p loci by CMA not present at diagnosis; the 17p deletion encompasses the *TP53* gene.

Molecular Studies

Peripheral blood: PICALM::MLLT10 fusion by RNA analysis and variants of strong clinical significance in the *ASXL2*, *PHF6*, *WT1* and *KRAS* genes by DNA analysis were detected. FLT3-ITD and TKD mutations were not detected.
Recurrent mediastinal mass: The previously detected *PICALM::MLL10* fusion and *KRAS* and *PHF6* variants of strong clinical significance were again detected. A *TP53* (p.Arg273His) variant of strong clinical significance (VAF 87%) not present in previous samples was detected. The previously identified *WT1* and *ASXL2* variants were not detected. Again, FLT3-ITD and TKD mutations were not detected.

Proposed Diagnosis

Acute leukemia with *PICALM::MLL10* fusion

Interesting Feature(s)

This unique fusion was initially reported in T-lymphoblastic leukemia/lymphoma (T-ALL) but was later also observed in AML in the mediastinum and even rare cases of B-ALL, underlying the stem-ness and lineage plasticity of this rare disease. Indeed, the recurrence in this case showed evidence of clonal evolution, including biallelic *TP53* alterations (mutation and copy number loss), as well as myeloid differentiation from an initially undifferentiated pretreatment phenotype.

Panel Diagnosis

AUL (WHO/ICC)

EA4HP24-BMWS-59

Early T-cell precursor subtype T-lymphoblastic leukemia (ETP-ALL) initially presenting with mixed/ambiguous phenotype

Prof. Jun Wang[1], Dr. Andrea Shields[1], Dr. Yan Liu[1], Dr. Michael Greas[1], Dr. Albert Kheradpour[2]

[1] Loma Linda University Health, Pathology, Loma Linda, USA; [2] Loma Linda University Health, Pediatric Hematology, Loma Linda, USA

Case Description

12-year-old male presented with a history of recent weight loss, progressive fatigue, decreased appetite, intermittent fevers, and intermittent joint swelling. Initial labs were significant for leukocytosis of 66.8 bil/L, anemia with Hgb of 5.0 g/dL, and

thrombocytopenia of 78 bil/L. Flow cytometry on the peripheral blood as well as a bone marrow biopsy was performed.

Biopsy Fixation Details

The marrow aspirate clot was fixed in 10% neutral buffered formalin and then embedded in paraffin, then sectioned at 3- to 4-mm thickness and stained with hematoxylin-eosin or counterstained with hematoxylin for immunohistochemistry.

Frozen Tissue Available

No

Details of Microscopic Findings

The peripheral blood smear showed marked leukocytosis with numerous circulating blasts (>90%), marked normocytic anemia and moderate thrombocytopenia. The hemodilute marrow aspirate smears showed sheets of blasts (>90%) with minimal residual hematopoiesis. These blasts were medium in size with very high N:C ratio, smooth chromatin, occasional nucleoli and scanty amount of deeply basophilic cytoplasm.

The marrow clot sections were marked hemodilution with only a tiny marrow elements present**,** consisting mostly of blasts.

Immunophenotype

By immunohistochemistry CD3 and PAX5 highlighted only scattered background T-cells and B-cells in marrow sections. CD34 stained blasts (>70%). CD117 and TdT highlighted ~40% of the blasts. Initial flow cytometry of both blood and bone marrow aspirate showed a blast population (~93% of total events) expressing CD45 variable, CD7, CD19 very dim, CD33 dim, CD34, CD117 partial, CD71, CD11b dim, CD38, CD58, CD235a subset, TdT dim and HLA-DR, but not CD20, CD2, sCD3/cCD3, CD14, CD13, CD15, CD22, CD42b, MPO, CD56 or CD123. An extensive flow cytometry showed the cells expressed cCD3 variable, CD5 increased, CD7 increased, CD13, CD19 increased, CD33 increased, CD34, CD38, CD45, CD71 increased, cCD70a increased, CD117 decreased, CD123, HLA-DR and SYTO16. The cells were negative for sCD3, CD4, CD8, CD14, CD15, CD16, CD48, CD56, CD64 and cMPO.

Cytogenetics

Chromosome studies showed 46,XY,ins(5;11)(q31;q21q13),der(10)add(10)(p12)add(10)(q22), ins(11;10)(q14;p13p11.2),der(12)t(10;12)(p11.2;p13), add(14)(q24)[14]/46,XY[6].

AML FISH panel was negative for rearrangements.

T-ALL FISH panel showed a t(10;11) MLLT10/PICALM fusion (85%) and deletion of 14q32(BCL11Bx1) in 95%.

Molecular Studies

NGS for AML (11 gene panel) showed no pathogenic genetic alterations.

T-cell receptor gene rearrangement studies were negative.

Proposed Diagnosis

Early T-cell precursor subtype T-lymphoblastic leukemia (ETP-ALL)

Interesting Feature(s)

This case is very interesting due to the complex immunophenotype and expression of markers of multiple lineages making the diagnosis very challenging. The blast population expressed CD7, CD19 dim, CD117 partial, CD71, CD11b dim and TdT dim, but not CD3 (surface or cytoplasmic), MPO or CD22 by conventional flow cytometry. The findings were initially considered an undifferentiated vs. an early vs. a mixed phenotype acute leukemia. Despite

expression of some B-cell markers and myeloid markers, but none of them was sufficiently expressed lineage-associated antigens to categorize them into those lineages. However, the special flow cytometry revealed expression of CD5, absent CD4/CD8 and variable cCD3 favoring a T progenitor population, i.e., early T-cell precursor subtype T-lymphoblastic leukemia (EPT-ALL). The detections of complexed chromosomal abnormalities and a t(10;11) MLLT10/PICALM fusion (85%) and deletion of 14q32(BCL11Bx1) by T-ALL FISH panel further supported a diagnosis of EPT-ALL.

Panel Diagnosis
ETP-ALL (WHO/ICC)

EA4HP24-BMWS-65

Acute Undifferentiated Leukemia with PHF6 mutation and clonal T-cell receptor and immunoglobulin gene rearrangement

Yi Sun

Robert Wood Johnson University Hospital, Pathology, New Brunswick, USA

Case Description
A 38-year-old man presented for evaluation of right upper quadrant pain. CBC revealed leukocytosis with many blasts. WBC: 49.19 K/uL, Hb: 13.6 g/dL, PLT: 112K/uL.
Biopsy Fixation Details
Bone marrow core biopsy was fixed in 10% formaldehyde (formalin) for at least 1 hour before putting in IMMUNOCAL for 3 hours for decalcification
Frozen Tissue Available
N/A
Details of Microscopic Findings
Peripheral blood smear shows Leukocytosis with 82% blasts that are variable in size, with irregular nuclei, high nuclear to cytoplasmic ratio, prominent nucleoli, pale basophilic cytoplasm and some contain cytoplasmic vacuoles. No Auer-rod is identified. Thrombocytopenia is also present.
Bone marrow aspirate smears and core biopsy show marked hypercellular bone marrow with extensive involvement by blasts, comprising more than 90% of total cellularity. Trilineage hematopoiesis is diminished. No significant dysplasia is seen.
Immunophenotype
Immunophenotying by flow cytometry identifies an abnormal blast population expressing CD34, CD33, partial CD123, HLA-DR, partial dim CD38, small subset CD36(~5%), small subset CD64 (~5%), small subset MPO (<3%). A minute population of blasts (about 1% of total blasts)

weakly express CD19, cCD79A, cCD22, partial CD33 and TdT. The blasts do not express other myelomonocytic or T-cell lineage markers tested.
By immunohistochemistry, the blasts are positive for CD34, CD33, subset TdT, small subset CD79A, while negative for CD117, PAX5, CD19, CD20, glycophorin A, E-Cadherin, CD3, OCT-2 and lysozyme.

Cytogenetics

47,XY,+4,t(11;12)(q21;p12),?dup(17)(q22q23)[18]/46,XY[2]
Abnormal male karyotype with trisomy 4, translocation between chromosomes 11 and 12, and a duplication or translocation of region 17q22 in 18 of 20 cells

Molecular Studies

Myeloid NGS analysis reveals PHF6-C99fs inactivating mutation (VAF 90.5%).
PCR for B-cell receptor and T-cell receptor gene rearrangements are both positive
Clonoseq study detects three dominant B-cell sequences (2 IGH and 1 IGK sequences, with ~6-8% frequency per total nucleated cells) and 1 dominant TCRG sequence (>99% frequency per total nucleated cells) suitable for clonality tracking.

Proposed Diagnosis

Acute undifferentiated leukemia

Interesting Feature(s)

Acute undifferentiated leukemia (AUL) is a very rare type of acute leukemia that lacks lineage-specific marker expression by current WHO classification. Due to its rarity, little is known about its genetic profile and cell of origin. Here we present a case of AUL that by next generation sequencing, positive for *PHF6* mutation, and interestingly, positive for both clonal T-cell and B-cell receptor (*BCR/TCR*) rearrangements. PHF6 (plant homeodomain finger 6) mutations were found in 38% of adults and ~ 16% of pediatric T lymphoblastic leukemias, 3% of acute myeloid leukemias, and reported to be one of the most frequent mutations in AULs (33%). PHF6 is speculated to function as a tumor suppressor or an oncogene, in a lineage-dependent manner. It is also shown to play a role in lineage plasticity of hematopoietic malignancies and skewed T cell differentiation. While whether the high frequency of rearrangement in the TCR gene loci indicate commitment to the lymphoid lineage of leukemic cells remains to be determined, the unique clonal sequences identified provide a more sensitive and accurate way to monitor minimal residual disease (MRD). More studies are needed to investigate the role of PHF6 in lymphoid lineage differentiation and frequency of TCR and/or immunoglobulin receptor rearrangement in AULs, which may provide valuable diagnostic, prognostic and therapeutic strategies.

Panel Diagnosis

AUL (WHO/ICC)

EA4HP24-BMWS-76

Hematopoietic neoplasm of ambiguous lineage

Dr. Nives Zimmermann, Dr. Anna Balog, Dr. Youssef Khafateh, Dr. Ahmad Mansour

University of Cincinnati, Pathology and Laboratory Medicine, Cincinnati, USA

Case Description

The patient is an 80-year-old man who presented with cervical lymphadenopathy and weight loss, found to have spontaneous tumor lysis syndrome. Imaging demonstrates diffuse marked lymphadenopathy. He initially had peripheral blood flow cytometry, lymph node and bone marrow biopsy performed at outside hospital. Peripheral blood flow cytometry showed 3% undifferentiated abnormal immature cells. Initial considerations on the lymph node and bone marrow biopsies included blastic plasmacytoid dendritic cell neoplasm and coexisting lymphoblastic leukemia/lymphoma. Following expert consult, the process was called “Immature hematopoietic neoplasm with evidence of monocytic and plasmacytoid dendritic cell lineage”. The patient transferred to our institution and underwent repeat lymph node excisional and bone marrow biopsy. The bone marrow had ~9% atypical immature cells with no obvious lineage differentiation, and the lymph node was involved by immature hematopoietic neoplasm, with molecular and immunohistochemical evidence "suspicious for chronic myelomonocytic leukemia" (per expert consultation). Six weeks after initial presentation, there were >20% circulating immature hematopoietic cells and based on peripheral blood flow cytometry the diagnosis of “acute leukemia of ambiguous lineage” was made, and the patient was treated accordingly.
Slides provided are those of the lymph node, as it has greater involvement by atypical cells. Peripheral blood flow cytometry images are in the attached presentation.

Biopsy Fixation Details

Formalin

Frozen Tissue Available

No

Details of Microscopic Findings

There is partial effacement of lymph node architecture with an atypical proliferation of mononuclear cells. The atypical cells have moderate to abundant cytoplasm, irregular nuclear contours with fine chromatin.

Immunophenotype

By immunohistochemistry of the lymph node, the atypical cells are positive for CD43, CD4, CD68(partial), TdT(?, partial), CD7(partial), and negative for CD3, CD20, CD123, CD56, CD30, CD1a, ALK.
Flow cytometric analysis of peripheral blood identified 39% are immature hematopoietic cells with the following immunophenotype: CD34+(partial), CD7+, CD5+(dim), CD11b+(dim),

CD33+, CD38+ and CD56+(subset) and negative for CD123, CD20, CD4, CD8, CD14, HLA-DR, CD117, CD2, MPO and CD64.

Cytogenetics

Karyotype (bone marrow): 46,XY[20]

Molecular Studies

Bone marrow NGS: *IDH2*.R140Q (VAF 6.1%), *NRAS*.G13R (VAF 3.1%).

Lymph node NGS: pathogenic *NRAS* mutations (G13R and G13D, VAFs of 21% and 3.4%, respectively), *IDH2*.R140Q (VAF 25%), and an out-of-frame alteration of SCOR at 2.7%. A *CSF2RA-CRLF2* fusion of uncertain significance was detected.

Proposed Diagnosis

Acute leukemia of ambiguous lineage, NOS

Interesting Feature(s)

This case highlights the difficulty of assigning lineage in some cases. Over 6 different specimens, multiple lineages were considered, including plasmacytoid dendritic cells, monocytes and lymphocytes. However, neither fit perfectly and thus we felt this case best fits under "ambiguous lineage" characterization. This difficulty in characterizing the process led to delay in diagnosis and treatment.

The other interesting feature of this case is its involvement of both the bone marrow/peripheral blood and lymphadenopathy. While molecular studies support it is the same process, the immunophenotype had some notable differences (for instance, CD4 and CD7). This may be due to differential recruitment of neoplastic cells that are differentiating towards different lineages to different organs, and/or the immunophenotype and/or differentiation program may be influenced differently in different microenvironments.

Panel Diagnosis

AUL (WHO/ICC)

EA4HP24-BMWS-109

Early T-cell Precursor (ETP) Acute Lymphoblastic Leukemia/ Lymphoma (ETP-ALL)

Dr. Swati Gite, Dr. Sucheta Malik, Dr. Nourhan Ibrahim, Dr. Sibel Sibel AK, Dr. Jacob Armstrong, Dr. Brenda Mai

University of Texas Health Science Center at Houston, Department of Pathology and Laboratory Medicine, Houston, USA

Case Description

31-year-old man who was a transfer to our facility for further evaluation and treatment of acute leukemia after presenting with bone and joint pain.

His complete blood count showed a white blood cell count of 4,800 ×109/L, hemoglobin of 14.9 g/dl, and platelet count of 60×109/L.

Biopsy Fixation Details

Formalin fixed formic acid-decalcified paraffin-embedded tissue

Frozen Tissue Available

None

Details of Microscopic Findings

- Examination of the peripheral blood smear revealed approximately 15-20% atypical blasts characterized by a notably high nuclear to cytoplasmic ratio, irregular notched nuclear outlines, fine chromatin, and minute nucleoli; the absence of cytoplasmic granules and Auer rods was noted.
- The bone marrow (BM) biopsy displayed hypercellularity (90-100%), predominantly composed of sheets of immature lymphoid cells and exhibiting reduced trilineage hematopoiesis.

Immunophenotype

- An aberrant T-lymphoblast population was identified (approximately 92% of events analyzed) with the following immunophenotype:

- Positive: CD45 (dim), cCD3, CD5 (subset, dim), CD7 (bright), CD10 (partial, dim), CD33 (partial), CD34, CD38, CD43, CD117 (subset), nTdT (dim)
- Negative: CD1a, CD2, sCD3, CD4, CD8, CD11b, CD11c, CD13, CD14, CD16, CD19, CD20, CD56, CD64, HLA-DR, cMPO

- The blasts were small (low to intermediate forward scatter) with low side scatter.

Cytogenetics

48,XY,+Y,+Y,der(16)t(16;17)(q22;q21)[14]/46,XY[6]

Cytogenetic analysis showed an abnormal male karyotype. Fourteen cells show two extra copies of the Y chromosome and a derivative chromosome 16, consisting of an unbalanced translocation between chromosomes 16 and 17.

Next generation sequencing (NGS) detected 3 mutations: ARID1A, PHF6, and WT1.

Molecular Studies

Next generation sequencing (NGS) detected three (3) mutations:

1. ARID1A,
2. PHF6, and
3. WT1.

Proposed Diagnosis

Early T-precursor lymphoblastic leukemia (ETP-ALL)

Interesting Feature(s)

- Early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) stands out as a distinct subtype within T lymphoblastic leukemia/lymphoma (T-ALL/LBL).
- ETP cells exhibit similarities with hematopoietic stem cells and myeloid progenitor cells.
- The classification of ETP-ALL is rooted in the immunophenotype of leukemic cells, typically characterized by CD1a−, CD8−, CD5− (dim), and positivity for one or more stem cell or myeloid antigens.
- ETP-ALL has been documented in approximately 10% of childhood T-ALL/LBL cases and around 7% of adult T-ALL/LBL cases.

- Its noteworthy distinguishing feature lies in its unique immunophenotypic and genomic profile.
- Earlier studies indicated a poor prognosis for ETP-ALL, marked by induction failure and a higher incidence of relapse/refractory disease.
- Recent strides in understanding genetic aberrations and molecular pathogenesis have been made, yet the diagnosis and management of ETP-ALL remain challenging.
- In this case of ETP-ALL, the pivotal aspect lies in its distinctive molecular profile.
- PHF6, situated on Xq26.2, encodes a protein involved in transcriptional regulation.
- WT1, located on 11p13, encodes a transcription factor and is present in 10% of T-cell acute lymphoblastic leukemia/lymphoma.
- Lastly, ARID1A, located on 1p36.11, possesses helicase and ATPase activities.
- ARID1A expression is notably downregulated in the bone marrow of acute myeloid leukemia patients, serving as both a predictor for therapeutic responses and a tracking marker for remission.
- The presence of ARID1A suggests an overlap of ETP-ALL with the myeloid lineage, necessitating further investigation for treatment and prognostic stratification.

Panel Diagnosis

ETP-ALL (WHO/ICC)

EA4HP24-BMWS-126

Mixedphenotype acute leukemia (MPAL) with BCR::ABL1 fusion

Dr. Sibel Ak, Dr. Nourhan Ibrahim, Dr. Levani Iashvili, Dr. Jacob Armstrong, Dr. Brenda Mai

The University of Texas Health Science Center at Houston, McGovern Medical School, Department of Pathology and Laboratory Sciences, Houston, USA

Case Description

A 37-year-old female with ovarian cancer status post total hysterectomy without chemotherapy or radiotherapy, presented with fatigue, shortness of breath, and chest pain. Her complete blood count showed leukocytosis (35.5 x 109/L), macrocytic anemia (Hb 6.9 g/dL), and thrombocytopenia (131x109/L). She had hepatosplenomegaly on abdomen computerized tomography scan.

Biopsy Fixation Details

Formalin-fixed, formic acid-decalcified, paraffin-embedded tissue.

Frozen Tissue Available

None

Details of Microscopic Findings

The peripheral blood smear showed approximately 20% circulating blasts and blast equivalents; some blasts were small to intermediate in size, with scant cytoplasm, fine to variably clumped chromatin, and occasional nucleoli while some blasts were intermediate to large in size, with abundant cytoplasm, folded/convoluted nuclei, fine chromatin, and prominent nucleoli. There were scattered immature granulocytes and occasional hypogranular neutrophils. No eosinophilia or basophilia was noted. The bone marrow aspirate revealed approximately 50% blasts with two distinct populations, similar to peripheral blood smear findings. Rare megakaryocytes had small hypolobated forms.The bone marrow biopsy was hypercellularity (>90%) with interspersed blasts and decreased granulocytic-predominant trilineage hematopoiesis.

Immunophenotype

Flow cytometric immunophenotyping of her bone marrow demonstrates two aberrant immature cells of B-cell and myeloid populations. The immature B cells (approximately 30% of analyzed events) were positive for CD45 (dim), CD10 (partial), CD13 (dim), CD19, CD20 (small subset, ~7%) CD33 (partial), CD38 (partial), CD56 (partial), CD117 (partial), CD123, HLA-DR, and nTdT. The aberrant immature myeloid population (approximately 17% of analyzed events) was positive for CD45 (decreased, brighter than B-lymphoid population), CD2 (partial), CD4 (dim), CD5 (subset), CD11c, CD13, CD33, CD34 (dim), CD38, CD56 (bright), CD64 (partial), CD117 (partial), CD123, HLA-DR and negative for CD3 (surface and cytoplasmic), CD7, CD10, CD11b, CD14, CD16, cMPO, and nTdT, consistent with monocytic differentiation.

Cytogenetics

Karyotype analysis showed an abnormal female karyotype
(46,XX,t(9;22)(q34.1;q11.2)[5]/46,idem,add(21)(q22)[13]/46,XX[2])
FISH analysis was positive for t(9;22) BCR-ABL1 fusion and IgH (14q32) rearrangement.

Molecular Studies

PCR was positive for p190 BCR::ABL1 fusion transcript.

Proposed Diagnosis

Mixed phenotype acute leukemia with BCR::ABL1 fusion

Interesting Feature(s)

·This case presents an interesting diagnostic dilemma.
·B-lymphoblastic leukemia (B-ALL) with BCR::ABL1 fusion can express myeloid-associated antigens and acute myeloid leukemia (AML) with BCR::ABL1 fusion may aberrantly express lymphoid antigens.
·In addition, distinguishing mixed phenotype acute leukemia (MPAL) with BCR::ABL1 from mixed-phenotype blast phase of CML is also challenging.
·In general, CML blast phase with a mixed phenotype is uncommon.
· It has been reported that patients might lack classic CML features like basophilia or a prominent left-shift, creating a diagnostic challenge, particularly in distinguishing it from MPAL with BCR::ABL fusion.
·In our case, the patient had hepatosplenomegaly but her peripheral blood revealed elevated blasts without eosinophilia or basophilia.
·Her bone marrow aspirate also had a subset of megakaryocytes that had small hypolobated forms, similar to that seen in CML.

·Classification of her acute leukemia was difficult, however, without a prior diagnosis of CML, a CML blast phase with mixed phenotype could not be diagnosed, but could not be entirely ruled out.

Panel Diagnosis

MPAL, B/myeloid (WHO/ICC) with BCR::ABL1 fusion

EA4HP24-BMWS-131

Mixed Phenotype Acute Leukemia with Monocytic Differentiation

Dr. Adelaide Kwon, Dr. Miguel Cantu, Dr. Rolando Garcia, Dr. Prasad Koduru, Dr. Jeffrey SoRelle, Dr. Franklin Fuda, Dr. Olga Weinberg

University of Texas Southwestern Medical Center, Pathology, Dallas, USA

Case Description

A 27-year-old male with no past medical history presented with two weeks of petechial rash and cervical lymphadenopathy. Peripheral blood demonstrated leukocytosis (white cell count $37x10^9$/L) with absolute neutropenia, anemia (Hgb 6.2 g/dL), thrombocytopenia (platelets $16x10^9$/L), and 85% circulating blasts. A bone marrow aspirate and biopsy were performed. The patient was started on induction chemotherapy.

Biopsy Fixation Details

The bone marrow biopsy was fixed in formalin and decalcified.

Frozen Tissue Available

No frozen tissue was available for this case.

Details of Microscopic Findings

In the peripheral blood, circulating blasts (85%) demonstrated variable size, immature chromatin, irregular nuclei, increased nuclear-cytoplasmic ratio, and scant pale cytoplasm. In the bone marrow aspirate, blasts comprised 96% of cells and demonstrated similar variable size, open chromatin, irregular nuclei, and scant pale cytoplasm, as well as occasional prominent nucleoli. However, a subset of the blasts were of larger size with ample cytoplasm. Bone marrow core biopsy demonstrated hypercellular marrow (~100% cellularity) consisting of sheets of blasts, with markedly decreased trilineage hematopoiesis.

