



EA4HP
2026



23RD MEETING OF THE EUROPEAN ASSOCIATION FOR HAEMATOPATHOLOGY

3–8 October 2026 | Berlin, Germany

BONE MARROW WORKSHOP BOOK



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Bone Marrow Workshop Part I | Hypoplastic MDS and hypoplastic AML (de novo) including cases presenting with germline disposition (limited to adult cases [≥ 18 years])

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Hypoplastic myelodysplastic syndrome, *TP53* mutated, in a background of prominent gelatinous transformation

Dr. Juanita Ferreira^{1,2}, Dr. Mutaz Amin^{1,3}, Dr. Majd Jawad^{1,2}

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Case Description

An 82-year-old male with a history of coronary artery disease presented for evaluation of pancytopenia. He reported episodic lightheadedness, night sweats, decreased appetite and weight loss, and was only able to eat sweets over the last 3 weeks.

Biopsy Fixation Details

10% neutral buffered formalin, following modified decalcification

Frozen Tissue Available

Not available

Details of Microscopic Findings

The bone marrow aspirate smears were extremely paucicellular and dilute, limiting accurate differential count or morphologic assessment of dysplasia. The bone marrow core biopsy demonstrated prominent gelatinous transformation (serous atrophy) highlighted by Alcian blue stain (pH2.5). The marrow was markedly hypocellular for age, ranging between 5% to 20% cellularity, with reduced trilineage hematopoiesis. There was moderate to severe fibrosis, highlighted by reticulin and trichrome stains. Megakaryocytes were scattered and included small hypolobated forms that were better visualized by immunohistochemistry.

Immunophenotype

Flow cytometric studies performed on the hemodilute aspirate demonstrated 2.3% CD34 positive without overt aberrancy. Granulocytes showed slightly decreased side scatter. B and T cells were normal. CD34 immunohistochemical stain highlighted approximately 5% positive mononuclear cells, consistent with blasts, and CD61 stain highlighted scattered small hypolobated megakaryocytes.

Cytogenetics

Cytogenetic studies showed a complex abnormal male karyotype with numerical and structural alterations: 39~40,XY, -5, -7, add(11)(p15), -16, -17, -18, del(20)(q11.2q13), -21,-22, +mar1, +mar2[2], +mar3[2][cp4]/46,XY[13]. Chromosome microarray confirmed these findings including loss in 12p13.3 region, encompassing the *ETV6*, *CDKN1B*, and *CREBL2* genes, loss of 17p13 comprising the *TP53* gene, and loss involving the long arm of chromosomes 5, 8 and 20, as well as multiple regions with gains and losses.

Molecular Studies

Molecular NGS analysis performed on the aspirate (OnkoSight NGS Myeloid Disorder Sequencing Panel) detected a single *TP53* pathogenic mutation (p.Ser215Gly; VAF: 28%).

Proposed Diagnosis

Myelodysplastic syndrome (MDS) with mutated *TP53* (per ICC 2022 classification), Myelodysplastic neoplasm (MDS) with biallelic *TP53* inactivation (per WHO 5th classification)

Interesting Feature(s)

The diagnosis of MDS is often challenging in the hypocellular setting and in this case is further complicated by the concurrent gelatinous transformation and fibrosis. Gelatinous transformation, also known as serous atrophy, is defined by replacement of normal marrow fat and hematopoietic elements with gelatinous extracellular material and is typically seen in the setting of prolonged malnutrition, infections or malignancies. Although the patient's cytopenias could be attributed to gelatinous transformation and malnutrition, given the increased CD34 positive blasts

and the presence of small megakaryocytes in the core biopsy, there was a high concern for involvement by a myeloid neoplasm. However, morphologic evaluation was very limited in this case. The detection of myeloid associated abnormalities identified by cytogenetic and molecular studies helped confirm the diagnosis of a myeloid neoplasm. Given the dysplastic (i.e. small hypolobated and non-clustered megakaryocytes) rather than proliferative features, a diagnosis of myelodysplastic syndrome/neoplasm was rendered, and was best classified as: myelodysplastic syndrome with mutated *TP53* (per ICC) or myelodysplastic neoplasm with biallelic *TP53* inactivation (per WHO 5th Edition).

Panel Diagnosis: MDS-biTP53 / MDS-TP53

EA4HP26-BMWS-31

Hypoplastic acute myeloid leukemia with MECOM rearrangement

PhD/MD Linda Zhang, PhD/MD Dita Gratzinger

Stanford University School of Medicine, Department of Pathology, Stanford, USA

Case Description

18-year-old male with history of adenoidectomy, asthma, respiratory allergies, eczema, and sinusitis now with 6 months of progressive pancytopenia. The patient reports increased frequency of bruising and nosebleeds, but denies fevers, night sweats, adenopathy, weight loss, or change in appetite.

Biopsy Fixation Details

The bone marrow core biopsy was placed in Bouin's solution for 1 hour, rinsed with water, covered with 10% formalin, and followed by Immunocal decalcification.

Frozen Tissue Available

N/A

Details of Microscopic Findings

Bone marrow aspirate: The bone marrow aspirate contains adequate paucicellular marrow particles. Erythroid precursors are present but proportionately increased, show left-shifted maturation, and rare dyspoietic features including binucleation and nuclear budding (<10%). Myeloid precursors show left-shifted maturation, normal nuclear segmentation, and decreased granularity. Megakaryocytes are present, are proportionately normal, and show dysplastic features (>10%) including forms with separated nuclear lobes and small hypolobated forms. 8.5% blasts are identified by manual differential.

Bone marrow core biopsy: The bone marrow core biopsy is markedly hypocellular at 10%. Erythroid precursors are proportionately increased, although overall decreased, and show left-shifted maturation. Myeloid precursors are decreased with an overall M:E ratio of 1:1. Megakaryocytes are decreased, show small, hypolobated forms, and show no significant clustering. Lymphocytes are decreased and show no lymphoid aggregates.

Immunophenotype

CD34 immunostaining showed 5-10% blasts.

E-cadherin highlights immature erythroid islands.

By flow cytometry, an abnormal myeloblast population was identified constituting 10% of CD45+ events, expressing CD34, CD38 (decreased), CD117, CD13, CD33, HLA-DR (decreased), CD71, CD200, CD43, CD81, CD61 (subset), and CD45; negative for CD19, CD15, CD64, CD4, CD14, CD16, CD10, CD7, CD56, CD11b, and B/T antigens.

Cytogenetics

Cytogenetic analysis of the aspirate demonstrated an abnormal karyotype, 46,XY,t(3;12)(q26.2;p13) in 15 of 28 cells examined, consistent with an ETV6::MECOM fusion.

Molecular Studies

Next-generation sequencing-based mutational profiling using the Stanford Actionable Mutation Panel for Hematopoietic and Lymphoid Malignancies (Heme-STAMP) was performed and revealed:

- BCOR Y497* mutation, VAF 23%
- SF3B1 K700E mutation, VAF 10%
- *ETV6:MECOM* rearrangement was identified by manual review of sequencing reads
- *IL7R* L35Q, Unknown significance, VAF 54%

Proposed Diagnosis

Hypoplastic acute myeloid leukemia, with *MECOM* rearrangement

Interesting Feature(s)

Hypoplastic AML is more commonly described in older adults. Here, we report an adolescent patient with pancytopenia documented as early as two years prior to bone marrow evaluation. This gradual onset suggests a more indolent course than typically seen in AML and raises the possibility of antecedent hypoplastic MDS, particularly given the multilineage dysplasia and presence of an SF3B1 mutation. In a young patient with a hypocellular marrow, the differential diagnosis includes hypoplastic MDS, aplastic anemia, childhood myelodysplastic neoplasms with increased blasts, and hypoplastic AML. Ultimately, comprehensive cytogenetic and molecular testing identified a *MECOM* rearrangement, which defines AML irrespective of blast count. This finding effectively upstages the process from a hypocellular marrow disorder to AML and underscores the critical role of molecular testing in evaluating hypocellular disease.

Panel Diagnosis: AML with *MECOM* rearrangement / MDS-EB

EA4HP26-BMWS-32

Hypoplastic myelodysplastic syndrome

Dr. Linda Zhang, Dr. Joshua Menke, Dr. Dita Gratzinger

Stanford University School of Medicine, Department of Pathology, Stanford, USA

Case Description

The patient is a 64-year-old female with a history of hereditary spherocytosis status-post splenectomy, presumed ITP without prior therapy, and mild pancytopenia with progressive macrocytosis and neutropenia. She has reported abnormal blood counts since the age of 17 and has had multiple prior bone marrow biopsies showing hypocellularity throughout her life.

Biopsy Fixation Details

The bone marrow core biopsy was placed in Bouin's solution for 1 hour, rinsed with water, covered with 10% formalin, and followed by Immunocal decalcification.

Frozen Tissue Available

N/A

Details of Microscopic Findings

Peripheral blood: the red blood cells are decreased and are normochromic and macrocytic with mild anisocytosis. Poikilocytosis is moderate with microcytes and target cells. Rare Pappenheimer bodies are seen. Polychromasia is mild, with rare circulating nucleated red blood cells. Leukocytes are decreased. Neutrophils show expected nuclear segmentation and decreased cytoplasmic granularity. Lymphocytes are heterogenous and mature. Platelets are decreased and are normal in morphology.

Bone marrow aspirate: the marrow aspirate contains adequate hypocellular marrow particles. Erythroid precursors are present, proportionately increased, and show left-shifted maturation. The morphology is predominately normal, but occasional erythroid precursors show morphologic atypia including nuclear budding (<10%). Myeloid precursors are present, proportionately decreased, and show normal nuclear segmentation and granularity. Blasts are not

increased by morphology. Megakaryocytes are present, proportionately decreased, and include a subset that are small and hypolobated (>10%). Lymphocytes are not increased and are predominately mature and heterogenous.

Bone marrow core biopsy: the bone marrow core biopsy is hypocellular at <10%. Erythroid and myeloid precursors are both proportionately decreased. Megakaryocytes are decreased and include small and hypolobated forms.

Immunophenotype

No abnormal blast population identified by flow cytometry.

Cytogenetics

Cytogenetic analysis of the aspirate demonstrated a normal karyotype, and the MDS FISH panel was negative.

Molecular Studies

Next-generation sequencing-based mutational profiling using the Stanford Actionable Mutation Panel for Hematopoietic and Lymphoid Malignancies (Heme-STAMP) was performed and revealed:

- *BCOR* I1290fs, VAF 21%
- *DNMT3A* Q249*, VAF 4%

A skin sample was collected and sent to Invitae to perform a “Bone Marrow Failure Syndromes” panel. The test identified two heterozygous variants of unknown significance.

Proposed Diagnosis

Myelodysplastic neoplasm, hypoplastic

Interesting Feature(s)

Here we present a case of hypoplastic MDS (hMDS) in a patient with longstanding cytopenias and bone marrow hypocellularity. This presentation raises concern for hMDS, aplastic anemia, or a possible inherited marrow failure predisposition. However, a germline bone marrow failure panel was performed and negative for any known pathogenic mutations. However, the clinical relevance of variants of unknown significance that were identified in our patient remains unclear and the classifications may be refined as additional functional studies and broader population data emerge. Notably, pronounced megakaryocyte dysplasia developed later in the patient’s course, helping to distinguish hMDS from aplastic anemia and suggesting dynamic marrow evolution. A second external review at an outside institution described the marrow as normocellular with erythroid hyperplasia and relative granulocytic and megakaryocytic hypoplasia, further highlighting the diagnostic complexity of this case.