Immunophenotype

By immunohistochemistry, blasts were variably positive for CD19 (50-60%), negative for MPO, and positive for TdT (>90%). A subset (5-10%) of the blasts were positive for lysozyme. 10-color flow immunophenotypic analysis revealed a 95% population of immature cells that were CD2(-), surface and cytoplasmic CD3(-), CD4(+), CD7(predominantly-/small subset partial+), CD9(+), CD10(-), CD11b(partial+), CD13(predominantly-), CD14(subset+),

CD15(partial+), CD19(+), CD20(-), CD22(partial+), CD24(+), CD25(predominantly-), CD33(+), CD34(partial+), CD36(partial+), CD38(dim+), CD45(partial+), CD56(-), CD64(small subset+), CD79a(+), CD81(+), CD117(-), CD123(partial+), HLA-DR(+), surface immunoglobulin light chains(-), MPO(-), and TdT(partial+).

Cytogenetics

Cytogenetic studies showed an abnormal male karyotype with isochromosome 7q, as follows: 46,XY,i(7)(q10)/46,XY. Fluorescent in-situ hybridization studies demonstrated no evidence of *CRLF2* rearrangement, aneuploidy of chromosomes 4 or 10, t(8;21), t(9;22), *KMT2A* rearrangement, t(12;21), *PML::RARA*, or inversion/translocation of chromosome 16.

Molecular Studies

Next generation sequencing studies revealed mutations in *PAX5, NRAS, JAK2*, and *U2AF1*; amplification of *BRAF*; loss of *IKZF1*; loss of heterozygosity in *NRAS* and *PIK3CD*; and a *DDX5::HMGB1* fusion.

Proposed Diagnosis

The patient was diagnosed with mixed phenotype acute leukemia, B/myeloid.

Interesting Feature(s)

This case demonstrates blasts with predominantly B-lineage markers but with a smaller subset (~10%) that are weak for CD19 and CD79a and strongly express myelomonocytic markers (lysozyme, CD11b, CD11c, CD14, and CD64). The identified *PAX5* and *IKZF1* variants are associated with B cell neoplasia while the *NRAS* and *U2AF1* variants often drive myeloid disease. Together, these findings support a bilineal mixed phenotype acute leukemia with rare monocytic differentiation.

Panel Diagnosis

B-ALL (WHO/ICC) with partial monocytic differentiation

EA4HP24-BMWS-132

Mixed Phenotype Acute Leukemia with Complex Karyotype

Dr. Adelaide Kwon, PhD/MD Mingyi Chen, Dr. Prasad Koduru, Dr. Jeffrey SoRelle, Dr. Franklin Fuda, Dr. Olga Weinberg

University of Texas Southwestern Medical Center, Pathology, Dallas, USA

Case Description

A 56-year-old male with a history of diabetes, coronary artery disease, and heart failure presented with four months of fatigue and pancytopenia (white cell count 2.7x10^9/L, Hgb 9.0 g/dL, platelets 89x10^9/L) with 25% circulating blasts. A bone marrow aspirate and biopsy were performed. The patient was started on induction chemotherapy.

Biopsy Fixation Details

The biopsy was fixed in formalin and decalcified.

Frozen Tissue Available

None.

Details of Microscopic Findings

In the peripheral blood, circulating blasts (25%) demonstrated medium to large size, scant cytoplasm, large nuclei, open chromatin, and prominent nucleoli. Erythrocytes demonstrated anisopoikilocytosis and frequent ovalocytes. In the bone marrow aspirate, sheets of blasts comprised 69% of cells and were of similar medium to large size with scant cytoplasm, large nuclei, open chromatin, and prominent nucleoli. Bone marrow core biopsy demonstrated hypercellular marrow (80-90%) consisting of sheets of blasts replacing 70-80% of marrow cellularity, with decreased trilineage hematopoiesis.

Immunophenotype

By immunohistochemistry, blasts were mostly positive for CD34 (60-70%) and negative for lysozyme and E-cadherin. Subsets of blasts were positive for CD117 (15-20%), TdT (40-50%), and PAX5 (40-50%). 10-color flow immunophenotypic analysis revealed a 49% population of immature cells that were CD5(-), CD10(-), CD13(mostly-to dim+), CD19(+), CD20(predominantly-), CD22(partial+), CD33(-), CD34(+), CD38(+), CD45(partial dim+), CD79a(+), CD117(-), CD123(-), HLA-DR(+), MPO(-), Lambda(-), Kappa(-), and TdT(+), along with a 13% myeloblast population that was CD7(-), CD11b(dim+), CD13(variably+), CD14(-), CD15(variably+), CD16(-), CD19(-), CD20(-), CD22(variably+), CD25(-), CD33(variably+), CD34(+), CD36(-), CD38(variably+), CD45(partial dim+), CD56(partial+), CD64(partial+), CD79a(-), CD117(+), CD123 (variably+), HLA-DR(variably+), MPO(+), and TdT(-).

Cytogenetics

Cytogenetic studies showed an abnormal complex male karyotype with three related clones demonstrating multiple structural and numerical abnormalities, as follows: 46~71,XY,add(1)(p22),add(4)(q21),der(4)t(4;9)(q31.1;q22),add(5)(q11.2),-7,der(9)t(1;9)(p22;q13),+10,
+ider(11)(q10)del(11)(q11q21)x3,t(12;15)(p13;q22),del(14)(q22),add(17)(p11.2),+22/46~53,idem,add(2 (q37)/49,idem,der(2;11)(q10;q10),add(18)(q23)/46,XY. Of note, this karyotype includes a partial loss of 5q, monosomy 7, and loss of 17p; the involvement of these chromosomes is often associated with myelodysplastic syndromes or acute myeloid leukemias. Fluorescent in-situ hybridization studies demonstrated amplification of *KMT2A* (11q23) in 87%, loss of *CBFB* (16q22) in 52.5%, and an extra copy of *BCR* (22q11.2) in 29.5% of cells examined, without evidence of t(8;21), t(9;22), *KMT2A* rearrangement, *PML::RARA*, or inversion/translocation of chromosome 16.

Molecular Studies

Next generation sequencing studies revealed mutation and amplification of *KMT2A*; mutation and loss of *TP53*; mutation of *BCORL1*; amplification of *IDH1*; loss of *ASXL1, CDKNB1, ETV6*, and *TET2*; losses of chromosomes 16, 5q, and 7q; and loss of heterozygosity of *FBXW7*.

Proposed Diagnosis

Mixed phenotype acute leukemia (MPAL), B/myeloid.

Interesting Feature(s)

MPAL with a complex karyotype is a rare, somewhat controversial entity with limited clinical and genetic data. Both the 5th edition of the World Health Organization classification and the International Consensus Classification of Myeloid and Lymphoid Neoplasms classification exclude MPAL with complex karyotype from the MPAL category to include them in the myelodysplasia-related categories of acute myeloid leukemia.

Panel Diagnosis

AML-MR (WHO/ICC) with MPAL immunophenotype

EA4HP24-BMWS-134

AUL with KMT2A rearrangement

Dr. Levani Iashvili, Dr. Nourhan Ibrahim, Dr. Sibel Ak, Dr. Hanadi El Achi, Dr. Jacob Armstrong, Dr. Brenda Mai

The University of Texas Health Science Center at Houston, Pathology and Laboratory Medicine, Houston, TX, Germany

Case Description

-This is a 56-year-old male presenting with persistent pancytopenia. He has alcoholic cirrhosis complicated by hepatocellular carcinoma status post chemoradiation and liver transplantation.

Biopsy Fixation Details

- Formalin fixed formic acid-decalcified paraffin-embedded tissue

Frozen Tissue Available

- None

Details of Microscopic Findings

Bone marrow:

- The bone marrow aspirate smears showed increased blasts that are small in size. The erythroid elements showed dysplastic features including binucleation, irregular nuclear contours, nuclear dyssynchrony, and megaloblastoid changes.
- The biopsy demonstrated a hypercellular marrow (80-90%) with blasts accounting for ~40-50% of marrow cellularity with erythroid hyperplasia.

Immunophenotype

- Flow cytometric evaluation of the bone marrow aspirate showed the blasts are positive for CD7, CD43, CD71 (dim), and CD117 and negative for CD1a, cCD3, CD7, CD10, CD11b, CD13, CD14, CD16, CD19, cCD22, CD33, CD34, CD41, CD43, CD45, CD61, CD64, cCD79a, CD117, CD123, CD235a, cMPO, HLA-DR, and nTdT.
- Immunohistochemical staining showed that the blasts were positive for CD123 (weak)

and negative for CD3, CD20, CD42b, CD61, CD71, PAX5, Tdt, MPO, factor VIII, E-cadherin, and glycophorin A.

Cytogenetics

- Cytogenetics: Karyotype analysis showed a 46,XY,t(11;19)(q23.3;p13.3)[2]/46,XY[15] with a KMT2A-MLLT1 fusion. Fluorescent in situ hybridization (FISH) panel detected a KMT2A (MLL) (11q23) rearrangement.

Molecular Studies

- Molecular studies: No abnormalities were detected on next genome sequence (NGS) studies.

Proposed Diagnosis

- Acute undifferentiated leukemia with KMT2A rearrangement, arising post-cytotoxic therapy.

Interesting Feature(s)

- Based on the dysplastic morphology, FISH abnormality, and abnormal karyotype, this case could be considered as an acute myeloid leukemia, myelodysplasia-related' or more specifically, 'myeloid neoplasm post cytotoxic therapy', based on the patient's clinical history, but the immunophenotype of the blasts did not have any myeloid expression.
- In addition, although the WHO 5th edition recognizes acute myeloid leukemia with KMT2A rearrangement as a separate entity, this case did not express myeloid markers and cannot be classified as such either. The t(11;19)(q23.3;p13.3) identified in this patient, involving the KMT2A (MLL) at 11q23.3 and the MLLT1 gene at 19p13.3, is a recurring abnormality in acute myeloid leukemia as well as acute lymphoblastic leukemia and is an indicator of poor prognosis.
- To our knowledge, this is the first reported case of acute undifferentiated leukemia with t(9;11).

Panel Diagnosis

ALAL with KMT2-MLLT1 rearrangement, post cytotoxic therapy (WHO/ICC)

EA4HP24-BMWS-135

Myeloid/lymphoid neoplasm with FLT3 rearrangement with simultaneous presentation of CML-like marrow proliferation and T-LBL/L of lymph nodes

Dr. Christine Heisen, Dr. Joshua Menke

Stanford University, Department of Pathology, Stanford, USA

Case Description

The patient is a 78 year old woman with B symptoms, lymphadenopathy and leukocytosis (50 K/ul) with neutrophilia, and eosinophilia (2.5 K/uL), normocytic anemia, thrombocytopenia. Bone marrow biopsy and left inguinal lymph node biopsy were performed. FISH and NGS showed *FLT3::ETV6* fusion. Myeloid/lymphoid neoplasm with FLT3 rearrangement was diagnosed. The patient was treated with gilteritinib and showed complete remission by imaging. Marrow 6 months post-diagnosis showed 5.5% *FLT3* rearrangement by FISH and negative NGS.

Biopsy Fixation Details

Marrow: Bouin's solution; Node: formalin

Frozen Tissue Available

No

Details of Microscopic Findings

Marrow sections are hypercellular for age (90%) with marked granulocytic proliferation and numerous eosinophils. Erythroid precursors show full spectrum maturation. The M:E ratio is increased (5:1). CD34+ blasts are <5%. CD61+ megakaryocytes are mildly increased, and show frequent small, hypolobated forms without significant clustering.

Lymph node sections show expansion of paracortical regions by intermediate-size blasts. Foci of increased eosinophils are seen. Lesional cells are positive for CD3, CD79a, TdT (subset) and CD34 (subset); and are negative for MPO and lysozyme. Ki67 index is ~80%. CD19 and PAX5 are negative in lesional T-cells.

Immunophenotype

Marrow flow: Small abnormal myeloblast population expressing dim CD38, dim HLA-DR, CD33, variable CD117; negative for CD13, CD34 and T-cell markers

Node flow: Abnormal immature T cell population expressing cCD3, CD4 (dim partial), CD5, CD7 (bright), CD2, TdT, CD1a (subset, 25%), CD45 (moderate), CD38, CD33 (dim), cCD79a, CD30 (partial), CD43 and CD81; negative for sCD3, CD8, CD57, CD56, CD16, TRBC1, TCRgd, CD34, CD10, CD117, CCR4, CD26, CD25, granulocytic markers, CD19 and other B-lineage markers

Cytogenetics

Marrow:

46,XX,t(12;13)(p13;q12)[20]

FISH showed 91.5% of cells with *FLT3* rearrangement and 82% of cells with *ETV6* rearrangements, and negative for *BCR::ABL1, PDGRFB, FGRF1, JAK2*

Molecular Studies

Heme-STAMP targeted NGS panel (203 genes) showed *ETV6::FLT3* rearrangement and *STAG2* A866fs.

Proposed Diagnosis

Myeloid/lymphoid neoplasm with *FLT3* rearrangement

Interesting Feature(s)

In this case of T-LBL/L with concurrent *BCR::ABL1* negative CML-like disease and eosinophilia, the defining feature is a tyrosine kinase (TK) fusion gene identified as *FLT3::ETV6*. As per the ICC and WHO 2022, the findings are best classified under myeloid/lymphoid neoplasm with *FLT3* rearrangement, but this case raises the question of the validity of the somewhat arbitrary current classification separating myeloid/lymphoid neoplasms with eosinophilia (MLN-Eo), defined by TK fusions, from mixed-phenotype acute leukemias (MPAL), defined primarily by phenotype. In this case of a bilineal, *BCR::ABL1* negative, *FLT3* rearranged disease, the genetic features trump the phenotypic features entirely, whereas this case could have been classified as MPAL (T/myeloid) by WHO 2022 had there been no such fusion gene identified, because of the simultaneous presentation of two abnormal progenitor populations (one T-cell and one myeloid) with a combined numerical blast count exceeding 20%. Instead, we propose that the MLN-Eo and MPAL categories be combined to reflect phenotypic similarities, while allowing for subcategorization based on genetics, such as the previously mentioned tyrosine kinase gene fusions like *BCR::ABL1, FLT3* rearrangements etc. that can be therapeutically targeted. Cases of MLN with *FLT3* rearrangement treated from diagnosis with FLT3 inhibitor are rare; our patient has shown an excellent treatment response to date.

Panel Diagnosis

Myeloid/lymphoid neoplasm with FLT3 rearrangement (WHO/ICC)

EA4HP24-BMWS-145

CD56+ Early T Precursor Acute Lymphoblastic Leukemia with RUNX1 rearrangement.

PhD/MD Amy Ku, Dr. Fernando Alekos Ocampo Gonzalez, Dr. Murty Vundavalli, PhD/MD Wen-Hsuan Wendy Lin, PhD/MD Rebecca Leeman-Neill, Dr. Govind Bhagat, Dr. Craig Soderquist, Dr. Bachir Alobeid

Columbia University Irving Medical Center, Department of Pathology and Cell Biology, New York, USA

Case Description

7-year-old male with no relevant medical history presented with abdominal pain, diarrhea, and 20-pound weight loss. CBC: WBC 100.41x10^3/uL, Hgb 5.5 and plt 172, with blasts comprising 95% of WBC. FC findings were compatible with CD56+ early T precursor acute lymphoblastic leukemia (ETP-ALL). The patient was enrolled in a clinical trial; after three months on therapy, BM was negative for disease by FC and cytogenetics.

Biopsy Fixation Details

BM biopsy fixed in Bouin's solution.

Frozen Tissue Available

N/A.

Details of Microscopic Findings

Bone marrow core biopsy:Dense, diffuse infiltrate of blasts with round to irregular nuclei, fine dispersed chromatin and scant cytoplasm, >80% of total cellularity.

Peripheral blood (PB) smear: Numerous blasts are seen.

Immunophenotype

PB FC analysis: CD45(dim)+ (low SSC gate), CD56+, CD34+, CD117+/-, TdT-, CD10-, cytoplasmic CD3+, surface CD3-, CD2+, CD5(dim)+ (<75%), CD7+, CD43+, CD38+, CD19(dim)+, CD4-, CD8-, CD25-, CD57-, CD103+, CD1a-, TCR-, CD16-, CD13-, CD33-, CD11b-, CD11c-, CD15-, CD14-, HLA-DR-, CD123-, MPO-, CD20-, cCD79a-, & CD22-.

Cytogenetics

Karyotype: Complex chromosome abnormalities in 2 sub-clones in 22 metaphases. Stemline clone showed t(2;21)(q11.2;q22), add(10)(p11.2) and homogenously staining regions (HSR) on both arms of chromosome 1. In the subclone, chromosome 1 with HSR formed a ring chromosome. FISH: RUNX1 rearrangement was detected in 93% of cells, consistent with t(2;21)(q11.2;q22) seen on karyotype. No evidence of ETV6 or KMT2A rearrangement.

Molecular Studies

TCR beta and IgH gene rearrangement analysis: polyclonal products.

Proposed Diagnosis

ETP-ALL.

Interesting Feature(s)

T lymphoblastic leukemia (T-ALL), ETP-ALL or near ETP (distinct variants of T-ALL) and NK lymphoblastic leukemia (NK-LL) can display significant immunophenotypic overlap,

making accurate diagnosis challenging, especially in CD56+ cases. T-ALL expresses surface and/or cytoplasmic CD3, and aberrant immature T-cell immunophenotype. Clonal TCR gene rearrangements are found in >90% of T-ALL; those lacking TCR rearrangements are largely ETP-ALL, which is postulated to arise from an early immature progenitor. Diagnostic criteria for ETP-ALL include expression of cytoplasmic CD3, absence of CD1a and CD8, dim CD5 expression by<75% blasts (>75% in near ETP), and expression of one or more myeloid and/or stem cell markers by ≥25% of blasts. NK-LL is rare and less well-defined. The revised 2017 WHO classification listed NK-LL as a provisional entity, and considered in cases with the following profile: CD56+, sCD3-, cCD3+, CD2+, CD7+, absence of B-cell and myeloid markers, and absence of TCR rearrangement.

While characteristic of NK cells, CD56 expression has also been noted in T-ALL and more frequently in the ETP-ALL subtype. Due to lack of precise diagnostic criteria and significant overlap with T-ALL/ETP-ALL, the 2022 WHO classification no longer recognizes NK-LL; however, it is still a provisional entity in the ICC categorization, but no definitive criteria have been listed.

In the current case, ETP-ALL criteria are met, and while the blasts are CD56+, dim CD19+ expression argues against NK-LL. CD19 has been described in T-ALL and has more frequently been reported in ETP-ALL (or near ETP-ALL), suggesting that ETP-ALL blasts have B/T/NK-cell and myeloid potential. The immunophenotype and lack of clonal TCR rearrangements support our diagnosis of ETP-ALL.

The t(2;21)(q11.2;q22) translocation involving RUNX1 has not been previously reported in ETP-ALL; though, case reports have described this translocation in two pediatric T-ALL patients, creating the novel fusion gene RUNX1-LAF4.

Panel Diagnosis

ETP-ALL (WHO/ICC)

EA4HP24-BMWS-154

Post cytotoxic chemotherapy-associated B/myeloid mixed phenotype acute leukemia

Dr. Meredith M. Nichols[1], PhD/MD Katelynn M. Wilton[1], PhD/MD Reuben Carrasco[1,2], Dr. Rahul Vedula[2], Dr. Sam Sadigh[1]

[1] Brigham and Women's Hospital, Department of Pathology, Boston, USA; [2] Dana-Farber Cancer Institute, Boston, USA

Case Description

A 55-year-old female with ovarian high-grade serous carcinoma diagnosed 3 years prior (treated with carboplatin, paclitaxel, nivaparib) presented with pancytopenia (WBC 2.09 k/µL [ANC 0.61], Hct 32.6%, Plt 142 k/µL) without circulating blasts. Bone marrow (BM) biopsy

was performed (very limited aspirate) and followed by a 2nd BM biopsy. She received Larson induction chemotherapy and had morphologic remission on a 3rd BM biopsy.

Biopsy Fixation Details

Biopsy 1: Formalin

Biopsies 2 & 3: Bouin's solution

Frozen Tissue Available

-

Details of Microscopic Findings

Biopsy 1: Aspirate: hemodilute; rare blasts. Biopsy: 50% cellularity; 40% composed of interstitial aggregates of intermediate-sized blasts with irregular nuclei, dispersed chromatin, variably distinct nucleoli and scant cytoplasm. Multiple lymphoid aggregates were present.

Biopsy 2: Aspirate: aspicular, hypocellular. Biopsy: 60% cellularity; 50% composed of blasts with features like those seen previously. Multiple lymphoid aggregates were seen.

Biopsy 3: Aspirate: paucispicular, hypocellular; 1% blasts. Biopsy: 40% cellularity without increased blasts. Lymphoid aggregates were not seen.

Immunophenotype

Biopsy 1: Flow cytometry (FC): negative for abnormal myeloid or lymphocyte population. Immunohistochemistry (IHC): immature cells expressed CD34, CD20, PAX5(subset[s]) and CD117 (dim[d],s). CD3 and CD5 showed T cell aggregates.

Biopsy 2: A subset of blasts expressed myeloperoxidase (MPO) cytochemistry. FC: 0.5% of total events positive for CD45([d]), HLA-DR, TdT, CD123(s), CD38(d,s), CD19, CD10(s), sCD22(s), CD13(variable[v]), CD33(v), CD15(s); negative for CD20, surface immunoglobulin kappa and lambda, MPO, and other myeloid, monocytic, and T-cell markers. Another population (0.8% of total events) was positive for CD45(d), CD34, HLA-DR, CD13, CD33; negative for monocytic, B, and T lymphoid markers. IHC: blasts expressed CD34, CD117(s), CD20(s), MPO(s), lysozyme(s), PAX5(s), TdT(s), CD19(s), CD79a(s) and CD33(s). 30% had dual expression of CD34/PAX5. Rare cells were dual positive for PAX5/CD33.

Biopsy 3: Measurable residual disease FC: 0.4% of total events were positive for CD45(d), CD34, CD117, CD13, CD33, HLA-DR; negative for CD64, CD14, CD56, CD123, and lymphoid markers.

Cytogenetics

Biopsy 1: None

Biopsy 2: 45,XX,-7[10]. FISH negative for *KMT2A* rearrangement.

Biopsy 3: 46,XX[20]

Molecular Studies

Next-generation sequencing (NGS) of peripheral blood 1 month after biopsy 1: mutations in *EZH2,PPM1D,PTPN11,RUNX1, TET2*.

Biopsy 2: NGS: mutations in *EZH2*, *PPM1D*, *PTPN11,RUNX1*, *TET2*. BCR::ABL1 p190/p210 negative. Clonoseq identified 2 trackable B-cell clones.

Biopsy 3: NGS: mutations in *PPM1D, TP53.*

Proposed Diagnosis

Mixed-phenotype acute leukemia (MPAL), B/myeloid, not otherwise specified, therapy-related (ICC)

MPAL, B/myeloid, post-cytotoxic therapy (WHO5)

Interesting Feature(s)

MPAL are unusual in a post-therapy setting. This lacked dysplasia and *TP53* mutations at diagnosis, features classically associated with therapy-related myeloid neoplasms (T-MN). MPAL can have *TET2* mutations, but often have a complex karyotype or structural abnormalities of chromosome 7; this only had monosomy 7. This case underscores the difficulty in establishing blast phenotype in the absence of a cellular aspirate. PARP inhibitors are an independent risk factor for T-MN, although association with MPAL is unknown. The significance of the lymphoid aggregates is unclear; disappearance after induction chemotherapy suggests that they may be paraneoplastic. Persistence of mutations in *PPM1D* and *TP53* after induction chemotherapy suggests possible underlying clonal hematopoiesis.

Panel Diagnosis

AML-MR (WHO/ICC) with MPAL immunophenotype, post-cytotoxic therapy

EA4HP24-BMWS-158

Acute Undifferentiated Leukemia

Dr. Ahmed A. Ahmed, Dr. Phuoc T. Christie-Nguyen, Prof. Jacob A. Armstrong, Prof. Brenda Mai

UT Health at Houston, Department of Pathology and Laboratory Medicine, HOUSTON, USA

Case Description

This is a 72-year-old male with history of prostate cancer status post prostatectomy and chemoradiation who presented with fatigue and shortness of breath. On admission, his complete blood count showed pancytopenia with a white blood cell count of 0.9 $\times 10^9$/L, hemoglobin of 4.3 g/dl, and platelet count of 63×10^9/L.

Biopsy Fixation Details

Formalin-fixed formic acid-decalcified paraffin-embedded tissue.

Frozen Tissue Available

None

Details of Microscopic Findings

Peripheral blood smear evaluation showed ~10-15% circulating blasts with a background pancytopenia. The aspirate smears showed increased blasts (~40-45%), dyserythropoiesis, granulocytic hypoplasia, and left-shifted granulocytic maturation. The blast population demonstrated scant agranular cytoplasm, smooth to mildly irregular nuclear contours, fine chromatin and occasional distinct nucleoli. The marrow cellularity was approximately 20-30%, and composed of sheets and large aggregates of small to intermediate blasts admixed with foci of erythroid-predominant hematopoietic elements.

Immunophenotype

Flow cytometric immunophenotyping of his peripheral blood showed ~30% blasts with expression of CD7 (subset), CD34, Tdt, and HLA-DR, and were negative for cCD3, sCD3, CD4, CD10, CD13, CD14, CD16, CD19, CD20, cCD22, CD33, CD56, CD64, cCD79a, and CD117. Immunophenotyping by bone marrow showed an identical immunophenotype. On immunohistochemical staining, the blast population was positive for CD34, Tdt and CD117 (subset), and negative for CD1a, CD3, CD61, CD71, CD79a, e-cadherin, factor VIII, glycophorin A, lysozyme, MPO, and PAX5.

Cytogenetics

Conventional cytogenetic analysis could not be performed as no metaphase cells were available for analysis.

Molecular Studies

No abnormalities were detected by myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) fluorescent in situ hybridization (FISH) panel. Next genome sequencing showed a RUNX1, BCOR and SRSF2 mutation. No alterations detected in BCR:ABL1, FLT3, NPM1, IDH1, or IDH2.

Proposed Diagnosis

Acute undifferentiated leukemia (AUL)

Interesting Feature(s)

AUL is a rare subtype of acute leukemia characterized by a lack of differentiation of the leukemia cells into specific blood cell lineage. Clinical, immunophenotypic, and genetic data is limited, and it is uncertain if acute undifferentiated leukemia is biologically distinct from other entities like acute myeloid leukemia with minimal differentiation, which also shows limited myeloid marker expression. The molecular profile seen in this case of AUL with RUNX1 and SRSF2 mutations suggests that this patient's acute leukemia may be genetically closer to AML than lymphoblastic leukemia (ALL). AUL has been reported to have a poor prognosis, however, individual outcomes can depend on factors such as age, overall health, and specific genetic features of the leukemia cells. The clinical presentation of AUL can be aggressive, and patients may experience symptoms such as fatigue (as in this case), weakness, fever, easy bruising, and frequent infections. The rapid proliferation of undifferentiated cells can lead to a rapid onset of symptoms. For all the previous reasons, accurate diagnosis of AUL is considered a real challenge. The treatment of AUL typically involves aggressive chemotherapy to eliminate the undifferentiated leukemia cells. Stem cell transplantation may also be considered, especially in cases with high-risk features or relapsed disease.

Panel Diagnosis

AUL (WHO/ICC), post cytotoxic therapy

EA4HP24-BMWS-161

Mixed-phenotype acute leukemia, T/myeloid (MPAL-TM)

Dr. Ion Prisneac, PhD/MD David Howell, Dr. Cara Randall

West Virginia University, Pathology, Anatomy and Laboratory Medicine, Morgantown, USA

Case Description

A 15-year-old male came to the emergency department (ED) with a significant sore throat for a week, severe upper abdominal pain radiating into the back, and vomiting. Streptococcus PCR, Monospot test, and COVID test were negative. ED laboratory workup was significant for a WBC of 139x10^3/uL with 11% blasts on the differential count and mild thrombocytopenia PLT of 163x10^3/uL. The basic metabolic panel was unremarkable and the hepatic function profile showed mild hepatitis on the hepatic function profile (AST = 42 U/L, and ALT = 30 U/L). Lipase and urine analysis were unremarkable. Computed tomography showed hepatosplenomegaly with multiple enlarged mesenteric lymph nodes.

Biopsy Fixation Details

Bone marrow:

- Bone marrow clot was submitted in formalin for fixation and histologic evaluation.
- Bone marrow core was submitted in Formical for decalcification and histologic evaluation.

Frozen Tissue Available

Not available

Details of Microscopic Findings

Complete blood count (CBC) showed marked leukocytosis (WBC = 130.1x10^3/uL) with increased circulating blasts (87%), mild normocytic anemia, and thrombocytopenia (Plt = 104 x10^3/uL). Most of the blasts were enlarged in size, with irregular, lobated nuclei, prominent nucleoli, smooth chromatin, and susbet promonocyte morphology; occasional blasts had bilobed nuclei and cytoplasmic granules, but no Auer rods were seen; few smaller blasts were seen with round nuclei, increased nucleus-to-cytoplasmic (N:C) ratio and small nucleoli.

The bone marrow aspirate is spiculated and is occupied by sheets of blasts (85%) composed of a dimorphic population: population 1 (majority) - large cell size with basophilic cytoplasm, subset clefted/irregular nuclei, prominent nucleoli, and subset promonocyte morphology and population 2 - smaller cell size, higher N:C ratio and "hand mirror" morphology. No Auer rods were identified. Background trilineage hematopoiesis was markedly decreased.

The bone marrow core biopsy sections revealed hypercellular-for-age marrow (90% cellularity) with sheets of medium to large-sized blasts with prominent nucleoli.

Immunophenotype
FC: **Pos:** cCD3 (subset), CD4 (subset), CD7 (het), CD11b (subset), CD11c (susbet), CD13, CD15 (subset), CD19 (het), CD22 (subset), CD33, CD34 (majority), CD38, CD64 (subset), HLA-DR, cTdT (het) and cMPO (minor subset); **neg:** CD1a, sCD3, CD5, CD8, CD10, CD20, CD36, CD56, CD61, cCD79a
IHC: **Pos:** CD34, TdT, MPO (subset), lysozyme (subset, CD3 (weak, minor subset); **neg:** PAX5, Factor VIII, E-cadherin
Cytogenetics
Chromosome analysis showed t(7;14)(q21;q32) [22/25]
BCL11B BAP negative for rearrangement
AML and eosinophilia FISH panels were negative
Molecular Studies
NGS: *FLT3*-ITD and pathogenic *WT1* variant
VUS: *NT5C2* and *ZFHX3*
Proposed Diagnosis
BONE MARROW BIOPSY, ASPIRATE AND TREPHINE CORE BIOPSY:
- Hypercellular marrow (90%) involved by mixed-phenotype acute leukemia, T/myeloid
Interesting Feature(s)
Certain 14q32 rearrangements result in *BCB11B* activation. Variants in *FLT3* and epigenetic genes (*WT1*, *DNMT3A*, and *TET2*) are associated with *BCL11B*-rearranged/activated cases. Our case findings, including immunophenotype, cytogenetic and molecular findings, raise the possibility of *BCL11B* activation/overexpression via a mechanism that does not result in a positive FISH BAP or gene fusion product by RNA sequencing. Without definitive evidence that the *BCL11B* relocation to chr7 in our case resulted in activation/overexpression, we were unable to formally classify this as MPAL with *BCL11B* rearrangement.

Panel Diagnosis
MPAL, T/myeloid

EA4HP24-BMWS-164

MIXED PHENOTYPE ACUTE LEUKEMIA, B/T/MYELOID

Dr. Shaoying Li, Dr. Guilin Tang, Dr. Wei Wang, Dr. L. Jeffrey Medeiros, Dr. Sa Wang

MD Anderson cancer Center, Hematopathology, Houston, USA

Case Description
•A 61-year-old man with pancytopenia and outside bone marrow aspiration and biopsy showed acute myeloid leukemia. The patient came for second opinion and treatment.
•CBC: WBC 2.7 K/ul, Hgb 8.0 g/dL, MCV 87 fL, Platelets: 45 K/ul; Differential count showed

absolute neutropenia, monocytosis, and 18% blasts
•In-house bone marrow aspiration and biopsy were performed

Biopsy Fixation Details

10% neutral buffered formalin

Frozen Tissue Available

N/A

Details of Microscopic Findings

BM biopsy showed a packed marrow with diffuse infiltrate of immature cells. Normal trilineage hematopoiesis is markedly decreased.
Aspirate smears showed many blasts of variable size, small to medium to large, and scant agranular cytoplasm. No Auer rods were identified.
Immunophenotype IHC: CD3 dimly stains a subset of blasts (strong in mature T cells)
Flow cytometry study showed:
Common phenotype of all blasts:•Positive: cyto-CD3 partial, CD10 partial, CD13, CD25, CD45, CD54, CD36 partial, CD56 minimal, CD123, CD133, HLA–DR decreased, and TdT;
•Negative: CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD14, CD15, CD66c, CRLF1, and MPO.
The blasts consisted of 2 subsets:•One subset (~50% of blasts) had mainly B lymphoblastic differentiation: Positive for CD19, CD20 partial, CD22 and CD79a; and negative for CD33, CD64, and CD117;
•The other subset (~50% of blasts) was partially positive for CD22, CD33, CD64 and CD117, and negative for CD19, suggestive of mainly myeloid differentiation.
In addition, monocytic cells were increased (23%) and showing an aberrant immunophenotype, with no CD4 expression and decreased expression of CD14, CD15, CD36, and HLA-DR.

Cytogenetics

•Karyotype: 44~47,XY,-3,del(5)(q15q33),-7,+11,+11,add(11)(p11.2),add(12)(p11.2),-18,+1~5mar[cp20]
•Optical Genome Mapping Analysis: Highly complex genome, including CHROMOANAGENESIS involving chromosome 3, 4, and 5, extensive COPY NUMBER ALTERATION including amplification of 11q/KMT2A, and COMPLEX REARRANGEMENTS.

Molecular Studies

•Myeloid neoplasm panel- 81 gene targeted NGS: •TP53 mutation: VAF 79%

Proposed Diagnosis

MIXED PHENOTYPE ACUTE LEUKEMIA, 73% BLASTS, B/T/MYELOID.

Interesting Feature(s)

•B/T/Myeloid trilineage mixed phenotype acute leukemia (MPAL) is extremely rare. Too few cases have been reported in literature and therefore there is currently no statement regarding its clinicopathologic features
•This case qualified for a diagnosis of B/T/Myeloid MPAL. A complete molecular cytogenetic work up has been done and therefore contributes to knowledge of this type of rare MPAL
•Optical Genome Mapping (OGM) showed that chromoanagenesis contributed to leukemogenesis in this case - not reported previously in MPAL.
•This case is unusual as none of the common mutations reported in MPAL, such as

DNMT3A, PHF6, and mutations in the JAK-STAT pathway, were detected. TP53 mutation was detected.

Panel Diagnosis

AML-MR (WHO)/AML with mutated TP53 (ICC) with MPAL immunophenotype

EA4HP24-BMWS-194

Acute myeloid leukemia, myelodysplasia related, with B/monocytic-dendritic phenotype.

Dr. Dapeng Wang, Dr. Xiaohui Zhang, Dr. Elizabeth Hyjek, **Dr. Ling Zhang**

Moffitt Cancer Center, Tampa, USA

Case Description

67-year-old female presented with pancytopenia, fatigue, and shortness of breath. Laboratory study showed Hgb 6.0 g/dL, WBC 0.77 x 10^9/L, and platelet 40 x 10^9/L, and normal metabolicand DIC profile. Bone marrow (BM) biopsy was performed to identify the etiology of the pancytopenia. The patient received mini-CVD + venetoclax + rituximab but did not respond well. She passed away 9 months after the diagnosis.

Biopsy Fixation Details

B Plus Fix fixative

Frozen Tissue Available

N/A

Details of Microscopic Findings

Peripheral blood shows severe neutropenia, moderate anemia, and thrombocytopenia. Circulating blasts are present. The BM aspirate comprised sheets of blasts with different morphologies, including small blasts with finely dispersed/speckled chromatin and scant cytoplasm, lager blastic cells with monocytic differentiation with more abundant agranular cytoplasm and spindly shaped blastic cells with plasmacytoid dendritic cell (pDC) morphology. Residual hematopoiesis is markedly reduced. The BM core biopsy shows approximately 100% cellularity with diffuse blastic cell infiltrate composed of two distinct cell populations with different immune-architectural distribution. One subset is small blasts, morphologically consistent with lymphoblasts, in paratrabecular distribution, and focally in interstitial distribution; the second subset of immature blastic cells is in intertrabecular distribution consisting of larger blasts admixed with many elongated or spindle cells with pDC morphology.

Immunophenotype

Immunohistochemical stains were performed on the BM core biopsy.There are two distinct blast populations. The firstpopulation withparatrabecular distribution: CD34+, CD19+, CD20+, PAX5+ and TdT+ , negative for immunoglobulin light chains, consistent with

immature B cell phenotype. The second blast population in the intertrabecular space: CD33+dim, CD4+, CD7weak +, CD68(PGM1)+, TCF4+, CD303/BDCA2+, CD163-, CD56-, CD34-, CD117-,CD3-, TCL1-, Lysozyme-, cytoplasmic MPO-, S100-, CD1a-, and cyclin D1-, consistent with early monocytic-dendritic cell differentiation. The concurrent flow cytometry revealed similar findings (Figures see PPT).

Cytogenetics

FISH for MDS panel detected del(5q) in 61.5% of nuclei examined . Karyotyping showed 46,XX,del(5)(q13q33)[17]/46,XX[3].

Molecular Studies

NGS detected pathological mutations of *FLT3*(p.V581_L601dup, c.1740_1802dup63, VAF % 35.9), *BCOR* (p.H866Tfs*13,c.2595_2596delinsC, VAF % 36.8) and potential pathological mutation of *RUNX1*(p.K110M, c.329A>T. VAF % 72.5). FoundationOne Heme panel also showed *FLT3-ITD* and *BCOR* mutation.

Proposed Diagnosis

Acute myeloid leukemia, myelodysplasia related (AML-MR), with B/monocytic-dendritic phenotype.

Interesting Feature(s)

Here, we present a case with AML-MR with B/myeloid phenotype, which is not uncommon.The interesting feature of this case is that the myeloid population exhibits monocytoid to dendritic cell differentiation (TCF4+ and CD303/BDCA2+), resembling pDC AML, which is often associated with cross-lineage antigen expression, $RUNX1^{mut}$ *and* TCF4 expression [PMID: 32871587, PMID: 33054062]. $RUNX1^{mut}$ is proposed to be a myelodysplasia related gene by ICC but not by WHO classification. It is unclear if pDC-AML is an independent entity that should be separated from AML-MR. It is also worthy of exploration if TCF4 alone could induce monocytic differentiation into pDC as well as macrophages [PMID: 10666185].

Panel Diagnosis

AML-MR (WHO/ICC)

EA4HP24-BMWS-216

Acute undifferentiated leukaemia with FLT3-ITD shows molecular signatures of AML

Dr. Ye Lin Hock[1], Dr. Hayder Hussein[2]

[1] Queen Elizabeth Hospital, Birmingham, University Hospitals Birmingham NHS Trust, Histopathology, Birmingham, UK; [2] Queen Elizabeth Hospital, Birmingham, University Hospitals Birmingham NHS Trust, Haematology, Birmingham, UK

Case Description

A previously healthy 36 year old male presented with pneumococcal meningitis. Peripheral blood showed Hb 84, WBC 18.5, platelet 40, neutrophil 6.27, lymphocytes 3.6 and circulating blasts. Bone marrow examination was undertaken (submitted sample). Due to the ambiguous lineage of his leukaemia, he was treated with FLAG Ida induction and achieved a first complete remission. Due to molecular genetics finding, he was consolidated with one cycle of DA & Midostaurin followed by high dose ara-C and Midostaurin. He then underwent HLA mismatched volunteer unrelated allograft. He developed GVHD and EBV reactivation, refractory to rituximab with development of monomorphic PTLD (DLBCL). However, he achieved complete metabolic response with clearance of EBV in blood after EBV-CTL therapy (TAB-CEL), with continuing metabolic & viral load remission.

Biopsy Fixation Details

AZF fixation & EDTA decalcification

Frozen Tissue Available

No

Details of Microscopic Findings

BM aspirate: Hypercellular with >90% undifferentiated blasts showing high NC ratio & agranular cytoplasm

BM trephine: Hypercellular (80% cellularity) with a diffuse (50% of TNC) blast infiltrate

Immunophenotype Trephine immunohistochemistry:

Positive:TDT, CD34, Pax5 (variable)

Negative:CD 117, CD2, CD3, CD5, CD7, myeloperoxidase, CD15, CD14, CD68(KP1), CD123, Glycophorin C, CD42b, CD19, CD20, CD79a

Flow cytometry:

79% blasts

Positive for:CD34, CD45, CD45 RA, weak CLL1, HLA-DR, CD38, weak CD 117(1/3), CD 123(1/3), nuclear TDT

Negative for: cytoplasmic myeloperoxidase, CD3, CD22, CD79a, CD13, CD33, CD19, CD4, CD7, CD 56

0.8% CD14+ monocytes, 3.5% CD11b+ mature myelomonocytic cells, 2.4% Glycophorin A positive early erythroid cells

No lymphoid abnormality

Cytogenetics

Trisomy 6 & trisomy 8

FISH: No BCR::ABL1, KMT2A, ETV6::RUNX1, IGH, TCF3 rearrangements, ABL class fusion or RUNX1 amplification

Molecular Studies

Fragment analysis: Clinically significant 69bp internal tandem duplication (1/5) within FLT3 (FLT 3-ITD to FLT3 wild type allelic ratio of 0.69 suggesting 41-81% clone)

NGS Myeloid 29 genes panel: Clinically significant variants in IDH2 (VAF 88%), RUNX1 (VAF 39%), 69bp FLT3 (ITD) (VAF 37%)

Proposed Diagnosis

Acute undifferentiated leukaemia with FLT3-ITD

Interesting Feature(s)

Acute undifferentiated leukaemia (AUL) is a category of acute leukaemia of ambiguous lineage (ALAL) which expresses haematopoietic stem cell markers but lack bona fide myeloid and lymphoid markers. In adults, AUL has the worst prognosis compared to AML & ALL. FLT3 signalling pathway is involved in the expansion of haemopoietic stem cells. Activating mutation of FLT3 is one of the most frequent pathological mutations in AML. In AUL, FLT3-ITD has not been well described even though a recent study reported 4 cases of ALAL, NOS with FLT3-ITD (1). Bone Marrow Pathology Group in 2018 described 2 cases of AUL with FLT3 but no cases with FLT3-ITD (2). Our case fulfils the criteria for AUL and does not fulfil criteria for AML with minimal differentiation with which it can overlap. Although AUL and ET-PLL can overlap, no T- antigen expression or molecular genetics associated with T-ALL is noted in our case. However, FLT3-ITD & other variants associated with AML were noted suggesting the molecular signatures of AML even though qualifying as AUL. FLT3-ITD is usually associated with an adverse outcome in AML but our patient responded to AML therapy. Identification of FLT3-ITD is important as targeted therapy against FLT3 tyrosine kinase has been proved to improve outcome in AML with FLT3-ITD.

Ref:

1.
Hematopathol 2022;7(2):1-10
1.
Modern Pathology 2019;32:1373-85

Panel Diagnosis

AUL (WHO)/ AML-MR gene mutations (ICC) with AUL immunophenotype

EA4HP24-BMWS-219

Mixed Phenotype Acute Leukemia (T/Myeloid) masquerading as Acute Myeloid Leukemia

Dr. Sarah E. DePew, Dr. John K. Choi, **Dr. Aishwarya Ravindran**

The University of Alabama at Birmingham, Department of Pathology, Division of Laboratory Medicine-Hematopathology, Birmingham, USA

Case Description

A 50-year-old woman with ADHD and kidney stones presented with palpitation, fatigue, and shortness of breath and was diagnosed with acute myeloid leukemia (AML) at an external facility. The patient was transferred to our institution, CBC revealed pancytopenia, and a repeat bone marrow (BM) biopsy showed AML with 40% blasts. A small population of myeloblasts with aberrant cCD3 expression was also identified but insufficient for a mixed phenotype subclassification. The patient was initiated on a 7+3 AML induction chemotherapy regimen. Day+14 post-induction chemotherapy BM showed persistent myeloid-lineage blasts and distinct cCD3 (bright)-positive T-lymphoblasts, supporting the diagnosis of a mixed phenotype acute leukemia (T/myeloid).

Biopsy Fixation Details

BM decalcified in hydrochloric acid-EDTA, formalin-fixed and paraffin-embedded

Frozen Tissue Available

None

Details of Microscopic Findings

Day+14 BM: 10-20% cellularity, >90% blasts

Immunophenotype

At diagnosis by flow cytometry: Blasts positive for myeloid lineage markers [CD45 (dim), CD34, CD117, MPO (subset), CD13, CD15 (subset), CD33, CD56 (subset), CD123, HLA-DR], aberrant expression of CD10 (partial), CD22 (partial), cCD3 (subset; MFI 571), CD2 (subset), CD5 (partial), CD7 (partial), and nTdT. Negative for sCD3, CD19, cCD79a, CD4, CD8, CD14, CD16, CD64, CD20

Day+14 two distinct lineage blasts: i) Myeloid lineage blasts positive for CD45 (dim), CD34, CD117, MPO (subset), CD13, CD33, CD56 (subset), CD123, HLA-DR, CD2 (subset), CD7 (partial); ii) T-cell lineage blasts: CD45 (dim), cCD3 (strong/bright, MFI 6314), CD2 (partial), CD4, CD5 (partial), CD7, nTdT, CD10 (partial); negative for sCD3, CD19, cCD79a, CD8, CD14, CD15, CD16, CD64, CD20, and CD23

Day +14 BM: extensive replacement by CD34+ blasts (>90% of BM cellularity), a substantial proportion represented T-lymphoblasts characterized by CD3 positivity

Cytogenetics

At diagnosis

Chromosome analysis: 47,X,-X,add(2)(p21),add(2)(q34),add(3)(p21),add(3)(q12),del(5)(q22q35), -8,+11,add(11)(p11.2),-

18,+21,+22,+mar[cp20]
FISH: EGR1 gene deletion at 5q31 (66.5%); One copy of RUNX1T1 gene at 8q22 (64%);
3 copies of KMT2A gene at 11q23 (64%); 3 copies of RUNX1 gene at 21q22 (64%)
Day+14: not done

Molecular Studies

At diagnosis, NGS revealed pathogenic biallelic TP53 mutations
Day+14: not done

Proposed Diagnosis

Mixed phenotype acute leukemia, T/myeloid

Interesting Feature(s)

i) This case highlights the practical challenges of application of defined criteria of level of cCD3 expression on lineage subtyping of blasts in mixed phenotype acute leukemia (T/myeloid). Per WHO (5th edition, beta version, 2022), cCD3 expression should be present in at least a portion of blasts with a level exceeding 50% of that seen on normal mature T-cells in the same sample
ii) Aberrant cCD3 expression can occur in approximately 7-10% of AML cases, and limited studies show low remission rates in such cases (PMID:17927977; PMID: 33603989); it is unclear if such poor prognosis is a consequence of diagnosing these cases as AML rather than MPAL (T/myeloid), therefore resulting in management with AML based regimens over ALL based regimens
iii) Immunophenotypic features of the second lineage of blasts in MPAL may not be apparent until after initiating treatment protocol (such as AML-based regimen in this case). This case underscores the significance of comprehensive flow cytometric analysis, especially in those acute leukemias with aberrant immunophenotype (i.e. alternate lineage-defining markers such as cCD3 in myeloblasts) noted at diagnosis to specifically evaluate the possibility of an emerging/evolving MPALs

Panel Diagnosis

AML-MR (WHO)/ AML with mutated TP53 (ICC) with MPAL immunophenotype

EA4HP24-BMWS-227

Chronic Myeloid Leukemia Presenting with Mixed Phenotype B/Myeloid Blast Phase: A Diagnostic Challenge

Dr. Daniel Rivera, Dr. Brenda Mai, Dr. Hanadi El Achi, Dr. Andy Nguyen, Dr. Amer Wahed, Dr. Jacob Armstrong

The University of Texas Health Science Center at Houston, Pathology and Laboratory Medicine, Houston, USA

Case Description

The patient is a 60-year-old man without significant past medical history who presented with fatigue, leukocytosis (36.7 $x10^9$/L), mild anemia (hemoglobin 12.2 g/dL), and a platelet count (193 $x10^9$/L). The differential revealed neutrophilia (10.3 $x10^9$/L) and 35% circulating blasts were identified.