Panel Diagnosis: Hypoplastic MDS /MDS-NOS

EA4HP26-BMWS-37

A case of hypoplastic AML with germline and somatic *DDX41* mutations

Dr. Maria Rozman, Dr. Irene Lopez-Oreja, Dr. Marta Garrote, Dr. Jose Ramon Alamo, Dr. Monica Lopez-Guerra, Dr. Elia Grau, Dr. Irene Madrigal, Dr. Francisca Guijarro, Dr. Jordi Esteve

Hospital Clinic Barcelona, Hematopathology, Barcelona, Spain

Case Description

A 71 year old woman without relevant medical history consulted in May 2024 for pancytopenia. She had progressive anemia since July 2023 not-associated to vitamin or iron deficiency, and developed neutropenia and thrombocytopenia. The blood counts revealed Hb 83 g/L, MCV 98 fL, platelets 50x10⁹/L, leukocytes 2.30 x10⁹/L (% n 12, bands 2, lymph 84, mono 1, myelo 1). Physical examination was unremarkable. Biochemistry was normal except for elevated erythropoietin 498 U/L (N: 4-20). After the diagnosis, a familial study was performed and a germinal *DDX41* variant was detected in two brothers, one daughter and one nephew.

Biopsy Fixation Details

B24-017206 A: fixation formalin 10%, decalcification formic acid 10%; B24-017206 B: fixation Bouin solution, decalcification hydrochloric acid 1-5%

Frozen Tissue Available

No

Details of Microscopic Findings

The bone marrow aspirate showed mostly acellular areas, absence of megakaryocytic and granulocytic precursors and scarce focal cellular areas with about 60% non-dysplastic erythroid precursors and about 14% blasts. The bone marrow biopsy was hypocellular (20%), with dysplastic hypolobated or non-lobated megakaryocytes, preserved erythroid precursors and scarce granulopoiesis; M/E ratio was inverted. Myeloid blasts (CD34 and CD117+) were increased (40-45%). Some reactive lymphoid aggregates were observed. Reticulin fibrosis was absent.

Immunophenotype

Flow cytometry (BM aspirate): 11% blasts positive for CD34, CD117, HLA-DR, CD33 weak, negative for CD56, CD7, Tdt and CD19.

Cytogenetics

BM karyotype:46,XX [20]

Molecular Studies

Mutational NGS study showed the following pathogenic/likely pathogenic variants: *ASXL1* p.Tyr974Ter (VAF: 4,7%), *ASXL1* p.Gly646TrpfsTer12 (VAF 3,9%), *DDX41* p.Arg525His (VAF: 7,1%), *EZH2* p.Pro132Ser (VAF: 7,1%), *ZRSR2* p.Arg295Ter (VAF: 5%).

DDX41 p.Arg525His variant was confirmed by digital PCR.

Germline study (hair follicle sample) showed an additional germline variant in *DDX41* (p.Gly230Arg) and confirmed the somatic nature of *DDX41* p.Arg525His .

Proposed Diagnosis

Acute myeloid leukemia with myelodysplasia-related gene mutations with germline *DDX41* mutation

Interesting Feature(s)

The present case exemplifies a prototypic case of a myeloid neoplasm with germline *DDX41* mutation.

Germline *DDX41* mutations have an estimated prevalence of 3% of myeloid malignancies. The development of myeloid malignancy presents typically at an older age with cytopenia, thus overlapping with sporadic MDS and AML. *DDX41*-associated AML is often presenting with bone marrow hypocellularity and a borderline increase in blasts and normal karyotype. These features make the initial diagnosis of this inherited AML more challenging than other hereditary hematologic predisposition syndromes. Myeloid neoplasia is often associated with biallelic *DDX41* mutations due to acquisition of a somatic mutation in the wild type allele, most commonly R525H, in combination with the germline mutation; thus, when a somatic *DDX41* mutation is found a hereditary hematologic predisposition testing is mandatory.

References:

- Rudelius M, Weinber OK, Niemeyer ChM, et al. The International Consensus Classification (ICC) of hematologic neoplasms with germline predisposition, pediatric myelodysplastic syndrome, and juvenile myelomonocytic leukemia. *Virchows Archiv* 2023 Jan;482(1):113-130.
- Makishima H, Bowman TV, Godley LA. *DDX41*-associated susceptibility to myeloid neoplasms. *Blood* 2023, 141(13):1544-1552.

Panel Diagnosis: AML-MR / AML with MR gene mutations and germline *DDX41*

Morphologically subtle hypoplastic MDS

Dr. Sheren Younes, Prof. Dita Gratzinger

Stanford University, Pathology, Palo Alto, USA

Case Description

The patient is a 78-year-old man with history of thrombocytopenia and macrocytic anemia with no overt dysplasia or increased blasts in a hypercellular bone marrow, normal karyotype, and NGS detected pathogenic variants in DNMT3a (6%) and U2AF1 (8%), rendering diagnosis of clonal cytopenias of undetermined significance (CCUS). Sequential bone marrow biopsies were taken for persistent cytopenias, with progression to hypocellular marrow, subtle dyspoiesis, and increasing VAFs of the pathogenic mutations supporting a diagnosis of hypoplastic MDS. Lymphoid aggregates and a clonal T cell population were also present. The patient was treated with thrombopoietic receptor agonists, Cyclosporin A, and transfusion support.

Biopsy Fixation Details

The bone marrow core biopsy was placed in Bouin's solution for 1 hour, rinsed with water, covered with 10% formalin, and followed by Immunocal decalcification.

Frozen Tissue Available

n/a

Details of Microscopic Findings

Serial bone marrow biopsies in at initial diagnosis, 4 months later, and again 14 months later

Peripheral blood: Normochromic macrocytic anemia (Hg 11.2 g/dL, MCV 108.8 fL) and marked thrombocytopenia (platelets 11 K/uL) and, at subsequent timepoints, progressive neutropenia (1.1K/uL, 0.7K/uL).

Bone marrow aspirate and biopsy:

Initial bone marrow biopsy was hypercellular for age (60-70%) with subtle <5% dyserythropiesis, and no increased blasts. Follow up bone marrow biopsies showed hypocellular bone marrow with few to no megakaryocytes for evaluation (overall 10% and 15% respectively). Scattered non-paratrabecular CD3+ T cell predominant lymphoid aggregates were noted.

Immunophenotype

No abnormal blast population or abnormal, TRBC1-restricted T cell population or light chain-restricted B cell population identified by flow cytometry. Paroxysmal nocturnal hemoglobinuria peripheral blood flow cytometry was negative.

Cytogenetics

Normal male karyotype

Molecular Studies

Next-generation sequencing using the Stanford Actionable Mutation Panel for Hematopoietic and Lymphoid Malignancies (Heme-STAMP) revealed progressive expansion of U2AF1 S34F and DNMT3A Q656fs mutant clones over time. The U2AF1 S34F variant allele frequency (VAF) increased from 6% to 23% over 2 years, while the DNMT3A Q656fs VAF rose from 4% to 22% over the same period. A clonal T cell receptor gamma rearrangement was also identified by NGS.

Proposed Diagnosis

At least clonal cytopenia of uncertain significance, favor hypoplastic myelodysplastic neoplasm.

Interesting Feature(s)

This case highlights several noteworthy features that underscore the diagnostic complexity of hypoplastic myelodysplastic syndrome (MDS). Both hypoplastic MDS and aplastic anemia present with pancytopenia and markedly hypocellular marrow, and their substantial clinicopathologic overlap often makes distinction challenging. Although hypoplastic MDS carries a worse prognosis and higher risk of leukemic progression, the profound hypocellularity in such cases can obscure morphologic evidence of dysplasia, placing greater emphasis on molecular

testing. Despite the absence of >10% morphologic dysplasia, we favor that this represents hypoplastic MDS. In this patient the presence of a U2AF1 mutation was especially suspicious as U2AF1 mutations are associated with a high rate and short latency of progression to MDS or acute myeloid leukemia (Pritzl SL et al, Clinical and molecular spectrum and prognostic outcomes of U2AF1 mutant clonal hematopoiesis: A prospective Mayo Clinic cohort study. Leuk Res. 2023. 125:107007. PMID: 36586169.)

Panel Diagnosis: CCUS

EA4HP26-BMWS-61

Hypoplastic acute myeloid leukemia arising from a DDX41 germline predisposition syndrome

Dr. April Chiu, Dr. Kaari Reichard

Mayo Clinic, Hematopathology, Rochester, USA

Case Description

68-year-old gentleman with a medical history notable for insulin-dependent diabetes, chronic kidney disease, atrial fibrillation on warfarin, and history of coronary artery disease status post CABG who presents for evaluation of pancytopenia. CBC - HGB 11.0 g/dL; RBC 3.06 x10¹²/L; MCV 112.4 fL; RDW 15.9 %; WBC 1.6 x10⁹/L; PLT 86 x10⁹/L Diff (%): neut 45, lymphs 47, monos 4, eos 4.

Biopsy Fixation Details

Biopsied decalcified

Frozen Tissue Available

NA

Details of Microscopic Findings

PB: Pancytopenia - macrocytic anemia, neutropenia and thrombocytopenia. No dysplasia; no circulating blasts

Aspirate: Increased blasts without Auer rods; no dysplasia

Core: Hypocellular for age; increased, sneaky, interstitial blasts; panhypoplasia

Immunophenotype

Flow cytometry: Blasts express CD34, CD45 (dim), CD13, CD33, CD117, HLA-DR, CD36 (partial), CD38 (dim), cMPO and do not express: CD19, CD10, CD3, CD15, CD16, CD2, CD7, CD56, CD64, nTdT, cCD22, cCD79a, cCD3.

IHC: Increased CD34-positive blasts in an interstitial pattern

Cytogenetics

46,XY[20]

Molecular Studies

NGS: Two DDX41 mutations only. All other genes negative

1. DDX41: Chr5(GRCh37):g.176943944C>T; NM_016222.2(DDX41):c.3G>A; p.M1? (also known as p.M1I) (51%)

2. DDX41:Chr5(GRCh37):g.176939370C>T; NM_016222.2(DDX41):c.1574G>A; p.Arg525His (3%)

NGS panel is detailed on the attached presentation slide set.