Biopsy Fixation Details

Formalin-fixed, paraffin-embedded tissue.

Frozen Tissue Available

None

Details of Microscopic Findings

Bone marrow biopsy and aspirate demonstrated a hypercellular marrow with a dimorphic blast population comprised of small blasts with scant cytoplasm, cytoplasmic tails, fine chromatin, and indistinct nucleoli, and large blasts with abundant cytoplasm, delicately folded nuclei, fine chromatin, and prominent nucleoli. Blasts comprised approximately 70% of cellularity, and background maturing trilineage hematopoiesis is decreased.

Immunophenotype

Two distinct blast populations are identified by flow cytometry, both of which are positive for CD45 (dim), CD13, CD34, HLA-DR and TdT, and negative for CD10, CD11b, CD14, CD16, CD20, cytoplasmic CD22, CD56, CD64 and pan-T cell markers. The major population (approximately 33%) is positive for CD4 (dim), CD33 (partial), CD38, CD117 and CD123, and a subset is positive for CD11c. The minor population (approximately 13%) is positive for CD19, CD38 (partial), cytoplasmic CD79a and dim CD123.

Cytogenetics

Conventional cytogenetic studies showed the following karyotype: 46,XY,t(9;22)(q34.1;q11.2)[17]/47,idem,+der(22)t(9;22)[2]/46,XY[1]. Interphase fluorescence in situ hybridization (FISH) showed the *BCR::ABL1* fusion in 94.5% of nuclei, including segmented nuclei.

Molecular Studies

A *BCR::ABL1* p190 transcript without *ABL1* domain mutations was identified. Additionally, frameshift mutations in *ASXL1* (p.G646Wfs*12, VAF 36.5%) and *RUNX1* (p.Q268Afs*332, VAF 32.2% and p.N159Kfs*51, VAF 28.6%) were detected.

Proposed Diagnosis

Mixed Phenotype Blast Phase of Chronic Myeloid Leukemia, B/myeloid.

Interesting Feature(s)

BCR::ABL1-positive leukemias are heterogeneous, including chronic myeloid leukemia (CML) and *de novo* B-lymphoblastic leukemia (B-ALL), acute myeloid leukemia (AML) and mixed phenotype acute leukemias (MPAL). Of these, CML is the most common, and the vast majority of these cases demonstrate the major p210 *BCR::ABL1* transcript while only approximately 1% harbor the minor p190 transcript. *De novo BCR::ABL1*-positive mixed phenotype acute leukemias are particularly rare, accounting for <1% of all acute leukemias, and conflicting reports show a prevalence of either the p210 or p190 transcript. Typically, CML can be easily distinguished from *de novo* acute leukemias by morphology as most cases present in chronic phase; however, rare cases of CML presenting in blast phase may be difficult or even impossible to distinguish from acute leukemia counterparts, particularly when background features of CML (e.g. left-shifted granulocytes, basophilia) are not apparent. Our case is interesting in that it represents CML presenting in mixed phenotype blast phase (B/myeloid) with a p190 *BCR::ABL1* fusion transcript, without distinct morphologic clues to distinguish from a *de novo BCR::ABL1*-positive MPAL. The diagnosis in this case is based on the discrepant number of *BCR::ABL1*-positive cells by interphase FISH and karyotyping in comparison to the blast count, and presence of the *BCR::ABL1* fusion in segmented nuclei suggesting mature neutrophils carry the fusion, highlighting the role ancillary studies play in distinguishing between these two morphologically and cytogenetically similar entities.

Panel Diagnosis

CML, blast phase (WHO/ICC) with MPAL (B/myeloid) immunophenotype; DD MPAL with bcr::abl1

EA4HP24-BMWS-228

Mixed-phenotype acute leukemia (MPAL), B/T

Dr. Nourhan Ibrahim, Dr. Swati S. Gite, Dr. Sibel Ak, Dr. Zubaidah Al-Jumaili, Dr. Levani Iashvili, Dr. Hanadi El Achi, Dr. Jacob Armstrong, Dr. Brenda Mai

University of Texas Health Science Center, Department of Pathology and Laboratory Medicine, Houston, USA

Case Description

A 65-year-old HIV-positive male with a past medical history of multiple malignancies including laryngeal carcinoma, melanoma, lung carcinoma, post-cytotoxic therapy, presented with leukocytosis

Biopsy Fixation Details

Formalin-fixed, paraffin-embedded tissue

Frozen Tissue Available

N/A

Details of Microscopic Findings

Peripheral blood smear showed leukocytosis (28,900 x109/L) with ~50% blasts, normocytic anemia (Hb 9.5 g/dL), and thrombocytopenia (37,000 x109/L)

The blasts were predominantly small and appeared lymphoid without cytoplasmic granules or Auer rods

Bone marrow examination showed increased cellularity, ~90%, with extensive involvement by acute leukemia with >90% infiltrating blasts

The megakaryocytes were increased with few dysplastic features including hypolobated and hyperchromatic forms; erythroid and myeloid elements were decreased

Immunophenotype

Flow cytometry analysis on the peripheral blood detected 51% blasts with the following immunophenotype: cCD3+ (subset), CD7+ (minor subset), CD10+ (minor subset), CD11b+ (subset), CD11c+(subset), CD13+(partial), CD14 (minor subset), CD11c+ (subset), CD19+ (subset), CD20+ (subset), cCD22+ (subset), CD33+ (subset), CD34+, CD38 (partial, dim), CD45+ (dim), CD56+ (subset), CD64+ (subset), CD71+, cCD79a+ (subset), CD117+, Tdt+ (subset), HLADR+, and negative for CD2, CD3, CD4, CD5, CD8, CD16, CD41, CD61, CD123, CD138, CD235a, MPO, kappa, and lambda

Flow cytometry analysis on the bone marrow aspirate with a similar immunophenotype

IHCs were performed on the core showed that a majority of the blasts were positive for Tdt, CD117, and CD34; a subset of blasts were positive for CD79a and CD20 and the other half were positive for CD3 and CD7. Myeloperoxidase (MPO), CD1a, CD4, CD5, and CD8, were negative

Cytogenetics

Karyotyping analysis showed a complex and composite male karyotype with multiple numerical and structural abnormalities in 19 of 20 analyzed metaphases: 43~45,XY,add(3)(q11.2),der(3)t(3;17)(q11.2;q11.2),del(5)(q13q33),add(7)(q32),add(11)(q21),add(13)(q14),-17,add(17)(p11.2),der(17;18)(q10;q10),-18,-20,psu dic(20;17)(q11.2;p13),-21,+1~2mar[cp19]/46,XY

Fluorescence in situ hybridization (FISH) detected a loss of chromosome 3q, del(5q), trisomy 6, trisomy 7, trisomy 10, amplification/gain of chromosome 11/11q, gain of chromosome 9/9q, trisomy 17, del17p, and gain of chromosome 22/22q

Molecular Studies

Next genome sequencing (NGS) detected a TP53 missense mutation, C-KIT, FLT3, IDH1/IDH2, NPM1 and CEBPA mutations were negative

Proposed Diagnosis

Mixed-phenotype acute leukemia B/T (MPAL-B/T)

Interesting Feature(s)

MPAL-B/T is a rare leukemia subtype as it represents only 6% of MPAL cases, according to literature review

Based on the dysplastic morphology, FISH abnormalities, and complex karyotypes, this case may be considered as an ‘acute myeloid leukemia, myelodysplasia-related’ or more

specifically, ‘myeloid neoplasm post cytotoxic therapy’, based on the patient’s clinical history, but the immunophenotypes of the blasts are consistent with a MPAL-B/T due to the lack of MPO expression. However, in the current 5th edition of the WHO Classification of Hematopoietic Tumors, one of the essential criteria of MPAL includes “no history of exposure to cytotoxic therapy”. This case serves as an interesting diagnostic dilemma as there is no equivalent ‘lymphoid neoplasm post cytotoxic therapy’
In addition, had the immunophenotype been myeloid, according to the International Consensus Classification, this case could have been classified as ‘AML with mutated TP53’, however, similarly, a category of ‘acute lymphoblastic leukemia with mutated TP53’, does not exist

Panel Diagnosis
AML-MR (WHO)/AML with mutated TP53 (ICC) with MPAL immunophenotype, therapy-related

EA4HP24-BMWS-232

A challenging case of *PICALM::MLLT10* rearranged acute leukemia with unusual immunophenotype and genotype

Dr. Jihee Choi, **Dr. Weina Chen**

University of Texas Southwestern Medical Center, Pathology, Dallas, USA

Case Description
A 9-year-old male presented with persistent fever for 2-3 weeks. Further evaluations on peripheral blood (PB) and bone marrow (BM) smears revealed bicytopenia and abundant lymphoid appearing blasts in PB/BM aspirate.
The patient was treated with B-ALL regimen with a poor response and then switched to AML regimen and in remission followed by first hematopoietic stem cell transplant (HSCT). His leukemia unfortunately relapsed with the immunophenotypic shift towards to AML. He was treated with AML regimen and in remission followed by second HSCT and is alive at the last follow-up (at 84th month).
Biopsy Fixation Details
Giemsa-stained smears on PB and BM aspirate and formalin-fixed clot section (no core biopsy performed)
Frozen Tissue Available
N/A
Details of Microscopic Findings
At diagnosis, leukemia blasts (75% in BM) have lymphoid appearance (predominantly medium-large sized cells with dispersed to moderately coarse chromatin, occasional nucleoli, and scant to moderate cytoplasm).

At relapse, leukemia blasts (47% in BM) have similar features with slightly more cytoplasm compared with blasts at diagnosis.

Immunophenotype

At diagnosis, leukemia blasts partially/variably express CD19/CD22/CD79a/TdT/CD34/CD13/CD33/CD117 but are negative for T-cell markers including CD3 (surface/intracellular), CD20, or myeloperoxidase (MPO). This immunophenotype is consistent with B-ALL (B-lymphoblastic leukemia) by 5th WHO/ICC or MPAL (B/myeloid) by EGIL scoring system.

At relapse, leukemia blasts express diminished B-cell antigens (only partial CD19, but absent CD22/CD79a) while maintaining a similar degree of myeloid differentiation (CD13 dim+/CD33+/CD117 partial+). The overall IP fits better for AML (acute myeloid leukemia).

Cytogenetics

At diagnosis: 46,XY,del(7)(q11.2q22),t(10;11)(p13;q21)[9]

At relapse - the same clone with clonal evolution:

46,XY,del(7)(q11.2q22),add(9)(p13),t(10;11)(p13;q21),t(17;17)(p11.2;q21)[1]

Molecular Studies

At diagnosis: *PICALM::MLLT10* (resulted from t(10;11)(p13;q21)), *STAT5B* N624H, *PHT6* E191*, *WT1* loss, and *NRAS* pGly12Val; this genomic signature including alteration in JAK/STAT signaling is commonly seen in T-lymphoblastic leukemia (T-ALL).

At relapse - similar findings with additional mutation in *DNMT3A* R882H

Genomic findings (at diagnosis/relapse) indicate the same leukemia clone with clonal evolutions despite immunophenotypic shift (diminished B-cell antigen expression) towards AML and persistent phenotype-genotype discordancy.

Proposed Diagnosis

Better classified as: Mixed phenotype acute leukemia, B/myeloid (by clinical course and EGIL)

Not favored: B-lymphoblastic leukemia by 5th WHO/ICC

Interesting Feature(s)

We report a rare case of acute leukemia harboring *PICALM::MLLT10,* classified as B-ALL by 5th WHO/ICC. Intriguingly, this case seems better classified as MPAL (B/myeloid) given extensive myeloid antigen expression (including CD117 but lack of MPO), which meets EGIL criteria but not 5th WHO/ICC criteria, and importantly a good response to the tandem chemotherapy (B-ALL to AML-based regimens). Additionally, the molecular features were more commonly seen in T-ALL (e.g., JAK-STAT*)* ("phenotype/lineage–genotype discordancy"). Awareness of such "discordancy" is important as both results are necessary for proper characterization of the disease and patients seemed to respond well to the lineage-matched treatment. Furthermore, our case poses a diagnostic challenge in MPAL regarding inclusion of CD117. Much further work is necessary to explore the impact of *PICALM::MLLT10* on the lineage differentiation and pathogenesis.

Panel Diagnosis

B-ALL (WHO/ICC)

EA4HP24-BMWS-233

Acute leukemia with trilineage differentiation, likely unrelated to reported prior MPN

Dr. Kseniya Petrova-Drus[1], Dr. Shenon Sethi[1], Dr. Filiz Sen[1], Dr. Umut Aypar[1], Robert Cimera[1], Dr. Yanming Zhang[1], Dr. Mark Ewalt[1], Dr. Eytan Stein[2], Dr. Mikhail Roshal[1]

[1] Memorial Sloan Kettering Cancer Center, Pathology and Laboratory Medicine, New York, USA; [2] Memorial Sloan Kettering Cancer Center, Leukemia Service, Department of Medicine, New York, USA

Case Description

77 year old woman with a reported history of "essential thrombocytosis (ET) several years" prior to presentation (no further information available) presented to an outside hospital (OSH) in February 2023 with an abnormal CBC and many circulating blasts (92%). No hepatosplenomegaly on physical exam. A bone marrow biopsy (BM Bx) was performed at OSH (reviewed at our institution) and showed acute leukemia with myeloid, B and T-cell differentiation. The patient was treated with mitoxantrone, etoposide, cytarabine (MEC), followed by 6 cycles of decitabine and venetoclax. Detailed treatment response is not available. In September 2023 she received low-intensity chemotherapy (cyclophosphamide, cytarabine, dexamethasone, methotrexate) with inotuzumab ozogamicin and blinatumomab iand IT chemotherapy for known CNS involvement (report/ slides not reviewed). A follow-up OSH BM Bx on 10/31/23 showed persistent disease (60% blasts) and came to MSKCC for second opinion and an in-house BM Bx was performed.

Biopsy Fixation Details

10% formalin and decal.

Frozen Tissue Available

N/A

Details of Microscopic Findings

Initial Diagnosis: OSH BM Bx 2/9/23: Hypercellular marrow (up to 100%) mostly replaced by blasts, and markedly reduced hematopoietic elements. Megakaryocytes are predominantly small and dysplastic. Aspicular and hemodiluted smears show numerous intermediate to large size blasts without Auer rods. Reticulin fibrosis is mild (MF-1)..
Follow up in course of treatment: OSH BM Bx: 10/31/23: Hypercellular marrow (50-6%) with markedly increased immature cells, and reduced hematopoietic elements.
Follow up: MSKCC BM Bx: 11/30/2023: Hypercellular marrow (80-90%) with increased blasts, reduced hematopoietic elements and dysplastic megakaryocytes. Reticulin shows mild fibrosis (MF-1).

Immunophenotype

OSH BM Bx 2/9/23: Blasts express: CD34, CD117, TdT, CD3,CD56, PAX5 (small subset), MPO (small subset); do not express: CD61, Factor VIII, TIA-1, granzyme B, perforin, CD20 and

CD15, P53.
OSH BM Bx: 10/31/23: 60% blasts by CD34.
MSKCC BM Bx: 11/30/2023: Blasts (~80% of cellularity) are positive for: CD34, CD117, TdT, MPO (small subset), lysozyme (small subset) and negative for CD61, which highlights megakaryocytes. Flow cytometry: Blast population (85.2%) with abnormal : CD5 , CD7 (partial), CD10 (partial dim), CD11b (partial dim), CD13 (bright), CD19 (partial), CD22 (partial, dim), CD24 (partial, dim), CD33 (variable), CD38 (dim), CD45RA (uniform, intermediate), CD56 (positive), CD117 (variable, bright to dim), CD123 (positive), cytoplasmic CD3 (partial), cytoplasmic CD79a (partial), cytoplasmic MPO (partial), cytoplasmic TdT (positive); normal expression of: CD4, CD34, CD45, CD71, HLA-DR; and negative for: CD1a, CD2, surface CD3, CD8, CD14, CD15, CD16, CD20, CD25, CD36, CD48, CD64, CD66b, CD73/CD304, CD86, CD105, CD90, CD99.

Cytogenetics

46,XX,t(6;11)(q21;q23)[1]/46,idem,t(4;14)(q12;q32),del(5)(q31q35)[15]/ 46,XX[4]
FISH: Del (5q) positive in 91.3% of cells. No KMT2A/MLL (11q23) rearrangement.

Molecular Studies

NGS Matched testing (IMPACT Heme): 27 somatic mutations (including SF3B1 p.K790E, STAT3 p.T500S, subclonal MPL p.W515L, complete list on slides), Deletion of IRF1 (5q31.1) (Fold Change: -2.6), Loss of 5q, Copy neutral loss of heterozygosity of 3p.
Negative for fusions by RNA-based NGS testing.

Proposed Diagnosis

Acute leukemia with T/B/ myeloid phenotype, favor acute myeloid leukemia with myelodysplasia-related (AML-MR) cytogenetic abnormality

Interesting Feature(s)

Challenging classification; unusual mutational profile; possibility of an antecedent myeloid neoplasm, however unclear history

Panel Diagnosis

AML-MR (WHO/ICC)

EA4HP24-BMWS-236

Acute myeloid leukemia without lineage-defining antigen expression

Dr. Genevieve Parecki[2,1], Dr. Dean Fong[1,2]

[1] Veterans Affairs Portland Health Care System, Pathology and Laboratory Medicine Service, Portland, USA; [2] 1Oregon Health & Science University, Department of Pathology and Laboratory Medicine, Portland, USA

Case Description

The patient is a 67 y/o male with a 1 year history of progressive anemia and neutropenia. He was found to be pancytopenic and a bone marrow biopsy was performed revealing acute leukemia most consistent with acute myeloid leukemia; however, the blast population lacked expression of lineage-defining markers. The patient completed 5 cycles of azacitidine, with venetoclax added on cycle 2. He passed away shortly after beginning cycle 6 of his chemotherapy regimen, 5 months after his bone marrow biopsy.

Biopsy Fixation Details

10% Formalin

Frozen Tissue Available

Not available

Details of Microscopic Findings

The peripheral blood smear shows pancytopenia with rare circulating blasts (1%). Red cells exhibit mild anisopoikilocytosis with rare nucleated forms. A subset of neutrophils exhibit dysplastic features including cytoplasmic hypogranularity and Pelgeroid nuclei. The bone marrow aspirate reveals a blast population comprising 54% of cells with increased N:C ratios, delicate nuclear chromatin, nucleoli, and pale blue agranular cytoplasm. Granulopoiesis is reduced with left-shifted maturation and morphologic dysplasia in a minor subset exhibiting cytoplasmic hypogranularity. Erythroid precursors are reduced and exhibit megaloblastoid change with rare dyspoietic features. A CD34-positive blast population comprising 40-50% of cells show co-expression of TdT and partial positivity for CD79a and CD117. They are negative for CD3, CD20, PAX-5, MPO, and lysozyme.

Immunophenotype

Flow cytometry revealed a blast population comprising 25% of cells with immunophenotype: dim CD13, CD25, CD34, CD38, partial CD117, dim CD123, HLA-DR, and TdT positive. This population is negative for CD3, MPO, CD19, and PAX5.

Cytogenetics

46,XY. One of 20 metaphase cells exhibited a nonspecific aberration.

Molecular Studies

Next generation sequencing was negative for FLT3 ITD or FLT3 D835/I836 TKD. A comprehensive genomic panel revealed 3 mutations in either RUNX1 or SRSF2. RUNX1 p.S388* was detected at a variant allele fraction (VAF) of 16%. RUNX1 p.P103fs*22 was detected at a VAF of 15%. SRSF2 p.P95L was detected at a VAF of 27%.

Proposed Diagnosis

Acute myeloid leukemia with ambiguous lineage

Interesting Feature(s)

The absence of lineage-specific myeloid, monocytic, B-cell, or T-cell markers made classification of the blast population challenging. The presence of partial expression of CD79a (by flow and IHC) and CD22 (by flow) raised thought for mixed-phenotype acute leukemia, but the absence of CD19 expression argued against this diagnosis. Acute undifferentiated leukemia and AML with minimal differentiation were also within the differential, although by WHO guidelines (2017) the expression of two myeloid-associated markers (CD13 and CD117) is best classified provisionally as acute myeloid leukemia. Cytogenetics and molecular studies were largely unrevealing, although the mutations in RUNX1 and SRSF2 are most commonly noted in acute myeloid leukemia. A small degree of morphologic dysplasia, particularly within granulocytic and megakaryocytic lineages was also noted, although definitive evaluation was limited due to paucicellular aspirate smears. This finding, although not specific, raised consideration for an underlying myelodysplastic neoplasm. The diagnosis of acute myeloid leukemia was rendered, although it is unclear whether this case represents a de novo acute leukemia or leukemia arising in the background of myelodysplastic neoplasm.

Panel Diagnosis

AML-MR gene mutations (WHO/ICC)

EA4HP24-BMWS-237

Extramedullary Mixed Phenotype Acute Leukemia Associated with Warthin's Tumor and Bone Marrow Involvement by Acute Myeloid Leukemia

Dr. Pooja Devi[1], Dr. Olga Pozdnyakova[1], Dr. Adam Bagg[1], Dr. Gabriel C. Caponetti[1]

[1] University of Pennsylvania Perelman School of Medicine, Department of Pathology, Hematopathology, Philadelphia, USA; [2] University of Pennsylvania Perelman School of Medicine, Department of Pathology, Hematopathology, Philadelphia, USA; [3] University of Pennsylvania Perelman School of Medicine, Department of Pathology, Hematopathology, Philadelphia, USA; [4] University of Pennsylvania Perelman School of Medicine, Department of Pathology, Hematopathology, Philadelphia, USA

Case Description

69 y/o male with parotid masses, status post left parotid FNA (2015) with findings compatible with Warthin's tumor (WT). CT scan revealed parotid masses and enlarged cervical lymph nodes (LN). Left parotid resection (2022) showed an encapsulated mass and a 1.7 cm lymph node (LN). A tandem CBC demonstrated anemia.

Biopsy Fixation Details

Formalin

Frozen Tissue Available

NA

Details of Microscopic Findings

Parotid:

An encapsulated mass with necrosis, histiocytes, and giant cells. Cysts lined by pseudostratified ciliated eosinophilic epithelium with aggregates of small lymphocytes. Intraparotid LN with partial architectural effacement by atypical small to medium-sized mononuclear cells with irregular nuclei, vesicular chromatin, occasional prominent nucleoli, and scant cytoplasm.

Bone marrow (BM):

Hypercellular BM (80%) with ~30% blasts. Erythroids and myeloids show full maturation without dyspoiesis. Megakaryocytes are adequate in number and unremarkable morphology.

Immunophenotype

IHC (Parotid)

A major subset of the atypical mononuclear cells express CD34, MPO, CD117, and CD33, while a smaller subset express TdT, CD1a, CD3, CD2, CD5, CD7, and CD4. No significant expression of CD19, CD20, PAX5, CD79A, cyclin D1, SOX11, CD10, BCL6, CD30, ALK1, EBER(ISH), or CD8 is seen.

Flow cytometry (BM)

Expanded CD45(dim)+ region with 25% blasts with following immunophenotype: CD34+ CD117(subset)+ HLA-DR(subset)+ CD33+ CD13+ CD16- CD123(dim)+ CD14(minor subset)+ CD64(variable)+ CD15- CD11b+ cMPO+ CD71- CD4(subset)+ CD7(subset)+ CD2 (subset)+ sCD3- cCD3- TdT-.