Proposed Diagnosis

Hypoplastic acute myeloid leukemia arising from a DDX41 germline predisposition syndrome

Interesting Feature(s)

1. Germline variants in DDX41 are the most common mutations predisposing to myelodysplastic syndrome/acute myeloid leukemia (MDS/AML in adults, with an increased risk observed in association with elevated red blood cell MCV and a co-occurring somatic DDX41 variant

2. DDX41 mutations occur in 0.5-4% of patients with adult-onset MDS/AML and may present as a germline and/or somatic event. In approximately 50% of germline cases, an acquired somatic variant is present in a biallelic fashion (as in this case)
3. From the pedigrees reported, familial MDS/AML with germline DDX41 mutations are associated with a relatively longer latency with a mean onset age of 62 years

Panel Diagnosis: AML, defined by differentiation / AML, NOS with germline DDX41

EA4HP26-BMWS-68

Familial GATA2 haploinsufficiency syndrome manifesting as WILD syndrome with acquired STAG2 mutation

Dr. Justin Kelley

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

Case Description

25 year old man presented with a family history of GATA2 haploinsufficiency. His history is remarkable for recurrent hand warts as a child and abnormal lymphoid subsets including decreased NK cells (2%) and inverted CD4:CD8 ratio (0.8). He has no other symptoms or syndromes. His mother was diagnosed with myelodysplastic syndrome 10 years ago when found to have pancytopenia during evaluation for fatigue. His maternal aunt was found to be affected after workup for miscarriages and also developed myelodysplastic syndrome. He has two half-siblings from his mother who are both affected and progressed to myelodysplastic syndrome in his brother and acute myeloid leukemia in his sister. All of these family members subsequently received allogenic stem cell transplants and remain well. This patient has been closely monitored without evidence of neoplasm. However due to persistent hypocellular bone marrow and increasing STAG2 mutation, he also recently received an allogenic stem cell transplant.

Biopsy Fixation Details

10% formalin (core and particle). B-Plus fixative (aspirate).

Frozen Tissue Available

None

Details of Microscopic Findings

The bone marrow is variably hypocellular for age (10-50%, overall approximately 20-30%) with maturing trilineage hematopoiesis including normal myeloid-to-erythroid ratio without dysplasia or increased blasts.

Immunophenotype

Flow cytometry showed no increase in blasts (0.1% of total cells).

Cytogenetics

The karyotype is normal (46,XY[20]) and MDS/AML panel is negative.

Molecular Studies

Myeloid NGS detected GATA2 mutation at approximately 50% allele frequency (c.351C>G, Thr117= at 51.60%). Additionally, a new STAG2 splice site deletion (c.1196_1196+1del) was also detected and increasing in allele frequency over the past 6 months (from 3.2% to 11.5%).

Proposed Diagnosis

Familial GATA2 haploinsufficiency syndrome with acquired STAG2 mutation

Interesting Feature(s)

The patient presents with a classically autosomal dominant familial syndrome characterized by GATA2 haploinsufficiency with increased risk of myeloid neoplasms, as seen in his maternal family members. While he does not present with prototypic immunodeficiency syndromes, the syndrome has a wide range of clinical presentation. However, his history of childhood warts and abnormal lymphoid subsets raises the possibility of WILD syndrome

(warts, immunodeficiency, lymphedema, anogenital dysplasia), although he has no known lymphedema or anogenital dysplasia. Furthermore, he was found to newly acquired and increasing STAG2 mutation six months ago. STAG2, in addition to ASXL1, SETBP1 and RAS mutations along with abnormalities of chromosome 7, are well-associated acquired genetic abnormalities in GATA2 haploinsufficiency syndrome. Despite the absence of myelodysplasia or acute leukemia, the patient is preparing for allogeneic stem cell transplant given his strong family history and new, increasing high-risk mutation.

Panel Diagnosis: Germline GATA2 predisposition with acquired clonal hematopoiesis (STAG2 mutation)

EA4HP26-BMWS-86

Hypoplastic acute myeloid leukemia arising in a man with a germline *DDX41* mutation

Dr. Meredith Nichols

Cleveland Clinic, Department of Pathology and Laboratory Medicine, Cleveland, USA

Case Description

A 67-year-old man with rheumatoid arthritis (RA) presented with ongoing neutropenia, for which he received G-CSF. A bone marrow biopsy was performed and demonstrated hypercellular bone marrow with erythroid hyperplasia and 5-10% blasts, the significance of which was unclear in the context of recent growth factor therapy. Molecular studies performed at that time were notable for a pathogenic variant in *DDX41*. Testing confirmed a germline pathogenic mutation in *DDX41* and heterozygous variants of unknown significance in *BRCA2* and *ETV6*. Follow-up bone marrow biopsy performed 1 year later demonstrated normocellular marrow with 12% myeloid blasts, consistent with a myelodysplastic syndrome (MDS). Subsequent bone marrow sampling 8 months later demonstrated hypocellular marrow with 8% blasts. The patient developed pancytopenia and underwent additional bone marrow sampling, which showed hypocellular marrow with 21% myeloid blasts, consistent with acute myeloid leukemia (AML). Molecular testing confirmed the presence of an additional pathogenic mutation in *DDX41* without other variants identified. The patient began chemotherapy and has had persistent disease with subsequent development of additional pathogenic variants on molecular testing.

Biopsy Fixation Details

The specimens were fixed in 10% neutral buffered formalin.

Frozen Tissue Available

None.

Details of Microscopic Findings

At the time of AML diagnosis, the bone marrow was overall mildly hypocellular for age with erythroid-predominant trilineage hematopoiesis. Blasts were 21% of total cells on a 500-cell manual differential count of the bone marrow aspirate. Occasional hypolobated megakaryocytes were present. The erythroid and granulocytic lineages were negative for dysplasia.

Immunophenotype

Flow cytometry demonstrated a blast population that expressed CD33 (dim), CD34, CD36 (dim), CD38 (dim), CD45 (dim), CD71 (dim), CD117, CD133, and HLA-DR and was negative for cCD3, CD19, myeloperoxidase, CD14, and CD64. By immunohistochemistry, lysozyme was positive in a subset of cells. Cytochemistry for myeloperoxidase and non-specific esterase alpha-naphthyl butyrate was negative in the blasts.

Cytogenetics

All bone marrows demonstrated 46,XY[20].

Molecular Studies

At the time of AML diagnosis, the following pathogenic variants were identified:

DDX41 p.0? VAF: 20.8%

DDX41 p.R525H VAF: 4.5%

Subsequent bone marrow testing in the setting of persistent AML identified the below additional pathogenic variants:

ASXL1 p.E635Rfs*15 VAF: 7.1%

CBL p.R420Q VAF: 12%

CUX1 p.T698Sfs*16 VAF: 19.1%

CUX1 p.Q194* VAF: 11.1%

Proposed Diagnosis

Acute myeloid leukemia, not otherwise specified, germline predisposition, arising from myelodysplastic syndrome (ICC 2022).

Acute myeloid leukemia with minimal differentiation, associated with germline *DDX41* variant (WHO 5th ed.).

Interesting Feature(s)

The patient had a classic presentation of a late-onset MDS with subsequent development of AML in the setting of a germline *DDX41* mutation, with acquisition of a second *DDX41* mutation, which is typical in this setting. Prior to marrow sampling, these cytopenias were contributed to the history of RA, emphasizing the importance of bone marrow biopsy refractory cytopenias. Blasts were increased at the time of initial bone marrow biopsy but the patient had recently received growth factor therapy. Clinical correlation and repeat bone marrow sampling after cessation of this therapy was required to clarify the findings. Classification of the AML by both the ICC and WHO5 was not straightforward; although there was a history of MDS, the marrow lacked other molecular or cytogenetic findings required for AML-MR.

Panel Diagnosis: MDS-IB2 / MDS/AML with germline *DDX41* predisposition, with subsequent progression to AML-MR / AML with MR gene mutations

EA4HP26-BMWS-88

Case of Hypoplastic Myelodysplastic Syndrome with Leukemic Transformation

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Case Description

The patient is a 61-year-old male diagnosed with rectal adenocarcinoma with metastasis in March 2024. He underwent chemotherapy with FOLFOXIRI and chemoradiation with capecitabine. His course was complicated by cytopenia and incomplete count recovery. He underwent a bone marrow (BM) biopsy at an outside institution in February 2025. The biopsy revealed hypocellular BM (10-15%) with dysgranulopoiesis, decreased erythropoiesis and megakaryocytes. BM blasts were not increased (1%). Cytogenetics revealed abnormal male karyotype with del(20q) and a marker chromosome (10/20 metaphases). Myeloid next-generation sequencing (NGS) was not performed. A repeat BM biopsy was performed 6 months later (provided case).

Biopsy Fixation Details

Received in B5 fixative, fixed in 10% buffered formalin, and decalcified.

Frozen Tissue Available

None.

Details of Microscopic Findings

The BM aspirate was sparsely cellular. The biopsy was markedly hypocellular for age (5 – 10%) with increased M:E ratio. Erythroid precursors showed atypical left-shifted morphology and occasional nuclear irregularities. Myeloid precursors showed atypical morphology including occasional hypogranular forms. Blasts were increased with fine chromatin, prominent nucleoli and moderate amounts of cytoplasm. Megakaryocytes were essentially absent.

Immunophenotype

Flow-cytometric immunophenotyping was performed:

6% blasts were positive for CD34, CD45 (dim), CD13, CD15, CD33, CD117 (partial), HLA-DR (partial), CD2 (partial), CD36 (partial) and CD38.

Immunohistochemical stain for CD34 demonstrated mildly increased blasts in mainly interstitial distribution (approximately 10%). CD61 showed near absence of megakaryocytes.

Cytogenetics

Conventional karyotyping was performed.

46, XY, del(20) (q11.2q13.3)[4]/47, idem, +8 [13]/47, idem +15 [3]

20 metaphases had 20q deletion. 13 metaphases with a 20q deletion had trisomy 8 and 3 had trisomy 15.

Molecular Studies

Pathogenic/likely pathogenic mutations were identified.

RUNX1: Chr21(GRCh37):g.36259202_36259203insCCCGCCAA; p.Phe97Leufs*28 (44%)

SRSF2: Chr17(GRCh37):g.74732959G>T; p.Pro95His (41%)

STAG2: ChrX(GRCh37):g.123184998dup; p.Thr349Asnfs*9 (81%)

Proposed Diagnosis

Myelodysplastic neoplasm, hypoplastic (WHO 5th).

Myelodysplastic syndrome, not otherwise specified / Myelodysplastic syndrome, hypoplastic (ICC 2022).

Interesting Feature(s)

Despite the BM hypocellularity and near absence of megakaryocytes, the morphologic dysplasia, increased blasts and genetic abnormalities support the diagnosis of a myelodysplastic syndrome. Although the cytogenetic findings are not definitively diagnostic of MDS in the absence of morphologic criteria, the findings of *RUNX1*, *SRSF2* and *STAG2* mutations support a diagnosis of primary MDS.

The molecular and cytogenetics can also be seen in MDS post cytotoxic therapy, however, the characteristic findings of complex karyotype and *TP53* mutation were not present. Another differential diagnosis, secondary MDS after aplastic anemia, is most commonly characterized by monosomy 7 or del(7q).

A month after the second biopsy, the patient had 35% peripheral blasts, consistent with leukemic transformation. BM biopsy showed 20% blasts and an additional *FLT3* tyrosine kinase domain D835 mutation was detected. Taken together, the initial BM biopsy was consistent with hypoplastic MDS, and the rapid evolution seen in the second and third biopsies indicate the high-risk nature of this neoplasm.

Panel Diagnosis: MDS-IB1, post cytotoxic therapy / MDS-EB, therapy related

Myelodysplastic syndrome with germline *DDX41* mutation and progression to increased blasts.

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Case Description

A 73 year-old woman presented with leucopenia and neutropenia for approximately 1 year. She had no history of hematologic malignancy. A CBC performed at our institution on presentation showed mild leucopenia but no neutropenia or other cytopenias: WBC 3.5 K/uL, Hgb 12.5 g/dL, MCV 99 fL, 199 K/uL, neutrophils 62%, 2.17 K/uL. Due to lack of significant cytopenias, the patient was initially observed until a follow-up bone marrow showed increased CD34-positive blasts, involving 15-20% of marrow cellularity, consistent with MDS/AML. She was then started on cladribine, low-dose cytarabine and venetoclax therapy.

Biopsy Fixation Details

Fixation: 3 hours in 10% neutral buffered formalin

Decal: 12 hours in 5% formic acid

Frozen Tissue Available

N/A

Details of Microscopic Findings

The initial bone marrow (BM) biopsy on 1/27/2025 was hypocellular (patchy, 0-25%, overall about 10%) with dysplastic megakaryocytes (small, hypo/monolobated). The cellular areas showed erythroid predominance. BM aspirate smears showed dysplastic megakaryocytes (small, hypo/monolobated, micromegakaryocytes). There was no significant dysplasia in the erythroid or myeloid lineages and no overt increase in blasts.

A subsequent BM biopsy on 11/13/2025 was variably cellular (5-60%) with dysplastic megakaryocytes. BM aspirate smears also showed dysplastic megakaryocytes and no significant dysplasia in the other lineages. Blasts were increased, approximately 15-20% by CD34 immunohistochemistry. The CBC at this time was WBC 2.6 K/uL, Hgb 11.3 g/dL, MCV 99 fL, platelets 153 K/uL, neutrophils 30%, 0.79 K/uL.

Immunophenotype

By immunohistochemistry in the initial BM, CD34 stain showed scattered blasts, less than 5% of cellularity. CD61 stains showed increased proportion of small hypo/monolobated megakaryocytes, including micromegakaryocytes. Flow cytometry detected aberrant CD34+ myeloblasts (2.3%) and immunophenotypic changes supporting a diagnosis of a myeloid neoplasm.

Cytogenetics

Chromosome analysis showed a normal female karyotype (46,XX[20]).

Optical genomic mapping did not detect structural or copy number variants.

Molecular Studies

A targeted NGS study using an 81-gene panel detected variants in *DDX41* [c.992_994del p.K331del; variant allelic frequency (VAF): 32%] and *WT1* [c.1559T>C, p.L520P; VAF: 48%]. These variants were also detected in the subsequent bone marrow specimen. Genetic testing confirmed the *DDX41* p.K331del as a pathogenic germline variant.

In addition, both specimens from 1/28/2025 and 11/17/2025 also showed a *DDX41* c.1037C>T p.A346V mutation but at very low level variant allelic frequency (<2.5%).

FLT3 mutation analysis was negative for ITD and TKD.

Proposed Diagnosis

WHO 5th ed: Myelodysplastic neoplasm with increased blasts and germline DDX41 mutation.

ICC: Myelodysplastic syndrome/AML with excess blasts and germline DDX41 mutation.

Interesting Feature(s)

Confirmed germline *DDX41* mutation in a patient presenting with myelodysplastic syndrome in a hypocellular bone marrow at an older age than other myeloid neoplasms with germline predisposition.

Different time points showing progression from a predominantly hypocellular bone marrow with low blasts to areas of hypercellularity and increased blasts at the time of disease progression. Both bone marrow specimens showed *DDX41* mutation [c.992_994del p.K331del] with VAF of 32%, less than heterozygous, but was confirmed to be a germline mutation on fibroblast testing. In addition, a second *DDX41* somatic mutation was also detected in both the bone marrow specimens, but at variant allelic frequency of less than 5%.

Panel Diagnosis: MDS-LB (WHO5) / MDS, NOS with *DDX41* mutation and subsequent progression

EA4HP26-BMWS-118

Hypoplastic MDS with germline *GATA2* mutation: A rare case report

PhD/MD Samah Kohla^{1,2}, Dr. Amna Gameil^{4,2}, Dr. Aliaa Amer¹, Dr. Amal S. Aly⁵, Dr. Imran N. Ahmed¹, Dr. Reem Al-Suliaman⁶, Author Contributions Dr. Samah Kohla: Made the primary diagnostic interpretation, conceptualized the case report, and led abstract preparation. Dr. Amna Gameil: Managed the clinical case and reviewed the abstract. Dr. Aliaa Amer: Reviewed the case and contributed to writing and refining the abstract. Dr. Amal Salem: Performed and interpreted the next-generation sequencing analysis. Dr. Imran aid in the diagnosis. Dr. Reem Al-Suliaman: Performed whole genome sequencing, contributed to genomic interpretation, and reviewed the abstract.

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Case Description

A 27-year-old male with chronic cytopenias since adolescence was referred for evaluation of bone marrow (BM) failure. At age 13 (2011), he was treated for pulmonary tuberculosis, during which persistent leukopenia and thrombocytopenia were documented. Over the following decade, he experienced intermittent neutropenia and thrombocytopenia with preserved hemoglobin levels and no transfusion requirements. In August 2023, he presented with fever, pneumonia, and worsening pancytopenia. BM examination in 2023 demonstrated hypocellularity with myelodysplasia and a complex karyotype. Cytopenias were refractory to cyclosporine. Laboratory values showed WBC $0.8 \times 10^3/\mu\text{L}$, hemoglobin 8.1 g/dL, platelets $88 \times 10^3/\mu\text{L}$, ANC $0.3 \times 10^3/\mu\text{L}$, MCV 94.7 fL, and absolute reticulocyte count $28 \times 10^9/\text{L}$.

Biopsy Fixation Details

Paraffin-embedded biopsy fixed in AZF.

Frozen Tissue Available

Not available.

Details of Microscopic Findings

Peripheral blood showed pancytopenia with normocytic normochromic anemia, reticulocytopenia, leukopenia with marked neutropenia, dysgranulopoiesis, lymphocytopenia, moderate thrombocytopenia, and 1% circulating blasts. BM aspirate was aparticulate and partially hemodilute, appearing hypocellular with scattered granulocytic and late erythroid precursors, few megakaryocytes, and 1% blasts. Dysgranulopoietic and dysmegakaryopoietic features

were prominent, with limited erythroid dysplasia. Iron stores could not be assessed and no ring sideroblasts were identified. BM biopsy demonstrated variable hypocellularity, with focal areas of 5–10% cellularity and others up to 45–55%. Granulopoiesis was decreased, erythropoiesis was left-shifted, and megakaryocytes were increased, pleomorphic, clustered, and hypolobated. CD34-positive blasts were not increased (approximately 2–4%).

Immunophenotype

Flow cytometry of the BM showed 37% T cells and 1% B cells, with no evidence of a monocytic B-cell population.

Cytogenetics

Karyotype analysis revealed a complex karyotype: 48,XY,del(1)(q21q42),+8,+mar[8]/48,idem,+r,-mar[11]/49,idem,+r[3]/46,XY[8]. Clonal abnormalities were identified in 22 of 30 metaphases.

Molecular Studies

NGS myeloid panel identified a frameshift mutation in *GATA2* with a variant allele frequency of approximately 41%. Whole-exome sequencing from buccal swab confirmed a heterozygous germline *GATA2* mutation, c.1021_1024dup p.(A342Gfs*43).

Proposed Diagnosis

Hypoplastic Myelodysplastic neoplasm with low blasts (MDS-LB) with *GATA2* germline predisposition

Interesting Feature(s)

This case highlights the importance of recognizing germline *GATA2* deficiency in young patients with hypocellular MDS, chronic cytopenias, recurrent infections, and characteristic cytogenetic abnormalities. Confirmation using non-hematopoietic tissue was essential to distinguish germline from somatic mutations, enabling appropriate family counseling and donor selection.

Conclusion

Germline *GATA2*-mutated MDS represents an overlap of inherited marrow failure, immunodeficiency, and myeloid malignancy. Early recognition is critical to guide surveillance and timely allogeneic hematopoietic stem cell transplantation, the only curative option.

Panel Diagnosis: MDS-LB / MDS, NOS with germline *GATA2*

EA4HP26-BMWS-126

Myelodysplastic Syndrome vs. Acute Myeloid Leukaemia with *MECOM* Rearrangement

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Case Description

The patient is a 48-year-old with no significant past medical history who presented with a 2-week history of extreme fatigue, dizziness, and blurry vision. A complete blood count showed pancytopenia; rare blasts were seen on the peripheral blood smear.

Biopsy Fixation Details

The core biopsy and clot specimens were fixed for at least 2 hours in 10% neutral buffered formalin. The core biopsy was then decalcified for 60 minutes in Formical 2000.

Frozen Tissue Available

No frozen tissue was available.

Details of Microscopic Findings

The peripheral blood smear was notable for rare circulating blasts and no significant dysplasia in neutrophils. The aspirate smear contained hypocellular spicules. Nucleated cells were predominantly small mature lymphocytes. Very few erythroid and granulocytic precursors were present – too few to evaluate dysplasia.

Megakaryocytes were predominantly small with hypolobated nuclei or separated nuclei. Blasts comprised 4% of total cells by morphology.

The clot section showed rare hypocellular particles with mostly stromal cells and rare small and hypolobated megakaryocytes.

The core biopsy showed markedly hypocellular marrow (<5% cellularity) with predominance of small, mature lymphocytes, few plasma cells, and markedly decreased trilineage hematopoiesis. A CD34 immunohistochemical stain demonstrated mildly increased scattered CD34-positive cells (5% of marrow cellularity). A CD61 immunohistochemical stain highlighted small and hypolobated megakaryocytes.

Immunophenotype

Concurrent flow cytometric immunophenotyping of the bone marrow aspirate demonstrated an abnormal myeloid blast population (1.5% of total events). The abnormal blasts were positive for weak CD2, CD7, CD13, CD33, CD34, CD38, bright CD45, CD56 (subset), CD71, CD117, CD123, and HLA-DR. They were negative for CD14, CD15, CD16, CD64, and additional lymphoid antigens.

Cytogenetics

44-46,XY,t(3;8)(q26.2;q24.2),-

7,add(11)(p13)[30].nuc ish(D5S721,EGR1)x2[188],(CEP7,D7S486)x1[83/200],(CEP8x2)[192],(D20S108x2)[190].

Cytogenetic analysis of the bone marrow aspirate revealed an abnormal male karyotype with a translocation between chromosomes 3q and 8q (involving breakpoints 3q26.2 and 8q24.2), loss of chromosome 7, and a rearrangement involving chromosome 11p.

The 3;8 translocation raised concern for *MECOM* rearrangement; subsequent *MECOM* break-apart FISH on interphase cells was positive for *MECOM* rearrangement in 31.0% of interphase cells.

Molecular Studies

Targeted next generation sequencing of 52 genes on the bone marrow aspirate demonstrated a pathogenic *PPM1D* mutation (VAF 17.2%), and likely pathogenic *GNAS* (VAF 47.9%), *KMT2A* (VAF 43.1%), and *TET2* (VAF 47.3%) mutations.

Proposed Diagnosis

Myelodysplastic syndrome with excess blasts (MDS-EB) (per ICC).

Acute myeloid leukaemia with *MECOM* rearrangement (WHO 5th edition).