Cytogenetics

BM

Karyotype: 47,XY,+?Y,t(2;11;7)(q37;q14;p13)[12]/46,XY[8]

FISH: Negative for 5p/5q deletion, deletion of 7q/monosomy 7, *TP53* deletion, *RUNX1T1*::*RUNX1*, CBFB rearrangements

Molecular Studies

NGS (Parotid)

Disease-associated variants (% VAF)

DNMT3A 36%

KRAS 30%

SRSF2 35%

TET2 36%

NGS (BM)

Disease-associated variants (% VAF)

DNMT3A 42%

KRAS 19%

SRSF2 42%

TET2 44%

Proposed Diagnosis

Parotid:

Mixed phenotype acute leukemia (MPAL), T/myeloid, NOS.

Warthin's tumor.

BM:

Acute myeloid leukemia (AML) with myelodysplasia-related gene mutations (ICC 2022)/ AML, myelodysplasia-related (WHO 2022).

The patient was refractory to induction therapy and is now s/p allogeneic HSCT and in continued remission.

Interesting Feature(s)

This acute leukemia presented as parotid masses in an otherwise asymptomatic patient with anemia. Acute leukemia associated with a WT was diagnosed incidentally and classified as extramedullary MPAL, T/myeloid, NOS. To our knowledge, only one case of WT with concurrent acute leukemia has been reported in literature (PMID: 31933816).Although the myeloblasts in the BM expressed some T-cell markers, their overall immunophenotype was insufficient for a diagnosis of MPAL in the BM, and a diagnosis of AML was rendered instead.

NGS done on the intraparotid LN and BM identified the same *DNMT3*, *SRSF2*, *TET2,* and *KRAS* pathogenic variants, which highlights the clonal relationship between the blasts in both organs. However, the VAF of the *KRAS* mutation in the BM is notably lower than that of the *KRAS* mutation in the LN, and much lower than the VAFs of other mutations in the BM than in the LN. This finding raises the possibility that the higher KRAS VAF in the LN is reflective of the T-cell component seen in this organ and that it may have mediated the expression of more T-cell markers by the blasts in the LN (in particular, CD3).

Our case highlights the immunophenotypic variability of acute leukemia across different sites of involvement and that, in some cases, this could be due to a difference in the VAFs of certain mutations.

Panel Diagnosis

AML-MR(WHO/ICC) with MPAL immunophenotype

EA4HP24-BMWS-238

B-lymphoblastic leukemia with aberrant MPO positivity

Dr. Genevieve Parecki[2,1], Dr. Dean Fong[1,2]

[1] Veterans Affairs Portland Health Care System, Pathology and Laboratory Medicine Service, Portland, USA; [2] Oregon Health & Science University, Department of Pathology and Laboratory Medicine, Portland, USA

Case Description

The patient is a 69 y/o male with a history of diffuse large B-cell lymphoma (DLBCL) s/p 3 cycles R-CHOP. 6 years later, he presented with pancytopenia and bone marrow involvement was suspected. A bone marrow biopsy showed involvement by B-lymphoblastic leukemia. He began therapy with vincristine/idarubicin/methotrexate/cytarabine, but his course was hindered by cardiac complications, and he became transfusion-dependent. He developed neutropenic fever and bacteremia with Gram positive cocci. He was transitioned to comfort care, passing away 1.5 months after his bone marrow biopsy.

Biopsy Fixation Details

10% Formalin

Frozen Tissue Available

Not available

Details of Microscopic Findings

The peripheral blood smear shows pancytopenia. No circulating blasts are seen. Red cells show moderate anisopoikilocytosis. Myeloid cells exhibit mature forms. Lymphocytes appear heterogeneous and mature. The aspirate smears reveal an increased population of morphologic blasts comprising 80% of nucleated cells. The blasts have markedly increased N:C ratios, delicate nuclear chromatin, occasional nucleoli, nuclear folding, and have scant agranular cytoplasm with occasional cytoplasmic vacuoles. No granulocytic precursors, erythroid precursors, or megakaryocytes are seen. The core biopsy and clot section are diffusely involved by similar immature mononuclear cell population. The blasts are positive for CD34, PAX-5, TdT, CD20 (partial) and MPO (weak); they are negative for lysozyme.

Immunophenotype

Flow cytometry revealed a blast population comprising 82% blasts with immunophenotype: CD9, CD10, CD19, dim CD20, CD22, dim CD33, CD34, dim CD38, CD58, cCD79a, HLA-DR, TdT, and dim MPO positive.

Cytogenetics

46,XY. FISH with an AML/ALL probe panel suggests the presence of hypodiploidy: 95% of cells had single signals for GATA2 (3q21)/MECOM (3q26.2), D7S486 (7q31)/CEP7, ABL/ASS/NUP214 (9q34), ETV (12q13), PML (15q24), CBFB (16q22)/MYH11 (16p13.1), and RARA (17q21). There is no evidence for rearrangements involving any of the probe regions.

Molecular Studies

A comprehensive hematopathology sequencing panel revealed a common pathogenic TP53 mutation: p.R273H at a variant allele frequency of 82%. Next-generation sequencing was negative for FLT3 internal tandem duplication.

Proposed Diagnosis

B-lymphoblastic leukemia

Interesting Feature(s)

In this case of lymphoblastic leukemia, the blast population showed dim MPO positivity, raising consideration for mixed phenotype acute leukemia (MPAL): B/myeloid. The blasts lacked bright positivity for any myeloid markers (CD13, CD33, CD117) by flow cytometry and a diagnosis of B-lymphoblastic leukemia was rendered. The patient had apparent hypodiploidy despite a normal karyotype. True hypodiploidy carries a poor prognosis in B-ALL, but the prognostic significance of the finding in our case is unknown. This diagnosis was made in the post-chemotherapy setting, raising the question of a therapy-related lymphoid neoplasm, which has been reported in up to 10% of patients having undergone cytotoxic therapy. Studies have noted the frequent presence of cytogenetic abnormalities represented in myeloid neoplasms post cytotoxic therapy, two of which (losses in 7q and 12q) were suggested by cytogenetics in our case. In short, our case represents a B-lymphoblastic leukemia arising in the post-chemotherapy setting, with weak aberrant expression of MPO.

Panel Diagnosis

B-ALL (WHO/ICC)

EA4HP24-BMWS-239

Myelodysplastic syndrome with biallelic TP53 inactivation exhibiting a T/Myeloid immunophenotype

Dr. Genevieve Parecki[2,1], Dr. Todd Williams[2], Dr. Dean Fong[1,2]

[1] Veterans Affairs Health Care System, Pathology and Laboratory Medicine Services, Portland, USA; [2] Oregon Health & Science University, Department of Pathology and Laboratory Medicine, Portland, USA

Case Description

The patient is a 70 y/o male with a complex medical history who presented with persistent fevers and malaise. He was found to be pancytopenic. Bone marrow biopsy revealed trilineage dysplasia and increased blasts expressing both myeloid and T-cell antigens. Cytogenetic studies revealed an abnormal karyotype with 17p/TP53 deletion, and molecular studies revealed a pathogenic TP53 mutation. The patient underwent chemotherapy induction with azacitidine/venetoclax. His course was complicated by infection and febrile neutropenia. A second bone marrow biopsy was performed, showing blasts with a similar

immunophenotype. The patient elected for hospice care and passed away just under a month after the second bone marrow biopsy.

Biopsy Fixation Details

10% Formalin

Frozen Tissue Available

Not available

Details of Microscopic Findings

The initial peripheral blood smear demonstrated pancytopenia and rare circulating blasts. Neutrophils exhibited left-shifted maturation and morphologic dysplasia with Pelgeroid nuclei and cytoplasmic hypogranularity. A subset of platelets exhibited cytoplasmic hypogranularity. The aspirate smears were myeloid-predominant trilineage hematopoiesis with left-shifted myeloid progenitors. Megaloblastoid change and dysplastic forms were present. Blasts comprised approximately 15% of nucleated cells and had increased N:C ratios, delicate nuclear chromatin, nucleoli, and scant cytoplasm. Erythroid and megakaryocytic components demonstrated dysplasia. Lymphoid cells were unremarkable. The core biopsy and clot section had similar findings to the aspirate smears. CD34-positive blasts were increased, present in atypical clusters and scattered single cells. The blast population co-express CD117, MPO, CD3, and CD79a. In the subsequent bone marrow, blasts comprised 4% of nucleated cells.

Immunophenotype

Flow cytometry on the initial marrow revealed 15% blasts: cCD3, dim CD5, CD9, CD13, variable CD22, CD33, CD34, CD38, CD56, dim variable CD79a, CD117, HLA-DR, variable MPO positivity. Flow cytometry on the second marrow revealed 5% blasts: cCD3, dim CD5, partial CD7, CD9, variable CD10, CD13, dim/partial CD19, variable CD22, CD33, CD34, CD38, CD56, CD117, HLA-DR positive.

Cytogenetics

Complex karyotype: 43~49, XY, +X, add(14) (P11.2), del(17)(p12), -18, del(20)(q12), ider(21) (q10) T(21;22) (q11.2;q22), +1~4mar [CP20]. FISH performed with an AML/MDS probe panel: TP53 deletion in 88% of cells, a 20q deletion in 82% of cells, and copies of RUNX1 on either side of the centromere due to the isoderivative chromosome and the abnormal chromosome 14.

Molecular Studies

No FLT3 D835/I836 TKD mutation was detected. A pathogenic TP53 p.C275Y mutation was detected at a variant allele frequency of 70%.

Proposed Diagnosis

Myelodysplastic neoplasm with biallelic TP53 inactivation

Interesting Feature(s)

This case raised consideration for myelodysplastic syndrome with excess blasts-2 (MDS-EB2) with features concerning for an evolving acute leukemia versus early evolving acute mixed phenotype acute leukemia (T/myeloid). The patient exhibited a complex abnormal karyotype with 17p deletion as well as a known pathogenic TP53 mutation, resulting in biallelic inactivation of TP53. In the context of myeloid dysplasia and less than 20% blasts, these findings are best classified as MDS with biallelic TP53 inactivation by WHO criteria. While the proportion of blasts fell short of criteria for acute leukemia, the presence of cCD3

expression suggested the possibility of an evolving mixed phenotype acute leukemia (T/myeloid).

Panel Diagnosis

MDS with biallelic TP53 inactivation (WHO)/MDS-AML with TP53 mutation (ICC)

EA4HP24-BMWS-240

Myeloid/B Mixed-Phenotype Acute Leukemia with MACROD1-RUNX1 Fusion

Dr. Genevieve Parecki[2,1], Dr. Dean Fong[1,2]

[1] Veterans Affairs Portland Health Care System, Pathology and Laboratory Medicine Services, Portland, USA; [2] Oregon Health & Science University, Department of Pathology and Laboratory Medicine, Portland, USA

Case Description

The patient is a 70 y/o male with a history of MDS, treated with 6 cycles of azacytadine, who presented with hypoxia and volume overload. He was found to have 15-20% circulating blasts, and bone marrow biopsy was performed. Bone marrow revealed 20% blasts, with two immunophenotypically distinct populations: one with myeloid and one with B-cell expression. Further chemotherapy was proposed to the patient, but the patient elected for home hospice and passed away less than one month after the biopsy.

Biopsy Fixation Details

10% Formalin

Frozen Tissue Available

Not available

Details of Microscopic Findings

The peripheral blood smear shows blasts with increased N:C ratios, delicate chromatin, multiple nucleoli, and scant basophilic agranular cytoplasm. Neutrophils exhibit left-shifted maturation and dysplastic with hypogranular forms. The aspirate smear shows two populations together comprising 41% of nucleated cells. One population consists of small forms with markedly increased N:C ratios, delicate chromatin, prominent nucleoli, and scant cytoplasm; the second consists of larger cells with similar nuclear features but with slightly more cytoplasm. Myeloid and erythroid precursors are decreased with left shift and dysplastic forms. Megakaryocytes exhibit mononuclear forms. The core biopsy and bone marrow clot section have similar findings as the aspirate smear. One blast population is positive for CD34 and TdT (negative for CD3, CD20, CD61, and CD71); and another population stains positive for MPO, CD117, PAX-5, and CD79a.

Immunophenotype

Flow cytometry reveals 2 bone marrow blast populations. The dominant population (25% of cells) with immunophenotype: dim CD9, CD11b, CD13, dim CD14, CD25, CD33, parital CD34,

CD38, dim CD64, CD117, dim HLA-DR, partial TdT, and MPO positive. The smaller population (10% of cells) with immunophenotype: CD13, CD19, CD22, CD34, CD38, bright CD58, CD79a, dim CD117, and TdT.

Cytogenetics

46XY, t(11;21)(q13;q22),der(18)t(11;18)(q22;23). These translocations resulted in 3 copies of the long arm of chromosome 11, 1 copy of the normal 11, and 1 each of the long arms of chromosomes 18 and 21. By FISH, 3 intact MLL (11q23) signals were observed in 80% of cells. One signal was seen on the normal chromosome 11 and one signal was seen on the der(18) and der(21). Three signals for RUNX1 (21q22) were observed in 74% of cells, with 1 signal on each of the chromosomes 21 and 1 signal seen on the der(11). FISH was performed with a RUNX1 break apart probe and 84% of cells had a split signal pattern, confirming a t(11;21) with RUNX1 rearrangement. This rearrangement most likely represents the fusion of MACROD1 (11q13) and RUNX1 (21q22).

Molecular Studies

Molecular studies were not performed on this specimen. An AML/MDS gene mutation panel run on a specimen 2 years prior found an SF3B1 mutation: p.D781G at a variant allele frequency of 39%. This was reported as a variant of unknown significance.

Proposed Diagnosis

Mixed-phenotype acute leukemia, Myeloid/B

Interesting Feature(s)

Although the patient has a history of MDS, this case revealed an acute leukemia consisting of 2 morphologically and immunophenotypically distinct blast populations: myeloblasts and B lymphoblasts, meeting criteria for mixed-phenotype acute leukemia. Notably, it exhibited a rare RUNX1 rearrangement, specifically a fusion between MACROD1 (11q13) and RUNX1 (21q22). This fusion has been identified in only a few cases.

Panel Diagnosis

AML-MR (WHO/ICC) with MPAL immunophenotype

EA4HP24-BMWS-247

Myeloid/lymphoid neoplasm with FGFR1 rearrangement: Mixed-phenotype acute leukemia (Acute leukemias of ambiguous lineage).

Dr. Nabil Tabish, Dr. Changlee S. Pang

University of Colorado School of Medicine, Department of Pathology/ Hematopathology, Aurora, USA

Case Description

A 66-year-old man presented with 2 months of progressive fatigue and peripheral lymphadenopathy. An axillary lymph node biopsy was performed on 6/8/23 and a bone marrow biopsy was performed on 6/29/2023.

Biopsy Fixation Details

10% neutral buffered formalin.

Frozen Tissue Available

N/A

Details of Microscopic Findings

Bone marrow biopsy:

The bone marrow is hypercellular for age (70-80%). Atypical lymphoid paratrabecular aggregates comprise ~30% of the cellularity with scattered eosinophils. Interspersed within and around the aggregates are small, mature lymphocytes and intermediate to large, immature blastoid cells with irregular contours, fine chromatin, prominent nucleoli, and scant cytoplasm.

Peripheral blood:

Severe neutropenia with 2% circulating blasts and absolute eosinophil count of 0.0 x10*9/L

Lymph node resection:

The architecture is diffusely effaced by an infiltrative densely cellular lymphoid process. These lymphoid cells are intermediate-sized to occasionally large cells with irregular nuclear contours, fine to condensed nuclear chromatin, conspicuous nucleoli, and scant cytoplasm. Scattered mitotic figures & a few apoptotic bodies are present throughout the lesion. Abundant intralesional vessels and focal sclerosis are noted.

Immunophenotype

Bone marrow biopsy:

CD34: 10-15% blasts

CD117: focally positive in the infiltrating CD34+ blasts

TdT/CD10/CD99: ~10% lymphoblasts in a pattern different from CD34+ blasts

PAX5/CD79a: focally positive in the CD99/TdT-positive infiltrating lymphoblasts (5-10%), indicating B-lymphoblasts

CD3: focally positive in the CD99/TdT-positive lymphoblasts (3-4%), indicating T lymphoblasts. Also stains abundant mature T cells in the atypical lymphocytic aggregates.

CD1a: negative
Reticulin: mild to focally moderate reticulin fibrosis (MF 1-2 of 3)
Bone marrow aspirate flowcytometry:
(1) 9% B-lineage lymphoid progenitor cell population:
- Positive for CD19 (mod), CD10 (bright), cCD79a (mod to bright), CD24 (bright), and TdT
- Negative for CD34, CD20, CD22, surface immunoglobulins, CD1a, cCD3, CD5, and CD7
(2) 6% T-lineage lymphoid progenitor cell population:
- Positive for CD2, cCD3, CD7 (bright), variable CD5 (neg to mod), and CD56 (major subset)
- Negative for CD34, sCD3, CD10, CD4, CD8, TdT, CD1a, CD16, CD19, and CD79a
(3) 5% myeloid progenitor cell population:
- Positive for CD34 (bright), CD33, CD117 (variable), CD4 (dim), CD7 (variable), and CD38 (decreased)
- Negative for CD10, TdT, CD1a, CD2, sCD3, cCD3, CD8, CD19, CD22, CD24, cCD79a, CD15, and cMPO
Lymph node resection:
The infiltrating lymphoid cells are positive for CD99, TdT, CD3 (with cytoplasmic accentuation), CD4 (diffuse), CD5, CD7 (strong, diffuse), TdT (patchy), and CD1a (patchy). The infiltrating lymphoid cells are negative for CD10, TCR Beta, TCR Delta, CD8, MPO, CD68, BCL6, CD25, ALK, CD20, PAX5 and CD34 (predominantly negative).

Cytogenetics

Complex karyotype with seven related abnormal clones including t(8;13) consistent with FGFR1 rearrangement

Molecular Studies

RUNX1 mutation in addition to *FGFR1-ZMYM2* fusion by NGS.

Proposed Diagnosis

Myeloid/lymphoid neoplasm with FGFR1 rearrangement: Mixed phenotype acute leukemia (acute leukemias of ambiguous lineage)

Interesting Feature(s)

Mixed phenotype acute leukemia, FGFR1 rearrangement, and RUNX1 mutation in a mixed phenotype leukemia

Panel Diagnosis

Myeloid/lymphoid neoplasm with FGFR1 rearrangement (WHO/ICC)

EA4HP24-BMWS-270

Blast Phase of Chronic Myeloid Leukemia, *BCR::ABL1* Positive, Presenting as B-Lymphoid/Myeloid Extramedullary Tumor

Dr. Samer Z. AlQuran[1], Dr. Robert Emmons[2], Dr. Mustafa Al-Kawaaz[1]

[1] University of Louisville, Department of Pathology, Louisville, USA; [2] University of Louisville, Department of Medicine / Medical Oncology & Hematology, Louisville, USA

Case Description

A 63-year-old woman who presented with right sided abdominal pain with associated shortness of breath and weakness for three weeks. Her pain was constant, non-radiating and sharp. Chest x ray showed left pleural effusion. Flow cytometric analysis (FCM) of pleural fluid showed a large myeloblast population (~61%) and a B lymphoblast population (~16%). Imaging studies showed a large mediastinal mass encompassing the aorta, esophagus with paraspinal extension, lymphadenopathy and splenomegaly (15.2 cm). A bone marrow biopsy was performed and showed cellular bone marrow with left shifted granulocytic maturation and BCR-ABL translocation without overt increase in blasts. Concurrent bone marrow FCM showed an abnormal myeloid blasts population (~1.7%) and an abnormal B blasts population (~0.7%). The peripheral blood showed circulating left shifted granulocytic cells, monocytosis with only few circulating myeloid blasts (~2%). A supraclavicular lymph node excisional biopsy was performed and showed proliferation of B-lymphoid and myeloid blasts. FCM of the lymph node showed abnormal blast population consisting of a myeloid and B lymphoid blasts. The patient was treated with HyperCVAD and showed excellent response with resolution of her chest mass and other lymphadenopathy. She is planned for another cycle of HyperCVAD and subsequent allogeneic transplant once deep remission has been achieved.

Biopsy Fixation Details

10% buffered formalin

Frozen Tissue Available

No.

Details of Microscopic Findings

The lymph node architecture was almost completely effaced by the diffuse proliferation of large blastoid cell with a brisk mitotic activity. The neoplastic cells had large nuclei, variable nuclear outline irregularities immature chromatin, and prominent single to multiple nucleoli.

Immunophenotype

Immunohistochemical studies performed on LN showed that the neoplastic cells are positive for: CD34, Lysozyme, CD33, C43, CD163 (subset), CD68 (subset), PAX-5(subset), CD19(subset), MPO(subset), CD79a(subset), OCT2(subset), CD15(minor subset) and TDT(subset), with few scattered CD117 positive cells. CD123 showed strong expression in few small clusters of cells and variable expression in blasts. CD20 is positive in scattered B

cells. CD3 is positive in T cells. CD71 and CD56 are negative in blasts.

Flow cytometric immunophenotyping of the lymph node demonstrated the presence of abnormal population of CD34(+) blasts, which appeared to be positive for CD38, CD13, dim CD45, dim CD123, CD33, HLA-DR, and partial dim CD7 with variable CD117 and CD71. The blasts appeared to be TdT(+) and included subset of CD19(+) cells with expression of cytoplasmic CD79a and subset with cytoplasmic MPO. They were negative for CD20, CD3, cyCD3, CD56, CD57, CD8, CD11b, CD15, CD64, CD14, CD16, CD61 and CD2

Cytogenetics

Bone marrow Karyotype: 46,XX,t(9;22)(q34.1;q11.2)[20]
Blood FISH: Positive for t(9;22)

Molecular Studies

NGS panel performed on the lymph node tissue showed *BCR-ABL1* translocation as well as a pathogenic RUNX1 mutation.

Proposed Diagnosis

Chronic myeloid leukemia, *BCR::ABL1* Positive, Blast phase, B-Lymphoid/Myeloid, in lymph node.

Interesting Feature(s)

Blast phase as initial manifestation of *BCR::ABL1* positive CML composed of mixed phenotype B/myeloid blasts and presenting as mediastinal mass with pleural effusion

Panel Diagnosis

CML, blast phase (WHO/ICC) with extramedullary MPAL (B/myeloid)

EA4HP24-BMWS-274

Mixed Phenotype Acute Leukemia

Dr. Olabisi I. Afolayan-Oloye, Vania Aikawa, Dr. Siddharth Bhattacharyya, Dr. Annapurna Saksena

Hospital of the University of Pennsylvania, Pathology and Laboratory Medicine, Philadelphia, USA

Case Description

71-year-old female presented with 2.5 months of gradually worsening fatigue, dyspnea with exertion, poor oral intake, easy bruising, with several large bruises over past few months. Labs were significant for normocytic anemia, lymphocytosis, neutopenia, thrombocytopenia prompting heme referral.

Biopsy Fixation Details

Bone marrow biopsy was decalcified.

Frozen Tissue Available

Not applicable.

Details of Microscopic Findings

On aspirate smear, blasts are increased (88%) and show a spectrum in morphology. Most of the blasts appear small to medium in size with scant cytoplasm, relatively condensed chromatin, and inconspicuous nucleoli. A subset of blasts appears larger in size, with scant to moderate blue cytoplasm, irregular nuclei, fine chromatin, and occasional prominent nucleoli. Residual trilineage hematopoiesis was significantly decreased. An iron stain shows storage iron; ring sideroblasts are not seen. On bone marrow biopsy, there is markedly hypercellular marrow (95%) for age. Marrow cellularity is replaced by sheets of blasts (>90%), medium-sized with dispersed chromatin and inconspicuous nucleoli. A reticulin stain shows grade 0 -1 fibrosis (European Consensus System).

Immunophenotype

Flow cytometry performed on peripheral blood showed an approximate differential of 42% lymphocytes, 3% granulocytes and 53% CD45 dim events based on CD45 versus side scatter gating. The lymphocyte region is comprised of 9.0% polytypic CD19+ B cells with a kappa:lambda ratio of 1.2, 87.4% CD3+ T cells with a CD4:CD8 ratio of 2.2 and 3.9% NK cells. The CD45 dim region shows a discrete population of immature precursors with the following immunophenotype: CD34+ CD19+ CD10+ CD20(subset +) CD22+ CD200+ CD38-HLADR+ CD123+ CD11b+ (n)TdT+ (c)CD79a+ CD71-CD64-CD14-. There is a separate CD45dim population with slightly higher SS that comprises approximately 10% of total events and expresses the following immunophenotype: CD34+ CD14+ CD64+ CD11b bright+ CD33bright TdT- MPO- HLADR+ CD200+CD117-CD16-CD20- CD79a-CD22-. Compared to the principal blast population, this population shows relatively dimmer expression of CD10 and CD19.