Interesting Feature(s)

This case highlights challenges in diagnosis of myeloid neoplasms with a markedly hypocellular marrow. Pancytopenia, megakaryocytic dysplasia, and a complex karyotype supported a diagnosis of at least myelodysplastic syndrome. Blast enumeration in hypocellular marrows can be challenging, as illustrated in this case. CD34 immunohistochemistry showed mildly increased blasts but <10% of marrow cellularity.

The presence of non-classical *MECOM* rearrangement, t(3;8)(3q26.2;8q24.2), is uncommon. The presence of *MECOM* rearrangement with <10% blasts resulted in significantly discrepant initial diagnoses between the ICC and WHO 5th edition, with impact on initial treatment. The disease eventually evolved into AML by both classifications.

Panel Diagnosis: AML with *MECOM* rearrangement/MDS-EB

Hypoplastic Acute Myeloid Leukemia with Possible Germline Predisposition

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Case Description

A 41-year-old man initially evaluated in 2023 for intermittent abdominal pain with clinical suspicion for celiac disease was found to have thrombocytopenia, anemia, and vitamin B12 deficiency, with normalization of anemia following supplementation. The patient was lost to follow-up while residing outside the country but reported persistent fatigue. In late May 2025, he was found to have pancytopenia with neutropenia, and a bone marrow biopsy was subsequently performed.

Biopsy Fixation Details

10% buffered formalin

Frozen Tissue Available

N/A

Details of Microscopic Findings

Peripheral blood smear:

- Pancytopenia with rare circulating blasts.

Bone Marrow:

- Markedly hypocellular (10-30% cellularity) with decreased trilineage hematopoiesis
- Differential count of hemodiluted imprints showed increased blasts; small- to medium-sized blasts with high N/C ratio, finely dispersed immature chromatin, distinct nucleoli, and basophilic agranular cytoplasm

Immunophenotype

- By immunohistochemistry: CD34 and CD117 show increased blasts ($\geq 20\%$).

Flow Cytometry:

- 62% CD34+ myeloblasts.
- Immunophenotype:
- **Positive:** CD7 (subset), CD13, CD15 (subset), CD33 (decreased), CD34, CD117 (dim), CD123, HLA-DR.
- **Negative:** sCD3, cCD3, CD19, CD11c, CD64, cMPO.
- A population of plasmacytoid dendritic cells

Cytogenetics

46,XY,del(6)(p23p21)[18]/46,XY[2]

FISH:

- Loss of *DEK* at 6p22.3 [60/100]
- MDS panel: No abnormalities detected involving chromosomes 5, 7, 8, 17p13.1, 20q, or 3q.

Molecular Studies

- *FANCD2* c.2715+1G>A (VAF 45%), *RUNX1* p.D62Rfs*75 (VAF 21.6%), *TERT* p.P704S (VAF 48.5%), *U2AF1* p.S34F (VAF 20.9%)

Proposed Diagnosis

Hypoplastic acute myeloid leukemia(AML)

Interesting Feature(s)

- AML with hypocellular bone marrow presentation
- An initial molecular finding involving *TERT* raised concern for a telomere biology disorder (dyskeratosis congenita); telomere length analysis showed shortened telomeres, though contribution from leukemic cells could not be excluded

- Subsequent germline testing identified a heterozygous pathogenic *FANCD2* variant, raising consideration of an inherited DNA repair pathway abnormality and predisposition to myeloid neoplasia
- Despite achievement of MRD–negative remission, the post-treatment marrow demonstrated persistent multilineage dysplasia, raising consideration of an underlying myelodysplastic neoplasm
- Increased number of plasmacytoid dendritic cells, a finding reported in association with *RUNX1*-mutated myeloid neoplasms

Follow-up: The patient underwent induction therapy with FLAG-Ida in combination with venetoclax, achieving morphologic remission, followed by consolidation therapy. He achieved an MRD- and molecular–negative remission, with only the *FANCD2* variant remaining detectable by NGS. Subsequent bone marrow biopsy, however, demonstrated multilineage dysplasia without increased blasts, with recurrence of low-level somatic mutations. Ongoing therapy with planned hematopoietic stem cell transplantation.

Przychodzen B, et al. Fanconi Anemia germline variants as susceptibility factors in aplastic anemia, MDS and AML. *Oncotarget*. 2018 Jan 5;9(2):2050-7. PMID: 29416752.

Nie D, Zhang J, et al. Fanconi anemia gene-associated germline predisposition in aplastic anemia and hematologic malignancies. *Front Med*. 2022 Jun;16(3):459-66. PMID: 34741701

Maung KZY, et al. Rare variants in Fanconi anemia genes are enriched in acute myeloid leukemia. *Blood Cancer J*. 2018 Jun 1;8(6):50. PMID: 29891941.

Panel Diagnosis: AML-MR / AML with MR gene mutations associated with germline predisposition (telomere biology disorder)

EA4HP26-BMWS-141

DDX41-mutated MDS, running in the family

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Case Description

Woman born 1947, presented in 1998 with persistent neutropenia, later pancytopenia. Due to persistent cytopenias and pathological bone marrow, diagnosis of MDS was made in 2002, with normal female karyotype; molecular studies were not routinely performed at that time. The patient received transfusions, then EPO and G-CSF, followed by azacytidine as the disease progressed. Following a good clinical response, allogeneic SCT was performed in November 2008, with graft from patient's brother (born in 1955), resulting in remission. In 2019 patient's cytopenias recurred, and the diagnosis of MDS of donor cell origin was made (100% donor chimerism), with ensuing conservative treatment. In 2022 the patient developed diffuse large B-cell lymphoma in advanced stage, even this disease was of donor cell origin. The patient expired in May 2023. Patient's brother developed cytopenias, was diagnosed with MDS in hypoplastic bone marrow in 2020 and underwent alloSCT in 2023; he is now in remission. Detailed molecular studies were first performed in 2019, revealing germline *DDX41* mutation in both subjects, with somatic *DDX41*

variant T227M at MDS diagnosis. The latter variant, in cells of donor origin, was detected in index patient after SCT and in her lymphoma tissue

Biopsy Fixation Details

Zinc buffered formalin, formic acid decalcifier

Frozen Tissue Available

No

Details of Microscopic Findings

Bone marrow at MDS diagnosis (2002, submitted): biopsy with irregular distribution of hematopoietic tissue, cellularity of 0-40%; clot material with cellularity of 30%. In both increase of mononuclear cells, and pathological megakaryocytes. No ring sideroblasts. In BM smears aberrations in erythropoiesis (megaloblastic change, irregular nuclear contours), myelopoiesis (large metamyelocytes, vacuolated monocytes) and megakaryocytes (hypolobated forms or cells with separate cell fragments). BM smear: 2.4% blasts, 26% lymphocytes, 22% monocytes, 28% erythropoiesis. PB smear: leukopenia (61% lymphocytes, 34% neutrophils, 5% monocytes) and mild anemia.

MDS progress 2008: BM cellularity 40-90%, trilineage dysplasia, 3.8% blasts

MDS 2019 (donor-derived): BM cellularity 0-30%, multilineage dysplasia, decreased megakaryocytes, 3.6% blasts

Immunophenotype

Flow cytometry BM 2002: 3% blasts CD34+ including 2.5% myeloblasts, 5% precursors CD117+, 7% monocytes, 62% lymphocytes IHC: not done 2002. At MDS progress 2008: 3-8% blasts CD34+, unevenly distributed megakaryocytes

Cytogenetics

BM 2002: 46,XX

BM 2019 (donor-derived MDS): 46,XY,inv(3)(p13q26),-20,+mar[10]/46XY[10]

Molecular Studies

BM 2002: not done (in retrospect: *DDX41* E256K 49%, T227M 29%)

BM 2019: *DDX41* E256K 51%, T227M 10% (male karyotype)

Proposed Diagnosis

WHO: Myelodysplastic neoplasm, hypoplastic

ICC: Myelodysplastic syndrome, NOS, with multilineage dysplasia

Interesting Feature(s)

The case exemplifies course of myelodysplastic syndrome developing in a carrier of germline *DDX41*-mutation. Both the index patient and her brother carried the same germline *DDX41* variant E256K and acquired a somatic T227K variant at MDS diagnosis, at 55 yrs and at 65 yrs of age, respectively. Bone marrow was in both individuals hypocellular at MDS diagnosis, and the disease slowly progressing. After 11 years from SCT, index patient developed donor derived MDS, with hypocellular BM, and later also NHL of donor cell origin.

Panel Diagnosis: Hypoplastic MDS / MDS, NOS with germline *DDX41* and post-transplant donor-derived MDS

EA4HP26-BMWS-175

Hypoplastic Acute Myeloid Leukemia with *TP53* and *IDH1* mutations

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Case Description

A 57-year-old man with a history of splenectomy following a motor vehicle accident presented with weakness of 2-3 days. Laboratory evaluation revealed severe pancytopenia (hemoglobin 5.1 g/dL, platelets $7 \times 10^9/L$, WBC of $10.5 \times 10^9/L$, ANC $1.06 \times 10^9/L$) with 43% circulating blasts. A bone marrow biopsy (bmbx) was performed, revealing strikingly hypocellular marrow (<1% cellularity) with no increase in blasts despite the high peripheral blast burden. Imaging studies demonstrated no lymphadenopathy or hepatomegaly, excluding possibility of compensatory extramedullary hematopoiesis (EMH). Given the discordance between the peripheral blood (PB) findings, a repeat bmbx was obtained five days later. The second biopsy again demonstrated profound hypocellularity (<5%) with rare focal areas of slightly increased cellularity, comprised entirely of myeloblasts, confirming the diagnosis of acute myeloid leukemia (AML). The patient was treated with decitabine and venetoclax but died approximately 4 months after diagnosis.

Biopsy Fixation Details

10% neutral phosphate-buffered formalin

Frozen Tissue Available

No

Details of Microscopic Findings

At initial bmbx, the PB contained 57% circulating blasts. Morphologically, the blasts frequently exhibited deep nuclear invaginations or bilobed appearance. Background neutrophils were dysplastic. Both bone marrow biopsies showed profound hypocellularity, with the limited cellularity represented by stromal elements, histiocytes, lymphocytes, and plasma cells. Maturing trilineage hematopoiesis was absent. In the second marrow, focal areas of increased cellularity were observed, composed entirely of blasts, meeting criteria for hypoplastic AML.

Immunophenotype

Flow cytometry revealed myeloblasts expressing CD13, CD34, CD117, HLA-DR, and partial MPO, negative for CD14, CD33, CD56, CD64, cytoplasmic CD3, and other T/B cell markers. Immunohistochemistry (IHC) showed CD34 positive blasts with strong, diffuse P53 expression; NPM1 IHC was negative.

Cytogenetics

Karyotyping demonstrated a complex karyotype: 44~45,XY,-

3,del(5)(q23q35),del(7)(q22q34),der(10)t(3;10)(q24;q24),del(20)(q11.21q13.2)[cp7]). Microarray analysis also confirmed multiple copy number abnormalities. FISH was negative for *BCR::ABL*, *PML::RARA*, and *RUNX1*, *RUNX1T1*, *KMT2A*, and *CBFB* rearrangements.

Molecular Studies

Next-generation sequencing revealed pathogenic mutations in *IDH1* (p.R132C, VAF 21%), *TP53* (p.Y220C, VAF 33%; p.M246V, VAF 33%) and *DNMT3A* (p.W305*, VAF 34%).