Immunohistochemical stains showed CD34+ population composed of two distinct populations: a CD14+ monocytic population and another CD79a+ B cell population.

Cytogenetics

Karyotype (ISCN Nomenclature): 56,XX,+X,+2,+5,+8,+10,+11,+14,+17,+18,+21[2]/46,XX[1].

FISH: Positive for gain of KMT2A.

Molecular Studies

DISEASE ASSOCIATED VARIANT [1 Total]: CBL p.Y371H; c.1111T>C; NM_005188 VAF: 86%

Proposed Diagnosis

Mixed Phenotype Acute Leukemia, bilineal (B/myeloid (monocytic) phenotype)

Interesting Feature(s)

·An interesting case of bilineal mixed phenotypic acute leukemia with two distinct populations of B cell and monocytic lineage by flow cytometry.

·Bone marrow aspirate shows two relatively discernable populations of blasts and IHC on bone marrow biopsy shows two leukemic populations

·Cytogenetic studies show complex karyotype with FISH studies positive for KMT2A gain but negative for KMT2A rearrangement and BCR::ABL1 rearrangement .

Panel Diagnosis

MPAL, B/myeloid (WHO/ICC)

EA4HP24-BMWS-278

Diagnostic Challenges in a Patient with Acute Leukemias: Myeloid or Lymphoid?

Dr. Zhihong Hu[1], Dr. Pei Lin[2], Dr. Brenda Mai[1]

[1] The University of Texas MD Anderson Cancer Center, Department of Laboratory Medicine, Houston, USA; [2] The University of Texas MD Anderson Cancer Center, Department of Hematopathology, Houston, USA; [3] The University of Texas MD Anderson Cancer Center, Houston, Germany

Case Description

A 65-year-old woman presented in July 2018 with fatigue, shortness of breath, significant weight loss, and lymphadenopathy. Her initial diagnosis was acute myeloid leukemia with minimal differentiation (AML-M0) and was treated with Fludarabine, Ara-C, G-CSF and Idarubicin and achieved the first complete remission (CR1). She had a relapse of acute leukemia in 8/2019 and was admitted to MDACC. She was diagnosed with AML with a small subset of T-lymphoblasts and received CPX351 (liposomal daunorubicin and Ara-C) + Venetoclax (VEN) followed by VEN and AZA and achieved CR2. She had a 2nd relapse in 9/2020 which was diagnosed with myeloid/T-lymphoblastic mixed acute leukemia (M/T MPAL) and was then treated with Enasidenib and VEN. In 11/2020 she was diagnosed with early T-precursor acute lymphoblastic leukemia (ETP-ALL) and was treated with Enasidenib and VEN with addition of Ara-C since 5/2021.

Biopsy Fixation Details

Formalin fixation.

Frozen Tissue Available

Not performed.

Details of Microscopic Findings

Peripheral blood smears revealed marked leukocytosis with many circulating blasts at initial diagnosis. No Auer rods were identified in the blasts. Bone marrow biopsies showed sheets of immature cells with blastic morphology and scant cytoplasm.

Immunophenotype

Initial diagnosis: The blasts were positive for CD5 (partial), CD7, CD10 (partial), CD11c (partial), CD33, CD34, CD38, CD71 (partial), CD117 (partial), HLA-DR (partial) and TDT, and negative for CD11b, CD13, CD15, surface/cytoplasmic CD3, and myeloperoxidase (MPO.
At first relapse: A small subset of T-lymphoblasts (a subset of blasts expressing cytoplasmic CD3 detected);
At 2nd relapse: M/T MPAL (MPO was detected in 6% of blasts by cytochemical staining)
Persistent disease: ETP-ALL (flow cytometry shows no signficant MPO)

Cytogenetics

46,XX (all samples before March 2021)
46,XX,del(11)(q13q23)[5]/47,XX,+8[1]/46,XX[14] (since March 2021)

Molecular Studies

NGS: at 1st and 2rd relapse

DNMT3A c.2370G>C p.R790S

DNMT3A c.2206C>T (variant allele frequency <2%)

DNMT3A c.2089G>T p.E697*

IDH2 c.419G>A p.R140Q,

ETV6 c.1045C>T p.L349F

JAK3 c.2873_2874insCCA p.E958delinsDQ

NOTCH1 c.4775_4776insTAC p.F1592_L1593insT

PHF6 c.464C>A p.S155*

PHF6 c.971_978delinsCCCC p.L324fs.

Proposed Diagnosis

Acute leukemia, mixed phenotype (Myeloid/T-lymphoblastic) presenting initially as undifferentiated versus early T-precursor acute lymphoblastic leukemia (ETP-ALL)

Interesting Feature(s)

- These cases illustrate the challenges in distinguishing between AML-M0, ETP ALL and MPAL, particularly post-therapy, as tumor heterogeneity and clonal evolution result in phenotypic shift over time.
- Undetectable cytoplasmic CD3 by flow cytometry using FITC fluorochrome at initial diagnosis led to a diagnosis of AML M0 with blasts expressing CD11b, CD11c, CD33 and CD117.
- Subsequent detection of a subset of cCD3-positive blasts using a brighter BV421 fluorochrome and MPO-positive blasts detected by cytochemical stain at relapse altered the diagnosis to MPAL (M/T).
- While gene mutations associated with both myeloid and T-ALL were detected by NGS, delineating the boundary between MPAL and ETP ALL remained challenging, and interpretation varied among different readers.

Panel Diagnosis

AML with minimal differentiation (WHO)/AML-NOS (ICC)

EA4HP24-BMWS-346

Acute leukemia with unusual phenotype and differentiation aspects

Dr. Laura Bandiera, Dr. Gabriella Daniela De Canal

ASST Grande Ospedale Metropolitano Niguarda Milano, Pathology Division, Department of Hematholgy, Oncology and Molecular Medicine, Milano, Italy

Case Description

Male, 54 year old. Mellitus diabetes type 2 (Metformin), hypersensitivity pneumonitis (Prednisone 15 mg/die).

He presented in ER with fatigue and B symptoms (weight loss of 10Kg in 4 months, fever >38°C and night sweats for 3 days).

WBC: 132.040/mmc (N 660/mmc, L 14.000/mmc, M 6.730/mmc, 83.8% blasts), Hb 10.3 g/dL, PLT 23000/mm3.

Multiple sites lymphadeopaties (lateral and posterior cervical, occipital, thoracic, inguinal bilateral, para-aortic), no splenomegaly, mild hepatomegaly.

Bone marrow aspirate and biopsy were performed in suspect of an acute leukemia.

Biopsy Fixation Details

10% buffered formalin and decalcification in EDTA with acid buffer (HCl).

Frozen Tissue Available

No

Details of Microscopic Findings

Smears showed immature cells with scant agranular cytoplasm (high N/C ratio), naked nuclei, dispersed chromatin and evident nucleoli, cytoplasmic vacuoles. Diagnostic orientation for a lymphoblastic acute leukemia.

Bone marrow biopsy showed almost 100% of cellularity with massive replacement (>80%) by population of immature/blasts cells, medium sized, round to irregular nuclei, finely dispersed chromatin with small nucleoli, scant cytoplasm.

In some areas, we observed aggregates of small-medium cells, irregular nuclei, fine chromatin, small nucleoli, with mitotic figures.

Immunophenotype

Flow citometry: 91% of blasts (unique polulation): CD45dim, CD5dim, CD7+, CD13dim, CD34+ CD99+, cytCD3+/-(27%), TdT+/-(40%), CD1a-, CD2-, sCD3-, CD4-, CD8-, CD10-, CD11b-, CD16-, CD19-, CD33-, CD117-, HLADR-.

Immunistochemical stains performed on bone marrow biopsy showed a prominent population (blasts): CD99+, CD34+/- (50-60%), TdT+ (40%), CD10-, CD2+/- (50-60%), CD3-/+(weak), CD1a-, CD4+, MPO-/+, CD15- (rare?), CD117-; aggregates resulted: CD303+, CD4+, CD56-.

Cytogenetics

45,XY,-7[20]

Nuc ish (KMT2Ax2,TCRADx2)[300], (5'TCL1,3'TCL1)x2(5'TCL1 sep 3'TCL1x1)[138/300]. ish 14q32(TCL1x2)[100]
Structural rearrangement involving TCL1 gene. No rearrangement in KMT2A and TCRAD.

Molecular Studies

NGS ongoing.

Proposed Diagnosis

ALL with aberrant expression of MPO and prominent blastic plasmacytoid dendritic cell proliferation DD acute leukemia with mixed phenotype/ambiguous lineage M/L (prevalence of lymphoblastic appearance and phenotype) and blastic plasmacytoid dendritic cell proliferation.

Interesting Feature(s)

Lymphoblast morphology of immature cells with unusual phenotype (single population in flow): expression of lymphoid (prevalent) and myeloid markers (MPO).
Considering a prevalent ALL phenotype (in accordance with clinical onset), unusual association with plasmacytoid dendritic cell proliferation, not only mature in appearance.
Do monosomy of 7 or rearrangement of TCL1 orient the diagnosis or not?
Could NGS studies be helpful/crucial to distinguish and classify this entity or not?
Patient started immediately a clinical trial with a ALL-scheme therapy (ongoing) with a good first response also in lymph nodes.

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Panel Diagnosis

AML-MR (WHO/ICC) with MPAL immunophenotype

EA4HP24-BMWS-350

Acute Leukaemia of Ambiguous Lineage or Acute Myeloid Leukaemia?

Dr. Yurina Miki[1], Dr. Mark Ong[1], Dr. Mina Mansy[1], Margeherita Panetta[3], Dr. Richard Dillon[2], Dr. Jennifer O'Sullivan[2], Hanan Perveen[3], **Dr. Anna C. Green**[4]

[1] Department of Cellular Pathology, Guy's and St Thomas' NHS Foundation Trust, London, UK; [2] Department of Haematology, Guy's and St Thomas' NHS Foundation Trust, London, UK; [3] Department of Special Haematology, Guy's and St Thomas' NHS Foundation Trust, London, UK; [4] Dept. Cellular Pathology, 2nd Floor, North Wing, St Thomas' Hospital, London, Germany

Case Description

74 year old male referred due to severe, isolated neutropenia.

No significant past medical history.

Full Blood Count: Hb 131, MCV 95, WBC 2.7, neutrophils 0.1, lymphocytes 2.6, monocytes 0.1, platelets 184.

Bone marrow aspirate and trephine performed.

Biopsy Fixation Details

5% buffered formalin; EDTA de-calcification

Frozen Tissue Available

No

Details of Microscopic Findings

BM aspirate:

Hypercellular; infiltration of large/ and medium size monomorphic population of myeloid immature cells in keeping with acute myeloid leukaemia.

BM trephine:

Hypercellular bone marrow. No fibrosis (WHO grade 0). Diffuse infiltrate of pleomorphic, medium sized blasts, with irregular nuclear contours and occasionally small nucleoli.

Haematopoiesis markedly reduced, with disrupted, left-shifted erythroid islands and occasional megakaryocytes. No micromegakaryocytes. Granulopoiesis markedly reduced and left shifted, with little, if any, maturation to neutrophils.

Immunophenotype

Immunophenotype by flow cytometry:

Two blast populations:

a) CD34+, CD117+, HLADR+, CD13+

b) CD34-/+, CD117-, HLADR+, CD123+/-.

Both populations negative for CD1a, CD10, CD19, CD2, CD3, CD4, CD7, CD8.

CD13 variable/dim

Intracellular TdT+, CD79a weak, CD22 weak.

Immunophenotype by immunohistochemistry:
TdT: Positive
CD117: Positive in 60-70%
CD34: Variable positivity, strong expression in 30-40%.
PAX5: weak expression in around 80%
CD79a: weak expression in 15-20%
MPO: expression in around 10%
Negative: CD19, CD20, CD33, CD15, CD14, CD61, CD10, CD2, CD3, CD5, CD7 or CD1a.

Cytogenetics

Normal karyotype.

Molecular Studies

Myeloid Gene Panel
Sample: bone marrow aspirate
Mutations:
IDH1, allele frequency 35%
RUNX1, allele frequency 70%

Proposed Diagnosis

Acute leukaemia of ambiguous lineage, NOS

Interesting Feature(s)

·Unusual immunophenotype: expression of weak CD79b and CD22 by flow, along with weak PAX5 and a subset with weak CD79a by immunohistochemistry, as well as a minority expressing MPO.
·Does not strictly meet diagnostic criteria of a B-cell acute lymphoblastic leukaemia, nor of mixed phenotype acute leukaemia.
·Evidence of B-cell lineage: weak CD79a, weak PAX5 and weak CD22
·Evidence of myeloid lineage: MPO only expressed in 10% by immunohistochemistry, negative by flow cytometry; CD117 positive.
·The expression of CD117 in a B-lymphoblastic leukaemia is unusual.
·IDH mutations are well recognised in acute myeloid leukaemia, but occur much less frequently in B-lymphoblastic leukaemia.
·Based on the immunophenotype along with IDH1, is this best regarded as acute myeloid leukaemia?

Panel Diagnosis

AML without maturation (WHO)/AML-MR (ICC)

EA4HP24-BMWS-366

Acute leukemia with PAX5 p.P80R and recurrence as a genetically-related histiocytic proliferation

PhD/MD Kemin Xu[1], Alana Wu[1], PhD/MD Liang Chen[1], Prof. Gail Vance[2], PhD/MD Jiehao Zhou[1], Dr. Kavita Umrau[1], Dr. Kenneth Ofori[1], Dr. Rohit Gulati[1], PhD/MD Shanxiang Zhang[1], Dr. Utpal Dave[3], PhD/MD Magdalena Czader[1]

[1] Indiana University School of Medicine, Department of Pathology and Laboratory Medicine, Indianapolis, USA; [2] Indiana University School of Medicine, Department of Medical and Molecular Genetics, Indianapolis, USA; [3] Indiana University School of Medicine, Department of Medicine, Indianapolis, USA

Case Description

This is a 27-year-old male with a history of congenital atrial septal defect presented with epistaxis and blurry vision with no focal weakness or sensory signs. Further workup showed he was anemic and thrombocytopenic with peripheral blasts. Peripheral blood flow cytometry was initially diagnosed as acute myeloid leukemia with monocytic differentiation. Bone marrow (BM) biopsy confirmed diagnosis of acute leukemia. Follow-up bone marrow exam three months after chemotherapy showed no morphological evidence of acute leukemia. Subsequent two bone marrow exams performed due to progressive cytopenias showed no evidence of acute leukemia, instead a prominent atypical histiocytic proliferation was seen. The patient remained pancytopenic and died of disseminated fungal infection.

Biopsy Fixation Details

Bone marrow were submitted in 10% formalin.

Frozen Tissue Available

N/A

Details of Microscopic Findings

The initial diagnostic bone marrow included a biopsy showing sheets of medium to large sized blasts with immature chromatin, frequent nuclear indentations, as well as the occasional prominent nucleoli and moderate basophilic cytoplasm.

Subsequent two bone marrow exams performed due to progressive cytopenias showed no evidence of acute leukemia but a prominent histiocytic proliferation with sheets of mature appearing histiocytes with abundant foamy cytoplasm, round to folded nuclei and occasional hemophagocytosis.

Immunophenotype

Initial flow cytometry (peripheral blood, PB): BM was inaspirable and flow cytometry was not performed.

Blasts positive for bright CD33, HLA-DR, CD14, bright CD64, partial CD123 and partial CD19. In addition, a minute population of B lymphoblasts positive for CD19, partial CD20, and dim TdT was seen.

Initial diagnostic BM:

Immunohistochemical stains were performed to clarify the presence of lymphoblasts on original PB immunophenotyping. PAX5 showed scattered positive cells, however TdT immunostain was negative.
Subsequent BMs with prominent histiocytic proliferation:
The histiocytic aggregates are diffusely and strongly positive for CD14, CD163, and cyclin D1. Ki67 staining is 5-10% within the histiocytic aggregates

Cytogenetics

Initial diagnostic BM:
Fluorescence in situ hybridization (FISH) showed markedly aneuploid population with the majority of probes of AML panel showing 3 to 5 signals (3q, 5q, 9q, 7q, 20q, 21q, 17q, and 16q).
Subsequent BMs with prominent histiocytic proliferation:
FISH studies demonstrated aneuploidy in the histiocytic nuclei.

Molecular Studies

PAX5 P80R and KRAS G13D were detected by next generation sequencing from initial diagnostic BM and subsequent BM with prominent histiocytic proliferation.
Microdissected histiocytic foci were positive for KRAS G13D mutation by PCR and showed clonal IGH gene rearrangements (FR1-FR3).

Proposed Diagnosis

Acute leukemia with PAX5 p.P80R

Interesting Feature(s)

PAX5 P80R mutation has been primarily reported in B-ALL/LBL and commonly associated with mutations of RAS pathways including KRAS. This mutation has not been reported to date in de novo acute myeloid leukemia.
The reported case demonstrates diagnostic caveats including a minute atypical B lymphoblastic population at the time of original diagnosis suggesting a possibility that original leukemia was B-ALL/LBL and rapidly transformed to acute myeloid leukemia with monocytic differentiation. Therefore, at the time of original diagnosis, primarily monocytic population was present.
Post-treatment bone marrows showed unusual features, genetically related mature histiocytic proliferation likely representing a recurrent disease.

Panel Diagnosis

AML defined by differentiation (WHO)/AML, NOS (ICC)

EA4HP24-BMWS-368

Mediastinal Mass with Myeloid Blasts: what's in a name?

Dr. Jan Bosch-Schips[1], Dr. Andrea Espasa[1], Dr. Enric Gamundi[1], Dr. Mónica Fernández[1], Dr. Clara Muntaner[2], PhD/MD Montserrat Arnan[2], PhD/MD Helena Pomares[2], PhD/MD Isabel Granada[3], PhD/MD Lurdes Zamora[3], Dr. Esmeralda De La Banda[1], Dr. Esther Alonso[1], PhD/MD Fina Climent[1]

[1] Hospital Universitari de Bellvitge, Pathology, L'Hospitalet de Llobregat, Spain; [2] Institut Català d'Oncologia (ICO), Hematology Department, L'Hospitalet de Llobregat, Spain; [3] ICO Badalona - Hospital Germans Trias i Pujol, Unidad de biología molecular, L'Hospitalet de Llobregat, Spain

Case Description

We present a case of a 21-year-old man with bronchial asthma, with no recent exacerbations. In August 2023, the patient presented with odynophagia, cough, and mucus production. There was a progression of symptoms, including facial and neck swelling, dyspnea, orthopnea, hoarse voice, and nocturnal sweating, leading to suspicion of superior vena cava syndrome - diagnostic tests included a chest X-ray and neck CT scans. Hemoglobin was at 141 g/L and Platelets at 280 x 10^9/L. Coagulation tests revealed PT at 1.34 and aPTT at 1.25. Biochemistry results indicated CRP at 30 mg/L, LDH at 261 U/L, with normal renal function. Comprehensive analysis showed Fibrinogen (Clauss) at 6.5 g/L and β2-microglobulin at 2.0 mg/L. Hemogram review revealed segmented neutrophils at 69%, lymphocytes at 28%, monocytes at 6%, eosinophils at 3%, and blasts at 4%.

Biopsy Fixation Details

FFPE

Frozen Tissue Available

No

Details of Microscopic Findings

BM aspirate:

Myeloid/monocytoid blasts (60-90%), large size, semilayered chromatin, many cells with nucleoli and ample cytoplasm with granulation; even some smaller ones with pseudo-Chediak granulation and isolated Auer rods.

Lymphoid (10-40%) of small size, irregular or semilaxial nucleus, and scanty agranular basophilic cytoplasm.

LN biopsy:

Infiltration by small-medium sized lymphoproliferation with nuclei of blast habitus, lymphoid phenotype. Isolated cells of myeloid phenotype

Immunophenotype

- 2% of undifferentiated myeloid blasts: CD34+/CD117+/CD33+/CD13+
- 22% differentiated myeloid/monocytoid blasts: MPO++/CD34-/CD117+/HLA-DR+/CD13-/CD33+/CD15+/CD56+
- 15% lymphoid T-cell blasts: cyCD3+/CD7+/CD1a+/CD4-/CD8-

Cytogenetics

45,X,-Y[20]/45,X,-Y,del(7)(q32q36)[6]/45,X,-Y,del(7)(p13p22)[4]/46,XY[12]

Deletion 7q36 by FISH

Molecular Studies

TP53	c.422G>A	p.Cys141Tyr	32%	Pathogenic
PHF6	c.939C>G	p.Tyr313Ter	45%	Probably pathogenic
ASXL1	c.2572C>T	p.Gln858Ter	23%	Probably pathogenic
NF1	c.5977C>T	p.Gln1993Ter	21%	Probably pathogenic
RUNX1	c.423_424insTAAG	p.Ala142Ter	18%	Probably pathogenic
EZH2	c.2215_2216dup	p.Lys740Ter	15%	Probably pathogenic
KMT2A	c.11834_11835insAAAGCA	p.Tyr3945delinsTer	8%	Probably pathogenic

Proposed Diagnosis

Mixed Phenotype Acute Leukemia, T/Myeloid, NOS (WHO 2017/ICC 2022/WHO 2022)

Interesting Feature(s)

- MPAL, T/Myeloid, NOS, uniquely exhibits characteristics of both T-lymphoid and myeloid lineages, either in separate cells (bilineal) or within the same cells (biphenotypic).
- Diagnosis is based on specific WHO and ICC criteria involving the expression of T-lymphoid and myeloid markers identified through immunophenotyping; integrating all disciplines is crucial
- MPAL often has various genetic abnormalities, although there are no recurrent genetic mutations
- Treatment is challenging, often requiring combinations of ALL and AML therapies. The prognosis is generally poorer compared to other leukemias and varies based on factors like age, health, and genetic markers.

Panel Diagnosis

AML-MR (WHO)/AML with mutated TP53 (ICC) and MPAL immunophenotype

EA4HP24-BMWS-394

A Diagnostic Dilemma for a Patient with Skin Lesions and Marked Leukocytosis: Mixed Phenotype Acute Leukemia or BPDCN with Aberrant CD3 Expression?

Zhihong Hu[1], Cheng C. Yin[2], Wei Wang[2], Sa A. Wang[2], Mingjian J. You[2]

[1] The University of Texas MD Anderson Cancer Center, Department of Laboratory Medicine, Houston, USA; [2] The University of Texas MD Anderson Cancer Center, Department of Hematopathology, Houston, USA

Case Description

The patient was a 79-year-old Caucasian male who presented with thrombocytopenia and leukopenia in February 2023. No bone marrow biopsy was performed at that time. His skin lesions first appeared in right jaw line in early October 2023 and increased in size over time. He developed numerous violaceous nodules along the upper trunk, right jawline and upper back that were progressively getting worse. A skin biopsy performed at an outside hospital showed that the tumor cells were positive for CD4, CD43, CD56, CD123, BCL-2 and TCL1 stains, and a diagnosis of “blastic plasmacytoid dendritic cell neoplasm” was rendered.

Biopsy Fixation Details

N/A

Frozen Tissue Available

N/A

Details of Microscopic Findings

Peripheral Blood Findings: There was marked leukocytosis composed predominantly of large atypical cells with blastic features that were negative for myeloperoxidase. Granulocytes were predominantly mature neutrophils. Monocytes were not increased in number. Lymphocytes were morphologically unremarkable. Rare nucleated erythroid precursors were present. Marked thrombocytopenia was noted.

Bone marrow aspirate smear revealed numerous neoplastic cells with a morphology similar to that observed in the peripheral blood smear. Bone marrow core biopsy was hypercellular (>95%) with trilineage hypoplasia and markedly increased immature cells with blastic morphology

Immunophenotype

Immunohistochemical studies showed that the neoplastic cells were positive for TCL–1, TCF4/CD123 (Dual-Color Multiplex Stain), CD117 (small subset), CD2 (partial), CD3, CD5 (subset), CD7(partial), TdT, and CD56 (subset), and negative for CD34, CD20, BCL11b, myeloperoxidase, and lysozyme. CD61 highlighted decreased megakaryocytes. Rare cells were positive for strong nuclear p53 stain.

Flow cytometric analysis of the marrow aspirate material showed an aberrant population of immature cells, positive for CD2 (partial), cytoplasmic CD3, CD4, CD5 (subset), CD7 (partial), CD36, CD38, CD10 (partial), CD45 (dim), CD54 (partial), CD56 (partial), CD117

(partial), CD122, HLA–DR, and TdT (small subset), and negative for surface CD3, CD13, CD14, CD15, CD19, CD25, CD8, CD1a, CD33, CD34, CD64, CD133, and myeloperoxidase.

Cytogenetics

Complex karyotype

41~46,XY,del(1)(q21),add(4)(q31.1),-5,-7,add(7)(q21),-9,add(9)(p13),-13,-14,-15,-17,-18,add(19)(q13.4),-20,-21,add(21)(p13),-22,+1~4mar[cp18]/46,XY,del(8)(p12),del(12)(p12)[1]/46,XY[1]

Molecular Studies

NGS mutation analysis: *PHF6* c.853C>T p.Q285* (variant allele frequency, VAF, 6%), *SF3B1* c.2122G>C p.A708P (VAF, 22%), *TET2* c.4546C>T p. R1516* (VAF, 48%), and *TET2* c.1677T>A p.Y559* (VAF, 47%)

TCR gene rearrangements: Negative for TCRβ or TCRγ gene rearrangements

NGS RNA translocation: Negative

Proposed Diagnosis

Blastic plasmacytoid dendritic cell neoplasm with aberrant CD3 expression

Interesting Feature(s)

Diagnosis of such an unusual case presents challenges. The neoplastic cells in this case display characteristics of both plasmacytoid dendritic cells and T-lymphoid phenotypes, resembling mixed phenotype acute leukemia. The identification of various PDC markers, including CD123, CD4, CD303, TCL1, and TCF4 in the neoplastic cells and the characteristic presentation of large, multiple skin lesions support the diagnosis of BPDCN. Notably, the absence of TCR gene rearrangement at the molecular level helps exclude a T-cell origin of the neoplasm. The expression of multiple T-lineage markers, including cytoplasmic CD3 in this BPDCN, may suggest a cell origin from a lymphoid-primed multipotent progenitor and may be attributed to a PHF6 mutation, which is frequently associated with T-lymphoid antigen expressions.

Panel Diagnosis

BPDCN (WHO/ICC)

EA4HP24-BMWS-399

Mediastinal germ cell tumor with associated haematological malignancy (B-and myeloid lineage leukemia carrying complex karyotype and sharing identical *TP53* mutation with extragonadal germ cell tumor)

Prof. Rose-Marie Amini, Dr. Peter Hollander, Dr. Panagiotis Baliakas

Uppsala University and Uppsala University Hospital, Dept of Immunology Genetics and Pathology, Uppsala, Sweden

Case Description

20 year old male was diagnosed with a mediastinal non-gonadal germ cell tumor treated with two cycles of PEI (cisplatin, etoposide, iphosphamide, uromitexan) and initially partial response, but quick progress and disseminated disease. Multiple lesions on PET were detected one year after germ cell tumor diagnosis. Hemoglobin: 100g/L, WBC: $22x10^9$/L, Plt: $30x10^9$/L

Diagnosed with an acute leukemia with a dominant B-lymphoblastic component and blasts of myelomoncytic lineage in addition to an abundance of dendritic cells.

Biopsy Fixation Details

Buffered 4% formalin

Frozen Tissue Available

Vital frozen cells

Details of Microscopic Findings

Hypercellular bone marrow with dysplastic features of megakaryocytes and dyserythropoesis. Bone marrow smears with 70% blasts of variable size-mainly two populations, where some blasts resemble lymphoblasts and other blasts are larger in appearance.

Immunophenotype

Flow cytometry:

A main population of blasts (40%) expresses a B-ALL phenotype and is positive for: CD45dim, CD19+, CD10+, TdT+, CD79A+, HLA-DR+, icCD22+, CD34+/-, CD38+, CD81+, CD24++. B-lymphoblasts are negative for CD66b/c-, CD304-, CD74-, ic/mCD3-, MPO-, CD117-, CD13-, CD33-.

Myeloblasts (17%): express CD34+, CD117+, CD13+, CD33dim, CD123+, CD7+ but not MPO-, B- or other T-cell markers.

Monocytic cells (20%) express CD36+, CD64+, CD33+, CD56+/- and half of this cell population is immature and negative for CD14- and IREM2-.

Dendritic cells (13%) express CD123++, CD38+, CD34dim and CD7+.

Cytogenetics

Cytogenetic analyses show a complex karyotype: 47~49,XY,+Y,add(1)(q3?2),-4,-5,-5,del(7)(q22q36),+8,+10,-12,-12,+14,-17,+3~5mar[cp18]/49, XY,+Y,+8,+10,add(11)(q2?5),der(14;15)(q10;q10)+mar[cp3]

Molecular Studies

Bone marrow: *TP53* mutation NM_000546.6:c. 919+1G>A p? VAF99%

Germ cell tumor: *TP53*: EXON B c.919+1G>A, p.? VAF96%

High allele frequency is explained by deletion of chromosome 17p (*TP53*)

No clonal rearrangments detected: *IGH, IGH* incomplete, *IGK-KDE, TCRD, TCRG* and *TCRB* genes. Minor TCRB-clone detected but not representative for PCR-MRD.

Proposed Diagnosis

Mediastinal germ cell tumor with associated haematological malignancy (B-and myeloid lineage leukemia carrying complex karyotype and sharing identical *TP53* mutation with extragonadal germ cell tumor)

Interesting Feature(s)

Mediastinal germ cell tumor with haematological malignancy evolving from a common shared precursor cell (clonally related).

Identical *TP53* mutation detected in extragonadal mediastinal germ cell tumor and subsequent hematological neoplasia with a complex karyotype and acute leukemia with mixed lineages.

How to classify the hematological neoplasm?

Myeloid neoplasm (MDS) (complex karyotype and *TP53* mutation) with transformation to acute leukemia (multilineage)?

Panel Diagnosis

AML-MR (WHO)/AML with TP53 mutation (ICC), with MPAL immunophenotype

EA4HP24-BMWS-400

An interesting case of Mixed Phenotype Acute Leukaemia B/myeloid

Dr. Timothy Farren, Dr. Hanine Medani, Prof. Maria Calaminici, Dr. Matthew Smith

NHS East and South East London Pathology Partnership, Barts Health NHS Trust, SIHMDS, London, UK

Case Description

A 50 year old female presented in October 2023 with a rash on both legs. Past medical history included recurrent chest infections and weight loss of 7kg in one month. Analysis was performed on blood, BM aspirate and trephine biopsies, in which a diagnosis of Mixed Phenotype Acute Leukaemia B/myeloid was made, formally assigned 'bilineage

leukaemia'. Cytogenetic analysis stratified as poor risk. Chemotherapy consisted of Cytarabine and Danuorubicin (3+10) in November 2023, with transfusion and G-CSF support. In December 2023, a bone marrow aspiration for disease monitoring showed no immunophenotypic myeloid progenitor or CD19+ B-cell progenitor expansion. In January 2024, the patient was admitted for cycle 2 of DA (3+8). A second outcome bone marrow was performed which showed no expansion of CD34+ CD117+ myeloid progenitors by immunophenotyping (0.7% of TNCs), however there was no AML MRD marker to follow for the AML component of this patient's disease. The B-ALL component of this patient's MPAL was still present.

Biopsy Fixation Details

Decalcification – immersion in 10% formic acid at room temperature for 4 – 5 hours. Embedded in mould with molten paraffin wax (+60°C) and solidified on cold plate (-5°C to -10°C).

Frozen Tissue Available

None

Details of Microscopic Findings

The diagnostic bone marrow trephine was markedly hypercellular at about 90-95% cellularity. Marked increase in blasts ~60-70% of total marrow cells present. In addition, medium sized lymphoblasts were present with fine dispersed chromatin and some show small nucleoli. Background haematopoieisis showed scattered good number of megakaryocytes with some hypolobated and micromegakaryocytes noted.
The diagnostic bone marrow aspirate was a packed hypercellular aspirate, with two populations of blast cells seen morphologically: (1) Large blasts with abundant cytoplasm; (2) smaller blasts with high N:C ratio (?more lymphoid in nature). No basophilia, reduced megakaryocytes. Residual neutrophils.

Immunophenotype

BM Trephine: Positive for CD79a, PAX5, CD34, TdT and CD10. About 30% of the blasts also expressed CD20.They were negative for CD117, CD68, HLA-DR and CD33.
CD117 highlighted fewer number of scattered blasts amounting to about 5% of total marrow cells, which likely co-expressed MPO. Raising suggestion of a second population of myeloid blasts. Background haematopoieisis showed scattered good number of megakaryocytes with some hypolobated and micromegakaryocytes noted on CD61. CD71 highlighted poorly formed colonies of erythroid cells with a prominent left shift in maturation. Reticulin shows focal grade 1 fibrosis (WHO score).

BM Aspirate: 10% of the TNCs were myeloid progenitors expressing the following phenotype: CD34+, CD117+, MPO+, cytCD3-, CD79a-, CD19-, CD13+ weak, CD33+/-, CD14-, CD7+ (78% weak) and CD5-. In addition, approximately 30% of the TNCs were abnormal, immature B cells which were: Positive for the lineage specific markers CD19 wk/mod and CD79a wk/mod. MPO and cytCD3 were negative. The lineage assciated markers were CD10+ strong, CD20-, CD22+ weak, CD13-, CD33-, CD117-, CD2-, CD5-, CD7- with CD34+ and Tdt+ strong and no smIg.
In addition, these B cell progenitors were: CD58+, CD66c/CD123-, CD24+ CD73/CD304-, CD81+ strong, CD79b-, CD103-, CD11c-, CD25-. The malignant features of these B cells were the under expressed CD45, CD19 and CD38 when compared to haematogones.

Cytogenetics
45,XX,-7
Molecular Studies
Missense variants detected in IDH2, DNMT3A, NF1 and EZH2 together with protein-truncating variants in ASLX1, NF1, RUNX1 and CSF3R.
No fusion transcripts detected.
Proposed Diagnosis
WHO 4R / WHO 5R/ICC: Acute Leukaemia of Ambiguous Lineage. Mixed-phenotype acute leukaemia, B/myeloid
Interesting Feature(s)
Bilineage phenotype rather than biphenotypic.
Discordance in the predominant blast lineage between aspirate and trephine.
The B-ALL component of this patient's MPAL was still present.

Panel Diagnosis
AML-MR (WHO/ICC) with MPAL immunophenotype

EA4HP24-BMWS-406

Acute undifferentiated leukaemia with low level FLT3 itd mutation and Trisomy 13 and discrepant immunophenotypes on flow cytometry and immunohistochemistry.

Dr. Bindu Vydianath, Hayder Hussain

University Hospital Birmingham QE, UK, Histopathology and Haematology, Birmingham, UK

Case Description
·76 year old with a 6 month history of weight loss and fatigue.
·Presented to primary care in September 2021 with a three week history of right knee bursitis. Blood-stained aspirate from right knee and found to have abnormal blood counts and referred to secondary care where he was admitted.
·Full blood count: White cell count 72.15x10*9/L, Haemoglobin 60g/L, Neutrophils 1.08x10*9/L, Lymphocytes 5.77x10*9/L , Monocytes 57.36x10*9/L, Blasts 7.22x10*9/L.
·CRP 222 mg/L, Cr 278 micromol/L, ALT 211 U/L, Alk phosphatase 463 U/L, bilirubin 37 micromol/L, Albumin23g/L.
·Diagnosed with acute myeloid leukaemia. Previous full blood counts in a different hospital showed high white cell counts since 2019(details not available); clinical notes suggest that this is therefore transformed disease from MDS/MPN.

Biopsy Fixation Details
Formalin fixed
Frozen Tissue Available
No
Details of Microscopic Findings
•Cellularity is markedly increased at approximately 95%. The
marrow is replaced with blasts which in areas appear myeloid with pale dispersed chromatin and inconspicuous nucleoli.
•Focally, undifferentiated blasts with slightly larger pale nuclei and multiple small indistinct nucleoli are noted.
Immunophenotype
•CD34: 60%
•TdT: 80%
•CD117 ~ 5% weak positive.
•MPO, CD19: 20%
•PAX5, CD79a: 30%
•CD14: 15%
•CD3, CD7, CD56, CD123: negative.
•Flow cytometry peripheral blood(marrow aspirate was prioritised for genetics and as the patient had peripheral blood involvement analysis of peripheral blood was done as surrogate for marrow due to limited material): 70% of nucleated cells are monocytic cells which are HLA DR, CD13, CD33 and CD11b positive with 54% TNC being CD4co- positive. There are 9% CD117/CD4 positive myeloblasts. Results consistent with acute monocytic leukaemia.
Overall phenotype:•CD45dim with increased SSC, CD33+, CD13+, HLADR+, CD14partial, CD4+, CLL1+
•Subset myeloblasts (7%) CD117+ CD34+ TdT+
•Negative for MPO, cyto CD79a, CD3, CD22
Cytogenetics
Trisomy 13
Molecular Studies
•DNA molecular analysis(fragment length)
•Significant 57bp internal tandem duplication(itd) in FLT3 with a FLT3 itd to FLT3 wild type ratio of 0.01. The assay does not exclude FLT3 tyrosine kinase domain(TKD) point mutations.
No evidence of insertion duplication variant within NPM gene
Proposed Diagnosis
•Acute leukaemia of ambiguous lineage.
•Flow cytometry of peripheral blood suggests acute monoblastic leukaemia.
Interesting Feature(s)
Acute leukaemia of ambiguous lineage/acute undifferentiated leukaemia with an aggressive clinical course, presenting with leukocytosis and monocytosis. Discrepant immunophenotype on trephine immunohistochemistry and flow cytometry of peripheral blood. Trephine showed diffuse CD34 and TdT expression as well as focal B cell marker

expression. CD14 was positive only in a minority of cells. Peripheral blood flow cytometry however suggests acute monoblastic leukaemia. A FLT3 itd mutation was detected on molecular genetics and karyotype showed trisomy 13. This is likely to represent transformed disease from a previous MDS/MPN(details on original disease are sketchy and only derived from clinical notes which states that leucocytosis was present for two years prior to presentation).

Panel Diagnosis

AML, defined by differentiation (WHO)/AML, NOS (ICC)

EA4HP24-BMWS-421

B-lymphoblastic leukemia with BCR::ABL1-like features (predicted *IGH*::*CRLF2* rearrangement) and partial monocytic differentiation

Alexandra Kovach[1,2], Annie Luong[3], Gerald Lu[1], Michael Lu[1], Sneha Chalfant[1], Chintan Parekh[3,4], Deepa Bhojwani[2,4], Gordana Raca[1,2], Brent Wood[1]

[1] Children's Hospital Los Angeles, Department of Pathology and Laboratory Medicine, Los Angeles, USA; [2] University of Southern California, Keck School of Medicine, Los Angeles, USA; [3] Children's Hospital Los Angeles, Flow Cytometry Core, Los Angeles, USA; [4] Children's Hospital Los Angeles, Department of Pediatrics, Hematology/Oncology, Los Angeles, USA

Case Description

A 14-year-old male presented with fever, fatigue and altered mental status. Complete blood count showed hyperleukocytosis (white blood cell count 1.1x10^6/mm^3), anemia and thrombocytopenia.

Biopsy Fixation Details

Bone marrow core biopsy fixed in formalin with decalcification

Frozen Tissue Available

No

Details of Microscopic Findings

Peripheral blood and bone marrow aspirate smears showed numerous variably sized blasts with scant cytoplasm, irregular nuclear contours, finely dispersed chromatin, and occasional cytoplasmic vacuoles (96% of circulating leukocytes, 91% of bone marrow aspirate cellularity).

Immunophenotype

By flow cytometry of peripheral blood, the blasts expressed CD4 (increased; dim, subset),* CD9 (uniform),* CD10 (variable),* CD13 (dim),* CD19 (increased),* CD20 (increased; dim, subset),* CD22,* CD24,* CD33 (dim, subset),* CD34 (variable),* CD38, CD45 (decreased),* CD58 (increased),* cCD79a, CD123 (variable),* CRLF2,* cytoplasmic MPO (variable,

approximately 50% of the population)* and HLA-DR (variable)* [* = abnormal expression compared to reference population of early B precursors (stage I hematogones)].

Cytogenetics

Karyotype was abnormal: 46,XY,t(2;7)(p12;q35)?c[20], with the t(2;27) interpreted as a likely benign constitutional variant. Fluorescence *in situ* hybridization (FISH) detected CRLF2 and IGH rearrangements, suggesting the presence of a cryptic t(X;14) or t(Y;14), predicted to cause CRLF2 overexpression in leukemia cells due to its juxtaposition to the IGH locus. CRLF2 rearrangement in the monocytic component was confirmed on the CD45dim/CD33bright/CD64bright cell-sorted fraction from archival ficolled fresh-frozen material.Chromosome microarray detected focal deletions involving the loci containing the following genes: *IKZF1*, *CDKN2A*/*CDKN2B*, *ADD3*, *RB1*, *VPREB1*, *WT1* and *CHECK2*. (All studies: bone marrow aspirate.)

Molecular Studies

Variants of strong clinical significance were detected in the *JAK2*, *NRAS* and *KRAS* genes on a next-generation sequencing panel (Hiemenz *et al*, 2018) from bone marrow aspirate.

Proposed Diagnosis

B-lymphoblastic leukemia (B-ALL) with *BCR::ABL1*-like features (Ph-like ALL, predicted *IGH::CRLF2* rearrangement) and partial monocytic differentiation

Interesting Feature(s)

CRLF2-rearranged B-ALL is a high-risk B-ALL subtype seen with increased prevalence in the Hispanic adolescent/young adult (AYA) population (Raca *et al*,2021). The biology underlying its relatively poor prognosis is not well understood. We have observed partial monocytic differentiation in several *CRLF2*-rearranged B-ALL at diagnosis, similar to that in one other case report (Choi *et al* 2017) and also to that seen in some B-ALL with *KMT2A*, *ZNF384* or *DUX4* rearrangement. In B-ALL with *KMT2A* or *ZNF384* rearrangement, the monocytic differentiation often meets diagnostic criteria for mixed phenotype acute leukemia (MPAL), B/Myeloid, underscoring the known propensity of these rearrangements for lineage switch from B-ALL to acute myeloid leukemia (AML). In B-ALL with *DUX4* rearrangement, classification as MPAL is best avoided because of the transience of the monocytic differentiation and the excellent prognosis with B-ALL-directed therapy. The significance of monocytic differentiation in B-ALL with *CRLF2* rearrangement at diagnosis is uncertain, but we hypothesize that it suggests stem cell-like biology and inherent chemoresistance.

Panel Diagnosis

B-ALL (WHO/ICC)

EA4HP24-BMWS-424

A difficult case of acute leukaemia

Dr. Elsa Poullot[1], Dr. Adrien Pecriaux[2], Dr. Bouchra Badaoui[3], Dr. Nicolas Freynet[3], Cyrielle Robe[1], Dr. Vincent Parinet[4], Prof. Philippe Gaulard[1]

[1] Henri Mondor University Hospital, APHP, Department of Pathology, Creteil, France; [2] Saint Antoine Hopsital, APHP, Department of Pathology, Paris, France; [3] Henri Mondor University Hospital, APHP, Hematology and immunobiology, Creteil, Germany; [4] Henri Mondor University Hospital, APHP, Hematology, Creteil, France

Case Description

A 75 year-old man with a history of cardiovascular disease was admitted in hospital in June 2022 for B symptoms, weight loss (10 kgs), polyadenopathy, and pancytopenia.
An axillar lymph node surgical biopsy and bone marrow biopsy were sucessively performed (slides submitted).

The patient died of disease 3 weeks after the treatment was initiated (Cytarabin + daunorubicin + vindesin + intra-thecal injections).

Biopsy Fixation Details

Formalin

Frozen Tissue Available

No

Details of Microscopic Findings

The lymph node was enlarged, and the normal architecture was effaced by a biphasic proliferation, with only rare residual B follicles. The 1st component was made up of small cells, with high N/C ratio, round nucleus, fine chromatin, inconspicuous nucleoli. The 2nd one consisted of aggregates of medium-sized cells, with abundant cytoplasm, and reniform nucleus with delicate lace-like chromatin.
The bone marrow was massively infiltrated by the same proliferation. Only rare residual erythroid islands and megakaryocytes were oberved.

Immunophenotype

The 1st population was CD34+, CD38+ (strong), TdT+, CD117+ low by IHC but not detected by flow cytometry (FCM), MPO+, CD33+, lysozyme+, CD7+, CD5+, cCD3+ (9% by FCM), CD123+(weak), CD56+(weak), TCL1-, CD68-, CD163-, CD2-, CD10-.
The 2nd population was CD34-, CD38+ (weak), TdT+, CD117-, MPO-, CD33-, lysozyme-, CD7+, CD5+ (weak), CD3-, CD123+, CD56+ (strong), TCL1-, CD68+, CD163-, CD2-, CD10-.
There were some discrepancies between flow cytometry (FCM) and immunohistochemistry (detailed in the submitted presentation).

Cytogenetics

Conventional Karyotype 46XY, del(7p) ; del(12p)

Molecular Studies·

PCR *IDH1* R132 was positive ; PCR *NPM1*, *FLT3*, I*DH2* R140 and *IDH2* R172 were negative.

Eight mutations in *IDH1* (p.Arg132Cys- VAF 35.5%), *DNMT3A* (VAF 26.5%), *NRAS* (VAF 22.2%), *BCOR* and *IKZF1* (4 variants – VAF 0.9 to 1.8%) were detected on the 42 genes myeloid panel performed in our institution.

Proposed Diagnosis

Mixed-phenotype acute leukaemia, T/myeloid – ICDO : 9809/3

Interesting Feature(s)

- This is a well documented case of acute leukaemia of mixed/ambiguous phenotype with lymph node and bone marrow biopsies, made of 2 morphologically distinct populations, with phenotypic variations, but likely a unique genetic profile.
- This unusual observation raises the following differential diagnoses :

Mixed-phenotype acute leukaemia, T/myeloid , BUT cytCD3 <10% (9% by FCM)

Acute leukaemia of ambiguous lineage, NOS , BUT MPO+, cytCD3 +

Acute myeloid leukemia (AML) BUT with aberrant expression of CD5 (66% by FCM)...and partial cytCD3 expression

AML with mature plasmocytoid dendritic cell proliferation (MPDCP) BUT dense patchy infiltrate in both BM and LN, phenotype : CD34-, TdT+, CD56+, TCL1-, no *RUNX1* mutation

- This case reflects the plasticity within the leukemic clone.
- It raises the discrepancies between FCM and immunohistochemistry, and classification issues.
- It shows that CD3+ TdT+ is not only detected in T-lymphoblastic leukaemia/lymphoma, especially in a lymph nodes of elderly people.

Panel Diagnosis

MPAL, T/myeloid (WHO/ICC)

EA4HP24-BMWS-445

MDS with Multilinear Dysplasia, Excess blasts and RUNX1-mutation with MixedmYEloidand early T-cell Precursor phenotype

Dr. Anna Blackö, PhD/MD Mats Ehinger

Skåne University Hospital, Lund, Lund, Sweden

Case Description

57 year old female, no medical history.

Pancytopenia. Macrocytic anemia, hemoglobin (Hb) 75 g/L, platelet count 45 x 10^9/L, WBC 1.0 x 10^9/L, absolute neutrophil count (ANC) 0.2 x 10^9/L.