Proposed Diagnosis

Hypoplastic AML, myelodysplasia-related (WHO-HAEM 5)

Hypoplastic AML with mutated *TP53* (ICC)

Interesting Feature(s)

This case illustrates a hypoplastic AML with profoundly low marrow cellularity despite high circulating blasts excluding the possibility of EMH. Repeat biopsies showed virtually absent hematopoiesis, highlighting the limitations of aspirates alone and the importance of repeat sampling, IHC, careful integration of clinical and radiologic findings, and molecular analysis. Hypocellular AML is biologically distinct from hypoplastic myelodysplastic syndrome (MDS) or aplastic anemia and typically harbor MDS-associated alterations and lack proliferative mutations such as *FLT3*, *RAS* pathway mutations, and *NPM1*, as seen here. In addition, this leukemia uniquely combined *TP53* mutations and complex karyotype with an *IDH1* mutation, a relatively uncommon combination. Morphologically, the blasts displayed pronounced nuclear invaginations, reminiscent of features described in *IDH2* R172- or *NPM1*-mutated AML or acute promyelocytic leukemia, but such morphology, to the best of our knowledge, has not been systemically characterized in *IDH1*-mutated AML.

Panel Diagnosis: AML-MR / AML with mutated TP53

Evolution of Myelodysplastic Neoplasm with Germline *DDX41* Mutation as a Sole Genetic Abnormality

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Case Description

An 80-year-old male with multiple pulmonary and cardiac diseases and prostatic adenocarcinoma had the first bone marrow (BM) biopsy on 8/4/2022 due to persistent anemia. There was no overt dysplasia on aspiration, and no increase in blasts by 500-cell differential count, flow cytometry analysis, or immunostains. Chromosome analysis revealed normal male karyotype. Molecular study by NGS identified a single pathogenic mutation: *DDX41*: Chr5(GRCh37):g.176940837_176940838del; NM_016222.2(*DDX41*):c.946_947del; p.Met316Aspfs*31 (47%). He received only intermittent packed red blood cell transfusion. On 9/17/2024, he underwent a second BM biopsy due to persistent anemia and newly onset of thrombocytopenia. Both aspiration and biopsy showed unremarkable morphology findings; and NGS revealed persistent *DDX41* mutation with VAF 49.6%. He developed pancytopenia including newly onset neutropenia, which was considered due to the sirolimus and tacrolimus. The neutropenia improved after adjusting the dosage of medicines. However, his continued fatigue and worsening pancytopenia prompted the third BM biopsy on 11/20/2025.

Biopsy Fixation Details

10% neutral buffered formalin with one-hour decalcification

Frozen Tissue Available

N/A

Details of Microscopic Findings

Peripheral blood (PB) and BM collected on 11/20/2025

The PB smear showed pancytopenia without morphological abnormalities. The BM showed hypocellular marrow. The M:E ratio was 2.5:1. Erythropoiesis was quantitatively and qualitatively normal. Granulopoiesis was normal with left-shifted maturation. The estimate of myeloblasts was 5.6% by 500-cell differential count. Lymphocytes were normal. Plasma cells were normal. Megakaryocytes were decreased without dysplasia.

Immunophenotype

The increased blasts are positive for CD34 and CD117 by flow cytometry analysis (8.69% by SSC/CD45 gating) and immunohistochemistry studies (10-15%). In addition, the flow cytometry analysis showed that blasts expressing CD13, CD33, CD38, CD45 (dim), CD56 (dim), HLA-DR, and negative for CD2, CD7, CD19, CD10, CD15, CD16, CD36, or CD64.

Cytogenetics

46, XY [20] and negative FISH for MDS panel in all three BM specimens.

Molecular Studies

Persistent single *DDX41* gene mutation c.946_947del by NGS study (VAF 47% in 2022, 49.6% in 2024 and 2025)

Proposed Diagnosis

Myelodysplastic neoplasm with increased blasts / Evolving acute myeloid leukemia with germline *DDX41* mutation.

Interesting Feature(s)

- The prevalence of germline *DDX41* mutations in patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) is between 2 to 8%, making *DDX41* mutations the most common germline predisposition for myeloid neoplasia.
- Unlike other germline predisposition syndromes, *DDX41* associated myeloid neoplasms (*DDX41*-MN) tend to arise in older individuals and have an incomplete penetrance of approximately 50%. *DDX41*-MNs have shown a faster rate of progression from MDS to secondary AML than in patients with wild type *DDX41*, specifically those

with truncating *DDX41* mutations. This patient's progression in BM findings and clinical course for the three-year duration illustrates the typical features of *DDX41*-MN.

- *DDX41*-MN with progression to MDS/ AML are often associated with additional somatic mutations or secondary cytogenetic abnormalities; however, our patient does not have any additional mutations or abnormalities on three bone marrow specimens beside the germline mutation.
- *DDX41*-MN with increased blasts is considered as indolent clinical course despite increased blasts and treated conservatively. However, our patient developed worsening symptoms and eventually started chemotherapy with modified regimens.

Panel Diagnosis: MDS-IB2 / MDS/AML with germline *DDX41*

EA4HP26-BMWS-199

Hypoplastic MDS in a germline *DDX41* mutation carrier

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Case Description

Woman born 1965, previously healthy. Isolated neutropenia was accidentally discovered in 2020 while the patient was investigated for sicca symptoms. Leukocytes $1,8 \times 10^9/L$, neutrophils $0,9 \times 10^9/L$. No previous infections. No lymphocytosis, negative for ANA, no signs of inflammation. Other blood values were normal, no macrocytosis and therefore clinically suspected to be autoimmune neutropenia rather than MDS. A first bone marrow biopsy in 2021 showed a cellularity of 50%, no significant dysplasia. Genetic analysis showed a *DDX41* variant of unclear significance with a VAF of 50%.

In 2022 slight macrocytic anemia developed (Hemoglobin 116 g/L) and also thrombocytopenia ($146 \times 10^9/L$). In 2023 a new bone marrow sampling (the submitted biopsy) showed slightly hypocellular bone marrow (30%) with morphologically atypical megakaryocytes and erythropoiesis. Genetic analysis showed normal female karyotype and the same variant in *DDX41*. Monosomy 7 was excluded.

In 2023-2024 gradually more anemia (Hemoglobin 113 g/L) and thrombocytopenia ($119 \times 10^9/L$) and neutrophils $0,7 \times 10^9/L$. Genetic analysis in 2023 showed that the *DDX41* variant was germline and a new, pathogenic, somatic variant in *DDX41* was discovered as well as a small pathogenic *TP53* mutation.

Biopsy Fixation Details

Buffered formalin

Frozen Tissue Available

No

Details of Microscopic Findings

Variable cellularity, in average 30%. No fibrosis. Iron positive, no ring sideroblasts. In bone marrow smears the granulocytopoiesis is 43% with 1% blasts and no dysplasia. Erythropoiesis is 29% with morphological dysplasia. Megakaryocytes small with monolobated nuclei.

Immunophenotype

CD34 <5% positive cells, p53 2% positive nuclei

Cytogenetics

Normal female karyotype, no monosomy 7

Molecular Studies

DDX41 c.736C>G; p(Gln246Glu); 48% in bone marrow and 50% in peripheral blood T-cells

DDX41 c.679A>G; p(Thr227Ala); 4% in bone marrow, < 0,7 in T-cells

TP53 c1146del; p(Lys382Asnfs*40); 2% in bone marrow, <0,7% in T-cells

Proposed Diagnosis

Hypoplastic MDS in a germline *DDX41* mutation carrier

Interesting Feature(s)

Germline mutations of *DDX41* promote the development of MDS and AML. Patients with germline mutations of *DDX41* may have a normal karyotype and the most frequent somatic mutation is a second *DDX41* mutation as in this case.

Panel Diagnosis: MDS-LB, MDS, NOS with germline *DDX41*

EA4HP26-BMWS-215

Hypoplastic MDS with features reminiscent of serous atrophy/gelatinous bone marrow transformation

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Case Description

- 42-year-old female with a history of normal counts presents with fatigue and pancytopenia
- WBC 2.7 k/uL, HGB 6.4 g/dL, HCT 19.8%, MCV 87.7 fL, platelets 56 k/uL
- No evidence of autoimmune disease or vitamin deficiency
- No history of cytotoxic chemotherapy, drugs, or excess alcohol
- No history of malnutrition or weight loss
- Two dry tap marrow biopsies were performed at an OSH without definitive diagnosis
- A third marrow biopsy was performed at our institution

Biopsy Fixation Details

10% neutral buffered formalin, decalcified

Frozen Tissue Available

No frozen tissue

Details of Microscopic Findings

The marrow was inaspirable and roll preparations of the cores showed scant cells

Marrow core biopsies showed a generalized loss of adipocytes and hematopoietic cells in a pattern reminiscent of serous atrophy/gelatinous transformation

Rare residual erythroid islands were present without definitive dysplasia, but erythroid dysplasia difficult to assess without aspirate smears

Scattered megakaryocytes and loose clusters of megakaryocytes with dysplastic nuclei were present

No increase in blasts on CD34 or CD117 immunohistochemistry
No evidence of increased or abnormal T cell infiltrate on CD3 immunohistochemistry

Mild diffuse reticulin deposition present, no fibrosis on trichrome stain

Immunophenotype

Marrow flow shows limited events with no convincing evidence of an abnormal blast population

Cytogenetics

Karyotype: 47,XX,+8,t(8;8)(q23;q24)[20]/46,XX[10]

FISH: Abnormal with one copy gain of centromere 8 in 67% of cells

Molecular Studies

Positive for clinically significant variants:

U2AF1 (p.S34Y, NM_001025203.1:c.101C>A, VAF 16%)

TP53 (p.R213Q, NM_000546.5:c.638G>A, VAF 3%)

No clinically significant variants were otherwise detected in other targeted genes.

Variant of unknown clinical significance:

U2AF1 (p.C154S, NM_001025203.1:c.460T>A, VAF 19%)

Proposed Diagnosis

Hypoplastic myelodysplastic neoplasm (hMDS) in the World Health Organization (WHO) classification of hematolymphoid tumors 5th edition

International Consensus Classification (ICC) also classifies hypoplastic MDS (MDS-h) as a distinct subtype

Interesting Feature(s)

This hypocellular MDS showed **features reminiscent of serous atrophy/gelatinous transformation**

Nakanishi et al. reported similar findings where hypoplastic MDS was diagnosed on a follow up marrow after an initial marrow showed prominent gelatinous transformation

This suggests that hypoplastic MDS should be a consideration when a marrow biopsy shows features of serous atrophy/gelatinous transformation, especially when the clinical setting is not typical of serous atrophy

Unusual karyotype, 47,XX,+8,t(8;8)(q23;q24)[20]/46,XX[10]

Translocation t(8;8) is not known to be a characteristic finding of any hematological neoplasms, but t(8;8) with variable breakpoints have been reported in solid tumors

Our case showed a p53 mutation but the frequency was low and would **not merit classification as a p53 related MDS**

No specific mutations or patterns of mutations have been associated with hMDS

In hMDS the mutations that are present are usually detected at lower variant allele frequencies (VAFs), likely due to the decreased cellularity of sampled marrow

Panel Diagnosis: Hypoplastic MDS / MDS, NOS

EA4HP26-BMWS-221

Hypoplastic Myelodysplastic Syndrome Associated with a Rare Germline *TERT* Variant

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Case Description

A 38-year-old male presented with an upper respiratory infection, profound fatigue, dyspnea on exertion, and palpitations. Subsequent evaluation revealed severe pancytopenia

On current admission, viral studies for HSV-1/2, CMV, and parvovirus were negative. Testing for paroxysmal nocturnal hemoglobinuria was negative. Given the low counts, he received Filgrastim and was transfused 2 units of platelets and 3 units of packed RBC. Bone marrow biopsy and flow cytometry were performed.