Biopsy Fixation Details

Formalin-fixed, standardized time period 24-36 hrs. 10% formaldehyde

Frozen Tissue Available

No.

Details of Microscopic Findings

•Blood smear with anisocytosis. Neutropenia, dysplasia. 2% blasts. Thrombocytopenia.
•Bone marrow aspirate: Low cellularity, no fragments. No megacaryocytes. 7% blasts of medium size with scant, basophilic cytoplasm.
Some larger blasts with more abundant cytoplasm. No granules or Auer rods. Decreased granulopoesis (7%), significant dysplasia. Expanded (42%) dysplastic erythropoiesis.
38% lymphocytes incl 10% granulated forms.•Bone marrow biopsy: Good representability, ordinary cellularity (50%). No reticulin fibrosis. Dysplastic megacaryopoiesis with micromegacaryocytes. Well represented granulopoesis, left shifted with lack of maturation dominated by early precursors. Expanded erythropoiesis. Blast cell count 10-15% (CD34+ and CD117+ cells).

Immunophenotype

Flow cytometric analysis of bone marrow with two abnormal blast
populations with lineage promiscuity, one resembling earlyT-cell precursor cells and the other myeloid blasts:
•Early T-cell precursor-like population (10%): CD45dim, CD7+, sCD3-, cytCD3+, CD5-, CD117+, CD34-, CD56+, CD10-, CD4-, CD38-. CD2+(partial), TdT-, MPO-, HLA-DR-. No
expression of the myeloid markers CD13 or CD33. Heterogenerous expression of CD8 (weak expression in 25% of cells). In addition, this population was CD11b+ and displayed heterogenous expression of CD16.
•Myeloid blast-like population (5%): CD34+/CD13+/CD38+/CD7dim, CD4dim, partial expression of CD117 and CD33. 2,5% expressed cCD3 with the same intensity as T-cells, the rest weakly positive/negative. Myeloid blasts negative for CD11b, CD56, CD16, CD36, CD10 and lymphoid markers sCD3, CD5, CD8, CD19, CD20 and TdT.
Non-lysis analysis 36% erythropoiesis with abnormal maturation and
decreased expression of CD71 and CD36.

Cytogenetics

G-banding detects trisomy 13

Molecular Studies

NGS detects RUNX1 mutation (VAF 61%), BCOR (19%), SRSF2 (33%) and IKZF1(18%) mutations. No germline mutations.

Proposed Diagnosis MDS-AML with myelodysplasia-related gene mutations (ICC)
MDS-IB2 (WHO-HAEM5)
MDS-EB2 (WHO-HAEM4)

Interesting Feature(s)

This is an unusual case of MDS (or MDS/AML) with several myelodysplasia-related gene mutations (RUNX1, BCOR and SRSF2)
and bilineage features with one lineage promiscuous
myeloid clone aberrantly expressing T-cell markers (partly cytCD3+ and CD7+),
and another equally lineage promiscuousearly T-cell clone with aberrant
expression of myeloid markers (CD117+ and CD11b+). The relatively large ETP-ALL-like T-cell clone is unusual for MDS.

There was an initial suspicion of imminent
mixed phenotype acuteleukemia (MPAL), but the disease kinetics was slow and the patient responded well to azacytidine/venetoclax.
This case illustrates the gray zone beween MDS and AML, reflected by the different designations in the ICC and WHO classification systems, respectively.
Clinically, the disease was considered more close to MDS than AML and the patient was treated with one course of
azacytidine/venetoclax and one additional course of azacytidine(because of profoud hypo plasia venetoclax was omitted) with good response, and
is now heading for allogeneic stem cell transplantation (february 2024)

Panel Diagnosis
MDS-IB (WHO)/MDS-AML (ICC)

EA4HP24-BMWS-458

Patient with blast phase multilineage (B and myeloid) neoplasm with *FGFR1* rearrangement and *RUNX1* mutation

Dr. Ashley Eckel[1], Dr. Nicholas Olson[2,3], Dr. Luise Hartmann[1], Dr. Hanyin Cheng[1], Dr. Dongbin Xu[1]

[1] Hematologics, Inc., Pathology, Seattle, USA; [2] Avera McKennan Hospital and University Health Center, Pathology, Sioux Falls, USA; [3] Physicians Laboratory Ltd., Pathology, Sioux Falls, USA

Case Description
A 44 year old man presented with leukocytosis (118 K/uL) with left-shift and 8% blasts, macrocytic anemia, and thrombocytopenia. Eosinophils were 4.7 K/uL. Bone marrow was hypercellular with 60% B lymphoblasts and 5% myeloid blasts. A t(8;22) translocation was identified suggesting *BCR::FGFR1*, as well as mutations in *DNMT3A* and *RUNX1*. After initial hydroxyurea, HyperCVAD/R-MA with rituximab was started. Bone marrow studies after course 2 showed no evidence of residual abnormal blasts. Approximately 4 months after diagnosis, the patient was found to have recurrent disease, with t(8;22) translocation by FISH and 1% B-lineage blasts by MRD flow cytometry. The patient received blinatumomab and ponatinib; however, ponatinib was held after symptomatic SARS-CoV-2. Approximately three weeks later, the patient presented with leukocytosis (90 K/uL) with 25% blasts on peripheral smear; flow cytometry indicated predominantly myeloblast phenotype in the blood and bone marrow. Therapies including MEC and FLAG-IDA were attempted; however, the patient was refractory and ultimately started on pemigatinib and hospice.
Biopsy Fixation Details
Formalin-fixed decalcified paraffin.

Frozen Tissue Available

No

Details of Microscopic Findings

The peripheral smear at diagnosis showed marked leukocytosis with leukoerythroblastic features including 8% blasts. Aspirate smears were aparticulate; however, marrow core biopsy was hypercellular (95% cellular), with increased morphologically immature cells. The majority of the immature cells were positive for CD20, PAX5, CD10, and TdT, dim to negative for CD34, and negative for CD3, CD5, CD138, CD61, CD117, and E-cadherin. CD34 and CD117 identified myeloblasts comprising 5% of the marrow. The patient's disease approximately 5 months later demonstrated similar peripheral blood features, with 25% circulating blasts; however, the marrow at that time demonstrated 26% blasts, found to be positive for CD34 and CD117 by immunohistochemical stains.

Immunophenotype

After initial rounds of therapy, the patient was found to have low-level MRD positivity with a B-lineage phenotype, demonstrating expression of CD19, CD10, and CD22, with dim CD34 and slightly decreased CD38, without CD20. After limited subseuqent therapy, the patient's disease quickly evolved to the predominantly myeloid phenotype, with myeloblasts expressing CD34, CD117, HLA-DR, CD38, CD13, CD33, CD7 (subset), CD56 (subset), and CD123, without CD11b, CD14, CD36, or CD19.

Cytogenetics

Karyotype at diagnosis demonstrated 46,XY,t(8;22)(p11.1;q11.2)[19]/46,XY[1]. Metaphase FISH studies using FGFR1 break-apart probe revealed transposition of 3'FGFR1 to the rearranged chromosome 22, and BCR(G)/ABL1(R)-ASS1(A) probes revealed the presence of BCR signal in both rearranged chromosome 8 and rearranged chromosome 22. Gain of a BCR signal was seen in 95% of interphase cells. Together, these results suggested *BCR*::*FGFR1* rearrangement.

Molecular Studies

NGS demonstrated *DNMT3A* c.958C>T;p.Arg320Ter (45% VAF)
and *RUNX1* c.820C>T;p.Gln274Ter (47% VAF). The specimen was negative for mutations in *JAK2 V617F,* CALR, MPL, and for *BCR-ABL1*.

Proposed Diagnosis

Blast phase of myeloid/lymphoid neoplasm with *FGFR1* rearrangement

Interesting Feature(s)

The case demonstrates presentation in multilineal blast-phase with predominantly B-lineage blasts and with *DNMT3A* and *RUNX1* nonsense mutations. Conventional karyotyping and metaphase FISH were utilized to identify alteration of *FGFR1*, including a break-apart probe for *FGFR1*. The case demonstrates an aggressive course of this entity and switching of the predominant lineage blast population over the treatment course.

Panel Diagnosis

Myeloid/lymphoid neoplasm with FGFR1 rearrangement (WHO/ICC), blast phase)

EA4HP24-BMWS-459

Acute leukemia with minimal (or ambiguous?) differentiation

Dr. Mihai Merzianu

Roswell Park Comprehensive Cancer Center, Department of Pathology, Buffalo, USA

Case Description

Patient is a 79-year-old man who presented with a seven-week history of enlarged cervical lymph nodes, fatigue and weight loss. A CT showed bilateral intraparotid and cervical lymphadenopathy and subsequent PET scan was interpreted elsewhere as “metabolically active lymphoma” in cervical and subdiaphragmatic lymph nodes, spleen and bone marrow. A cervical neck lymph node biopsy was performed elsewhere. Lab results at presentation: WBC 4.4k/dL, Hb 12.8, platelets 190, and LDH 194 [121–224]. One month later, WBC increased to 78k/dL. Patient was referred to our institution, where a bone marrow biopsy was performed.

Biopsy Fixation Details

Formalin-fixed, paraffin embedded tissue

Frozen Tissue Available

No

Details of Microscopic Findings

Blood with marked lymphocytosis of immature cells with small-medium sized nuclei, high N:C ratio, and scant agranular cytoplasm with rare vacuoles. A second population of small lymphocytes with CLL appearance including smudge cells is seen. Marrow aspirate smears show similar morphology except increased numbers of large blasts. Marrow space is diffusely involved by medium-sized blasts with many tingible body macrophages (starry sky pattern), decreased erythroid and granulocyte precursors with dysmegakaryoiesis. Lymph node biopsy showed paracortical involvement by a small -medium sized lymphoid proliferation interpreted elsewhere as T prolymphocytic leukemia.

Immunophenotype

Combined flow cytometry/immunohistochemistry: main neoplastic population expressed CD4s, CD5s, CD11bd, CD14s, CD15s, CD25, CD33s, CD38, CD45d, CD52d, CD64s, CD71, CD99, CD117d, CD123, CD200, HLA-DR, TdTs, lysozyme (-/+); and is negative for CD1a, CD2, CD3, CD7, CD8, CD10, CD14, CD19, CD20, CD23, CD30, CD36, CD56, CD57, CD68, CD79a, CD163, BCL1, gamma delta TCR, EBER and cyto MPO, CD79a, CD22 and CD3. A second population with typical CLL immunoprofile (positive for CD5, CD19, CD20d, CD200, lambda restriction) accounted for 2% of total cells. By histochemistry, blasts were negative for MPO and CAE/ANBE. Neoplastic cells reportedly expressed CD3, CD4, CD5, TCL1, CD123 with dim/absent CD2 and CD7 by immunohistochemistry, evaluation hampered by poor tissue preservation.

Cytogenetics

45,XY,i(17)(q10),-21 [17/20 cells]; Deleted TP53 (17p13.1) (isochromosome formation); Deleted RUNX1 (21q22). No alterations: TCRAD (14q11.2), TCRB (7q34), KMT2A (11q23.3), BCR/ABL1, CDKN2A (9p21.3), ETV6

Molecular Studies

NGS positive for ETV6 E197* (44.4%) and RUNX1 R201* (82%). NGS showed clonal rearrangements in TRB (23% reads) and negative in TRG; and B-cell clonality reflecting incidental MBL-CLL population.

Proposed Diagnosis

Acute myeloid leukemia with minimal differentiationMonoclonal B-cell lymphocytosis, CLL type

Interesting Feature(s)

- Unusual presentation of acute leukemia with lymphadenopathy and initial normal CBC, small-medium sized blasts, T cell markers and clonality may pose diagnostic challenges
- Differential considerations include acute undifferentiated leukemia, AML-myelodysplasia related changes and AML, NOS with minimal differentiation. In view of several myeloid markers expressed by flow, albeit with minimal, dim, and/or heterogeneous expression, the latter was favored
- AML-myelodysplasia related changes would be supported by dysplastic megakaryocytes and/or RUNX1 mutations and i(17)(q10) (per ICC classification)
- Distinction between AUL and minimally differentiated AL is somewhat arbitrary when lacking currently accepted lineage defining markers
- "Stem cell" markers: CD99, TdT, CD33, CD34, CD38, HLD-DR may be expressed in both ALL and AML. T cell clonality may be seen in AML

Panel Diagnosis

AML with minimal differentiation (WHO)/AML-MR (ICC)

EA4HP24-BMWS-464

Trilineal mixed-phenotype acute leukemia

Dr. Alex Jenei

Semmelweis University, Department of Pathology and Experimental Cancer Research, Budapest, VIII., Hungary

Case Description

In 2020 a then 43 years old male presented with pancytopenia and pleural fluid.

After the diagnosis, induction treatment with Clofarabine + Venetoclax + ARA-C + Daunorubicin containing chemotherapy was started. After induction treatment bone marrow examination revealed 0.19% residual leukaemic cells. Subsequently, the patient

received FLAG+Venetoclax consolidation treatment. Again, a crista biopsy was performed, which confirmed relapse with 20-30% blasts predominantly of T/B phenotype. Due to relapse, augmented treatment with Hyper-CVAD + Venetoclax was started, with control examination demonstrating hypocellular bone marrow and residual acute leukaemia. The patient then received Nelarabin + HD MTX salvage treatment. In the next control bone marrow biopsy 47% persistent T-lymphoblasts were detected. He received CLAG-M regimen, after which severe aplasia developed. Gravis thrombopenia resulted in a hemorrhage of the temporal lobe and lateral ventricule and the patient died as a result of intracranial pressure elevation and foraminal hernation.

Biopsy Fixation Details

Bone marrow biopsy was fixed for 10–16 h in Schaefer's fixative (4% neutral buffered formaldehyde containing methanol and glucose), then decalcified overnight in 10% EDTA-Na2 and embedded routinely into paraffin wax.

Frozen Tissue Available

Frozen tissue is not available.

Details of Microscopic Findings

Bone marrow cellularity was 100% and composed of monomorph blast population.

Immunophenotype

Bone marrow aspiration confirmed nearly 100% blasts, which were divided into two populations by flow cytometry: **49%** CD45dim, CD34poz, CD117het, HLA-DRpoz, CD33dim/poz, CD14neg, CD7neg/dim, CD19dim, CD36neg, CD64neg, CD11bneg, CD15neg, NG2neg/dim, CD133dim, CD4neg, CD99dim, CD13poz, CD71poz, CD2neg, CD22dim, CD56poz, CD38poz, CD5neg, sCD3neg, cyCD3neg, TdTdim, MPOneg/dim, CD20neg, CD10neg, CD58dim, IgMneg, CD1aneg and **48%** CD45dim, CD34het, CD117neg, HLA-DRpoz, CD33neg, CD14neg, CD7br, CD19neg/dim, CD36neg, CD64neg, CD11bneg, CD15neg, NG2neg/dim, CD133neg, CD4neg, CD99dim, CD13dim, CD71poz; CD2poz, CD22dim, CD56neg, CD38poz; CD5neg/dim, sCD3neg, cyCD3neg/dim, TdTdim, MPOneg, CD20neg, CD10poz, CD58dim, IgMneg, CD1aneg phenotype blasts.

In the bone marrow biopsy, proximately 5% of blasts were MPO-positive and 20-30% of cells show expression of CD3. With CD79a antibody, at least 80% of cells show variable labeling, some with stronger, some with weaker intensity. Also with PAX5, about 80-90% of cells show faint expression.

Cytogenetics

In the absence of metaphases, karyotiping wasn't successful. With FISH probes for t(9;22), t(12;21), t(18;21) and MLL, no abnormal signals were detected.

Molecular Studies

Monoclonal T cell receptor gene rearrangements and monoclonal IgH gene rearrangements were identified in the sample. The sample was negative for NPM1, FLT3-ITD, FLT3-TKD, IDH1, IDH2, CEBPA mutations.

Proposed Diagnosis

Mixed-phenotype acute leukaemia, B/T/Myeloid (MPAL-B/T/M).

Interesting Feature(s)
The interesting feature of this case is the mixed phenotype involving B-, T- and myeloid lineages which is a rarity even among acute leukemias with a mixed phenotype. The case also demonstrates the unfavourable course of MPALs.

Panel Diagnosis
MPAL, B/T/myeloid (WHO/ICC)

EA4HP24-BMWS-467

Early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) with high mutation burden

Matthew Ye, Dr. Zhuang Zuo, Fengxi Ye, Ellen Dai, Dr. Yaling Yang, **Dr. M. James You**

MD Anderson Cancer Center, Hematopathology, Houston, USA

Case Description
The patient is a 68-year-old man with no significant past medical history who was found to have thrombocytopenia during a work-up for arterial thrombosis in lower leg. A complete blood cell count showed white blood cell 1.9 K/uL, hemoglobin 10.2 g/dL, MCV 105 fL, and platelets 202 K/uL. No circulating blasts were noted. A bone marrow (BM) biopsy showed T-lymphoblastic leukemia with an early T-cell precursor immunophenotype. The patient was treated with Hyper-CVAD and venetoclax, achieved complete remission, and then received allogeneic stem cell transplantation. He remained in complete remission at the last follow-up, 72 months after initial diagnosis.
Biopsy Fixation Details
The BM core biopsy was decalcified, and the biopsy and aspirate clot specimens were fixed in formalin.
Frozen Tissue Available
No
Details of Microscopic Findings
The BM core biopsy and aspirate smear showed hypercellular (70%) BM with trilineage hypoplasia and increased blasts (37%). The blasts were intermediate in size with vesicular chromatin, occasional prominent nucleoli, and scant cytoplasm, and were negative for myeloperoxidase by cytochemistry.
Immunophenotype
Flow cytometry immunophenotypic analysis revealed a population of blasts; positive for CD2, cytoplasmic CD3, CD7, CD13, CD34, CD38, CD45, CD117, CD123, HLA-DR, TdT (partial), and negative for CD1a, surface CD3, CD4, CD5, CD8, CD10, CD14, CD15, CD19, CD22,

CD25, CD33, CD36, CD41, CD56, CD64, myeloperoxidase, consistent with an early T-cell precursor immunophenotype.

Cytogenetics

47,XY,+4[1]/46,XY,del(9)(q12)[1]/46,XY[18]

Molecular Studies

Monoclonal T-cell receptor beta gene rearrangement was detected by PCR.

Next generation sequencing (NGS) using a panel of 81 genes showed:

FLT3 c.2503G>T p.D835Y (variant allele frequency, VAF 20.4%)

FLT3 c.2028C>G p.N676K (VAF 2%)

RUNX1 c.419delins p.Y140fs (VAF 6.9%)

WT1 c.1124_1125insGGGTACG p.V376fs (VAF 23.9%)

WT1 c.1121_1122insGGGGGTCCCG p.R375fs (18.5%)

WT1 c.1153delins p.R385fs (VAF 8.8%)

Proposed Diagnosis

Early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) with high mutation burden

Interesting Feature(s)

ETP-ALL is a distinct subtype of T-ALL with a unique immunophenotype and highly heterogeneous mutation landscape. The molecular genetic abnormalities and their prognostic impact on patients with ETP-ALL are poorly understood. We present a case of ETP-ALL with six mutations detected by NGS, including genes that are commonly mutated in myeloid neoplasms such as *FLT3* and *RUNX1* and genes that are more commonly mutated in T-ALL such as *WT1*, in keeping with the early/undifferentiated nature of this precursor cell neoplasm. The presence of variable allele frequencies among different mutations suggests a subclonal structure with some mutations as driver mutations and founding clones, whereas others as subclones. Despite the high mutation burden and the fact that these mutations have been associated with poor prognosis in acute myeloid leukemia and T-ALL, with appropriate treatment, such as stem cell transplantation in this case, patients may achieve and attain a durable complete morphologic, immunophenotypic and molecular remission.

Panel Diagnosis

ETP-ALL (WHO/ICC)

EA4HP24-BMWS-476

Acute leukemia, mixed myeloid/plasmacytoid dendritic cell phenotype involving bone marrow and lymph node.

Dr. Sara Niyazi, Prof. Ling Zhang, Prof. Lynn Moscinski

H Lee Moffitt Cancer Center, Pathology, Tampa, USA

Case Description

52-year-old male presenting with a 1-month history of fevers, chills, dyspnea, hemoptysis, and newly noted painful lymphadenopathy. CT of the abdomen & pelvis demonstrated splenomegaly (15 cm), and enlarged bilateral iliac and inguinal lymph nodes (LN) measuring up to 2.7 cm. Complete blood count was notable for a white blood cell count of 18.8 x 10^9/L, hemoglobin of 8.2 g/dL, and platelet count of 33 10^9/L. Mild eosinophilia (1.7 x 10^9/L) is noted. LN and BM biopsies were performed.

Biopsy Fixation Details

B Plus Fix fixative, BM and 10% buffered formalin, LN.

Frozen Tissue Available

NA

Details of Microscopic Findings

Peripheral blood shows leukocytosis with mild eosinophilia and 16% blasts. The BM aspirate and core biopsy shows 90% cellularity with a marked increase in blasts. Notably, there are two subset populations. One subset is comprised of large blasts, with morphology consistent with myeloblasts; the second subset is comprised of small blasts admixed with many elongated or spindle cells with plasmacytoid dendritic cell (pDC) morphology. LN biopsy demonstrated complete replacement by diffuse sheets of atypical cells with similar blast and pDC morphology.

Immunophenotype

Immunohistochemical (IHC) stains performed on the BM core demonstrate two distinct populations. The firstmajority population with the following IHC profile: CD34+, CD117+, CD4 +/-, CD56- CD123-, TCF4-, and TCL1-, consistent with a myeloblast phenotype. The second minor population with the following IHC profile: CD4+, CD56-, CD123+, TCF4+, TCL1-, CD34-, and CD117-, consistent with pDC differentiation. IHC stains were also performed on the LN biopsy and demonstrated the same dual populations. However, in the LN it showed predominantly CD123+, CD4+, and TCF4+ cells in big clusters, demonstrating more involvement by the pDC population when compared to the BM. Aslo refer to the concurrent flow cytometry in PPT.

Cytogenetics

FISH AML panel performed on the BM showed no evidence of *PML::RARA*, *RUNX1::RUNX1T1*, *BCR:ABL1*, *CBFB* or *MLL/KMT2A* rearrangement, del(5q)/-5, del(7q)/-7, +8, del(17p), or del(20q). Karyotyping showed 46,XY[20].

Molecular Studies

NGS detected Tier IA pathological mutations of *RUNX1* (p.V90lfs*51, VAF 47.1%), *SRSF2* (p.95H, VAF 45.1%), and *FLT3* (p. D835Y, VAF 1.2%), Tier IIC mutations of *DNMT3A* (p.R882H, VAF 46.2%), *CSF3R* (p.Q766*, VAF 4.8%), *KRAS* (p.G13D, VAF 4.1%), *NRAS* (p. G13C, VAF 3.4%), *PTPN11* (p. A72V, VAF 19.3%), *PTPN11* (p.D61H, VAF 4.5%), and Tier III mutation of *SH2B3* (p.395K, VAF 51%).

Proposed Diagnosis

Acute leukemia - mixed myeloid and pDC phenotype; with BM and LN involvement.

Interesting Feature(s)

Here, we present a unique case of acute leukemia, with mixed myeloid and pDC phenotype involving both LN and BM. The most interesting feature of this case is that these blasts show distinct immunophenotypes found in LN and BM. In the bone marrow, the CD34+/CD117+ myeloblasts are dominant, intermingling with small clusters of pDC, which could be missed without additional IHC staining; whereas in the LN there is more prominent nodular pDC proliferation and the CD34-/CD117+ blasts are focally retained. Similar to reported pDC AML, our case also shows cross-lineage antigen expression, *RUNX1*^{mut}and pDC marker expressions, e.g., CD123 & TCF4 [PMID: 32871587, 33054062], however, in our case, CD34+ cells appear more prevalent in BM but absent in LN, while CD123+ cells are limited in BM but confluent in LN, suggesting increased pDC differentiation in peripheral location. Sorting cell strategy for deep sequencing is warranted for further insight into the molecular fingerprints of this particular variant of acute leukemia.

Panel Diagnosis

AML-MR (WHO/ICC)