Biopsy Fixation Details

2 mL of bone marrow aspirate was collected in a lavender-top tube, smeared onto slides, and stained with Wright's stain.

One core bone marrow biopsy (1.0 cm) was received in formalin and fixed in B5. Subsequently, decalcified, embedded in paraffin, sectioned, and stained with routine H&E.

Frozen Tissue Available

Not available

Details of Microscopic Findings

The peripheral smear shows marked anemia. The platelet count is markedly low with occasional giant forms. The white cell count is decreased with neutrophils hypogranular and hypolobated.

The bone marrow smears show "The erythroid series shows dysplastic changes, including nuclear-cytoplasmic asynchrony and bi-nucleated forms. Neutrophils show Pelgeroid forms. Megakaryocytes are markedly decreased with small hypolobated forms."

The bone marrow core biopsy shows "Hypocellular marrow with an approximate cellularity of 20-30%. The myeloid series is left-shifted and shows slightly increased blasts. Erythropoiesis is decreased. Megakaryocytes are markedly decreased with small hypolobated forms. Plasma cells are increased, scattered and in small clusters. Background contains mild fibrosis. Iron stores are increased, as indicated by the iron stain. Aspirate clot was aparticle. Reticulin staining highlights mild fibrosis."

Immunophenotype

CD34 immunostain highlights increased blasts, accounting for 6-8% of the cellularity

The flow cytometry of the bone marrow showing "The CD45-dim population (10.1% of the total) consists of an abnormal blast population (6% of the total) which is positive for CD11b(dim), CD13, CD33 (dim), CD34 (bright), CD38, CD64, CD117, and HLA-DR."

Cytogenetics

Karyotype: 46,XY,+1,der(1;7)(q10;p10)[19]/46,XY[1]

FISH: ABNORMAL results with deletion 7q and trisomy 8.

Molecular Studies

1. NGS molecular detected mutation of *IDH1*, *RUNX1*, *PHF6*, *ETV6*
2. Skin biopsy tissue culture : Sanger sequencing detected a c.421C>G (p.L141V) variant in the *TERT* gene.
3. Telomere length Assay: The patient's Telomere length was very low (<1st percentile) compared with the normal median Telomere Length at age

Proposed Diagnosis

Hypoplastic Myelodysplastic Neoplasm with increased blast 1 (MDS-IB-1), arising in the context of a germline *TERT* variant

Interesting Feature(s)

Germline pathogenic variants in the *TERT* (telomerase reverse transcriptase) gene are associated with telomere biology and predispose individuals to conditions such as dyskeratosis congenita, pulmonary fibrosis, and various solid tumors, as well as the development of myelodysplastic syndromes (MDS).

In our case, the patient did not exhibit any phenotypic manifestations of dyskeratosis congenita; instead, they presented with hypoplastic MDS in their second decade.

The Germline ***TERT* p.Leu141Val** missensevariant detected in our patient has not been previously reported as a pathogenic variant-phenotype association in the genomic database or published literature

Shorter telomere length in pre-transplant blood is independently linked to increased mortality in MDS. Our patient's telomere length was less than 1 percentile of the matched telomere length at the matched age

Panel Diagnosis: MDS-IB1 / MDS-EB with germline *TERT*

Hypocellular, MECOM-rearranged myeloid neoplasm

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Case Description

A 65-year-old male, with a 6 month-history of anemia and thrombocytopenia. Blood cells count at admission in the Hematology Department: WBC 2850/mm³ (N 1580, Ly 1140, Eo 0, Mo 0), Hb 9.5 g/dL, Plt 47000/mm³.

Bone marrow aspirate (BMA) deemed inadequate (highly hypocellular, with few dysplastic cells and no blast); flow cytometry, unremarkable. Bone marrow biopsy (BMB) consistent with hypocellular myeloid neoplasm, featuring trilinear morphologic dysplasia and very low blast count, except for a scattered CD61+ small cells consistent with megakaryoblast. Full karyotype demonstrated t(3;21)(q26;q22), consistent with MECOM rearrangement. Follow up: the patient received upfront myeloablative haploidentical hematopoietic cell transplantation (HCT), complicated by grade 2 acute graft versus host disease responsive. At last follow up (+3 months from HCT), complete remission, with normal karyotype and full donor chimerism.

Biopsy Fixation Details

10% buffered formalin fixed, EDTA decalcified

Frozen Tissue Available

No

Details of Microscopic Findings

BMA: at diagnosis, paucicellular. Pretransplant, myeloid and erythroid dysplasia; scattered rare megakaryocytes; 6% monocytoïd cells; 2% agranular blasts.

BMB: 5-10% cellularity, with morphologic atypia mostly appreciated in the thrombopoietic lineage, including small-sized, hypolobated megakaryocytes and CD61+, scattered and clustered small cells, consistent with megakaryoblasts (4-5% of nucleated cells).

Immunophenotype

Flow cytometry: unremarkable.

BMB: 4-5% CD61+/CD34-/CD117- megakaryoblasts; 1% CD34+/CD117+ myeloid precursors.

Cytogenetics

46,XY,t(3;21)(q26;q22)[21]/47,idem,+8[3]/46,XY[1]

Molecular Studies

Targeted NGS (DNA): DNMT3A p.(Arg882Cys) VAF 29.3%; TET2 p.(Ser1286Phe) VAF 4.7%.

Proposed Diagnosis

Hypoplastic myeloid neoplasm with MECOM rearrangement and megakaryoblastic features.

Interesting Feature(s)

The differential diagnosis between hypocellular myelodysplastic syndromes (hMDS) or hypocellular acute myeloid leukemia (hAML), including pauciblastic variants, and non-clonal bone marrow hypoplasia remains inherently challenging, due to overlapping clinical and morphologic features. Hypocellularity reduces diagnostic yield from conventional morphology, as dysplastic changes may be subtle and blast count inconspicuous. Moreover, pauciblastic AML may lack the definitive excess of blasts expected in classical presentations, further complicating distinction from hypocellular states. Key diagnostic features in the current case include megakaryocyte atypia on BMB, with increased megakaryoblasts, and molecular features, including CHIP-like mutations at NGS and disease defining (MECOM-rearranged [MECOM-R] AML) at karyotype. As well, megakaryoblastic differentiation is expected in a subset of MECOM-R AML.

Given the hypocellularity and the low blast count, no AML-like induction treated was deemed necessary by the clinical team and an upfront myeloablative transplant strategy was preferred.

The diagnosis of AML with leukemia-defining genetic alterations in the setting of a blast count <20% raises substantial conceptual and practical challenges. Current WHO and ICC classifications emphasize that specific genetic lesions, including MECOM-R, enable a diagnosis of AML, although it's not fully clear whether a <10% blast percentage warrants such a conclusion. In the specific setting of MECOM-R myeloid neoplasm, a recent series [PMID 40983567] highlighted that a threshold <20% blasts identifies cases with better 3-year OS, but still with a 55% 3-year OS for < 5% blast count cases.

Panel Diagnosis: Hypoplastic MDS / MDS, NOS

EA4HP26-BMWS-245

ELANE-Driven Cytopenia: A Gateway to Hypoplastic MDS and Amagakaryocytic Thrombocytopenia Associated with Monosomy 7 Acquisition

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Case Description

A 34-year-old Asian man, presented with heavy gum bleeding after dental procedure. He was found to have significant severe pancytopenia: (WBCx10³/ul:2.2, Hb g/dl: 8.8 and platelets of 42: x10³/ul) and severe reticulocytopenia (16.0x10⁹/L). First BM biopsy was inadequate for morphologic assessment with normal karyotype.

Biopsy Fixation Details

Formalin-fixed, paraffin-embedded, and decalcified using standardized protocols.

Frozen Tissue Available

NA

Details of Microscopic Findings Peripheral blood shows moderate macrocytic anemia, mild neutropenia and marked thrombocytopenia.

Bone marrow (BM) aspirate is partially hemodilute and shows preserved granulopoiesis with orderly maturation and significant dysgranulopoiesis mostly small hypolobated forms. Erythropoiesis is prominent with significant dyserythropoiesis, mostly represented by intracytoplasmic bridging, increased early precursors, cytoplasmic vacuolation and megaloblastic chromatin. No megakaryocytes seen with no increase in blasts. Iron stain showed increased iron stores with increased sideroblasts and no ring sideroblasts.

BM biopsy is hypocellular for age, roughly estimated at 20-30% alternating with few relatively cellular patches with decreased granulopoiesis, relatively prominent erythropoiesis and markedly suppressed megakaryopoiesis

Immunophenotype

IHC: No increase in CD34 positive precursors.

CD117 immunostain: highlighted increased mast cells (with no clusters).

Flow cytometry: rare cells with PNH phenotype in granulocytes (0.05%). No increase in blasts and no immunophenotypic evidence of monoclonal B-cells

Cytogenetics

Karyotype : On first BM: Normal male KT.

Karyotype on the second BM: 45,XY,-7[13]/46,XY[7]

Molecular Studies

Next generation sequence: No clinically significant variants identified using a targeted panel of thirty-three genes associated with myeloid neoplasia and forty-three fusion genes.

Whole genome sequencing was initiated to assess for inherited bone marrow failure and revealed ELANE germ line mutation.

Proposed Diagnosis

Myeloid neoplasm with germ line predisposition: ELANE-related bone marrow failure evolving into hypoplastic MDS with monosomy 7

Interesting Feature(s)

ELANE-related cytopenias commonly manifest as isolated severe Congenital Neutropenia or Cyclic Neutropenia. These conditions are characterized by a distinct maturation arrest of the myeloid lineage, typically occurring at the promyelocyte or myelocyte stage. However, these distinctive morphologic features are not encountered in the case presented here.

This case exemplifies a rare phenotype of ELANE-related cytopenia transitioning into hypoplastic MDS, associated with the acquisition of monosomy 7 and accompanied by amegakaryocytic thrombocytopenia. Instances of ELANE-associated amegakaryocytic hypoplasia, aplastic anemia or pancytopenia are infrequently reported in the literature. ELANE mutation was listed in the WHO classification (2022) in association with congenital neutropenia with no definitive correlation with MDS or AML. We propose that a somatic acquisition of monosomy 7 occurred in a patient with an ELANE germline mutation, led to mix phenotype with overlapping features between hypoplastic MDS and amegakaryocytic thrombocytopenia.

Panel Diagnosis: MDS-LB / MDS, NOS with germline ELANE

EA4HP26-BMWS-277

Hypocellular/hypoplastic Myelodysplastic syndrome with germline *DDX41* mutation

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Case Description

A 70-year-old Asian man with a history of hypertension, asthma, hyperlipidemia, primary hypertriglyceridemia, and diabetes first presented approximately 10 years ago with mild cytopenias, including thrombocytopenia (~120 K/ μ L) and neutropenia (~1.2 K/ μ L). Over subsequent years, he developed progressive cytopenias with macrocytic anemia (hemoglobin 12.8 g/dL, MCV 101 fL), neutropenia (0.9×10^9 /L), and thrombocytopenia (110×10^9 /L), without constitutional symptoms or hepatosplenomegaly. Given his stable clinical status, he was initially monitored.

In June 2024, worsening neutropenia prompted repeat bone marrow evaluation. CBC showed hemoglobin 10 g/dL, MCV 103 fL, WBC: 1.3×10^9 /L, neutrophils 0.46×10^9 /L, and platelets 79×10^9 /L.

Biopsy Fixation Details

The core biopsy is formalin fixed and decalcified in Formical 2000 for one hour.

Frozen Tissue Available

None

Details of Microscopic Findings

The bone marrow showed a variably hypocellular marrow (0–20% cellular) with erythroid hyperplasia (M: E 1:4), marked dyserythropoiesis (megakaryoblastoid change, nuclear budding), left-shifted granulopoiesis, and approximately 7% blasts.

Immunophenotype

Flow cytometry identified 6.1% myeloid blasts with a mildly atypical phenotype expressing CD13, CD33, CD34, variable CD38, CD117, HLA-DR, and weak-variable CD7 (subset).

Cytogenetics

Abnormal male karyotype; 46,XY,del(13q)(q14q22)[1]/46,XY[19]

Molecular Studies

Molecular studies identified somatic *KRAS* (V14I, 3.8%), *NRAS* (G12D, 1.0%), *TP53* (Y220C, 1.7%), and *DDX41* T377I (14%), and a possible germline mutation in *DDX41* E256K (47.5%).

Proposed Diagnosis

Hypocellular/hypoplastic myelodysplastic syndrome associated with germline *DDX41* mutation

Interesting Feature(s)

Germline *DDX41* mutations are associated with late-onset MDS/AML; *DDX41*-mutated myeloid neoplasms are recognized as a distinct entity by both WHO 5th edition and ICC classifications. While truncating germline variants confer higher risk of leukemic progression, non-truncating germline variants such as E256K, reported more frequently in Asian populations, are associated with progression rates similar to *DDX41*-wild-type disease. Co-occurring mutations, including RAS pathway genes and *TP53*, generally do not adversely impact outcomes. Earlier molecular testing in this patient failed to detect *DDX41* variants due to limited gene coverage, highlighting the importance of including germline predisposition genes in NGS panels for hematologic malignancies.

Panel Diagnosis: MDS-IB1 / MDS-EB with germline *DDX41*

EA4HP26-BMWS-323

Hypoplastic Myelodysplastic Syndrome Associated with Germline *GATA2* Mutation: When Subtle Cytopenias Signal Inherited Risk

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Case Description

A 38-year-old Bangladeshi patient, with history of recurrent UTI, pneumonia, referred to hematology for evaluation of severe neutropenia. Leukopenia and neutropenia are fluctuating between (1.4-2.6) and (0.4-1.3), respectively with no clear cyclic pattern recognized. Thrombocytopenia is subtle and fluctuates between 122-150 x10³/μl. There is consistent severe monocytopenia (0.0 x10³/uL). No family history of myeloid malignancy. Bone marrow examination was done to assess cytopenia

Biopsy Fixation Details

The trephine biopsy was formalin-fixed, paraffin-embedded, and decalcified using standard laboratory protocols.

Frozen Tissue Available

N/A

Details of Microscopic Findings

Peripheral smear shows marked neutropenia with few hyposegmented neutrophils. It was noted that lymphocytes including large granular lymphocytes are smaller than usual.

Bone marrow aspirate is hypocellular. Megakaryocytes exhibit significant dysmegakaryopoiesis; in the form of hypolobated forms and forms with widely separated nuclear lobes. Erythropoiesis is relatively increased and exhibit

significant dyserythropoiesis (karyorrhexis, bridging, nuclear budding, cytoplasmic vacuolation and nucleocytoplasmic asynchronism). Granulopoiesis with evident dysgranulopoiesis (hyposegmentation, hypogranulation, vacuolated cytoplasm, abnormal segmentation and abnormal chromatin pattern). Blasts are not increased (<1%). Deeper core biopsy sections revealed multiple hypocellular areas ranging from <5 -<25% with suppressed trilineage hematopoiesis, relatively prominent erythropoiesis with prominent small hypolobated megakaryocytes seen. No lymphoid aggregates noted. No increase in CD34-positive precursors.

Immunophenotype

Flow cytometry shows approximately 1% B-cells with no definitive immunophenotypic evidence of a monoclonal B-cell population. T-cells comprise 37% T-cells with increased CD4/CD8 double-negative gamma/delta T-cells (4%) and increased proportion of CD57-positive T-LGL (30% of T-cells). NK cells comprise approximately 6%. Decreased granulocytes at 37% and monocytes at 2%. Blasts are <1%. No evidence of PNH clone based upon analysis of a variety of GPI-linked antibodies on red blood cells, granulocytes and monocytes.

Cytogenetics

Karyotype: 46,XY,+1,der(1;7)(q10;p10)[19]/45,XY,-7[3]/46,XY

Molecular Studies

NGS: No clinically significant variants identified using a targeted panel of thirty-three genes associated with myeloid neoplasia and forty-three fusion genes.

Whole genome sequencing: GATA2 mutation

Proposed Diagnosis

Myeloid neoplasm with germ line predisposition-hypoplastic MDS.

Interesting Feature(s)

Mutations in *GATA2* causing *GATA2* deficiency is linked to variable cytopenias, BM failure, and myeloid neoplasms associated with germ line predisposition (MDS/AML). In this case, symptoms appeared during adulthood and the degree of cytopenia was not pronounced enough to prompt a hematology referral. This case emphasizes the importance of maintaining a strong suspicion and screening for germline disorders even when cytopenia is mild but accompanied by notable symptoms, especially recurrent infections resulting from severe monocytopenia and impaired neutrophil function. Although the diagnostic criteria for hypoplastic Myelodysplastic Neoplasm (WHO 2022) were only partially met during bone marrow evaluation, the clinical presentation and pathological findings align with hypoplastic myelodysplastic neoplasm which also confirmed by cytogenetic findings. Detection of monosomy 7 and der(1;7)(q10p10) and bone marrow hypocellularity raised the suspicion of possible germ line predisposition and prompt testing.

Panel Diagnosis: Hypoplastic MDS / MDS, NOS with germline *GATA2*

EA4HP26-BMWS-330

Uncertain bone marrow findings in a patient with heterozygous germline *FLT3* mutation

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Case Description

A 42-year-old man with no relevant history came to our hospital for a two-year-history of anemia and thrombopenia (Hb 2.9 g/dL, platelet $18 \times 10^3/\mu\text{L}$ at presentation). He also presented hemorrhoidal bleeding with high-grade intraepithelial dysplasia. Splenomegaly of 15 cm was detected, without evidence of liver disease. Autoimmune, immunodeficiency, and infectious etiologies were excluded. He did not response to either Immunoglobulins nor corticosteroids nor eltrombopag. Ascites, hepatomegaly, and non-cirrhotic portal hypertension developed, and a liver biopsy was performed. By the moment, the patient continues to exhibit marked cytopenias, and he is receiving transfusions and treatment for portal hypertension, waiting for stem cell bone marrow transplantation.

Biopsy Fixation Details

Bone marrow tissue sample was fixed in 10% neutral buffered formalin and embedded in paraffin. Fixation time was 24-28 hours. Formol 10%.

Frozen Tissue Available

Yes

Details of Microscopic Findings

-Bone marrow smears showed hypocellularity without dyserythropoiesis, reduced but morphologically normal megakaryocytes, and granulocytic preserved maturation, not suggestive of dysplasia.

-Bone marrow biopsy showed one hypocellular area and other normocellular. In the latter, there was an increase of erythroid lineage with paratrabecular location. Myeloid series preserved. Megakaryocytes were decreased with presence of small hypolobulated megakaryocytes. There was an increased dense reticulin framework with intersections (MF1-2).

-Liver biopsy showed preserved architecture, and extramedullary hematopoiesis obliterating the liver sinusoids along with mild perisinusoidal fibrosis.

Immunophenotype

-Flow cytometry study showed no abnormalities in granulation. Myeloid precursor cells accounted for 0.11%. Mature monocyte population was observed with normal proportion of non-classical forms.

-Immunohistochemistry of bone marrow biopsy showed no increase in CD34+ blasts, highlighting intrasinusoidal hematopoiesis. CD61 highlighted small megakaryocytes. CD61 and CD71 highlighted extramedullary hematopoiesis within the sinusoids of the liver.

Cytogenetics

Cytogenetic showed a normal karyotype with no structural abnormalities.

Molecular Studies

-Next generation sequencing study (Haematology OncoKit Dx) identified a pathogenic variant in *FLT3* gene (FLT3Q577*; Variant c.1729C>T; VAF 47%), which was also detected in hair follicle, confirming its germline origin.

-Whole exome sequencing looking for genes related to storage disorders, congenital bone marrow failure syndromes, and telomeropathies showed no abnormalities.

Proposed Diagnosis

Histologic findings were suggestive of hypoplastic myelodysplasia with fibrosis. However, multidisciplinary committee review (including cytologic and flow analyses) did not support a diagnosis of myelodysplastic syndrome (MDS), favoring reactive findings.

Interesting Feature(s)

-We submit a case of a young male with histologic findings suggestive of myelodysplasia with myelofibrosis. Germline *FLT3* variants have been reported to be associated in the current literature with aplastic anemia and a spectrum of myeloid disorders, including MDS.

-Extramedullary hematopoiesis of liver sinusoids and perisinusoidal fibrosis are reported in myeloid pathologies. This, together with the patient's evolution, supports the pathological condition of this case to our criteria. However, a diagnosis of MDS could not be conclusively established. We would greatly appreciate the expert committee's opinion.

Panel Diagnosis: Bone marrow with expanded erythropoiesis and fibrosis, favor reactive

Germline *DDX41*-mutated myelodysplastic syndrome

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Case Description

-A 71-year-old man was referred to our hospital in July 2025 for evaluation of mild normochromic anemia (hemoglobin 10.8 g/dL) persisting for one year, thrombocytopenia (platelet count 92,000/μL), and neutropenia (1,000/μL). He had a history of colorectal adenocarcinoma diagnosed in 2018, treated with chemotherapy and radiotherapy. The patient remains in remission to date.

-Treatment with azacitidine in combination with venetoclax was initiated in July 2025, and the patient actually continues to exhibit mild cytopenias.

Biopsy Fixation Details

-Bone marrow tissue sample was fixed in 10% neutral buffered formalin and embedded in paraffin. Fixation time was 24-28 hours. Paraffin embedded tissue. Formol 10%.

Frozen Tissue Available

No

Details of Microscopic Findings

-Bone marrow smears were insufficient for diagnostic evaluation. Nevertheless, 6% blasts with myeloid appearance were observed.

-The bone marrow biopsy was mildly hypercellular for the patient's age. An increased megakaryocytic lineage with frequent hypolobated forms was observed. The myeloid-to-erythroid ratio was preserved, with numerous immature forms. There was a mild increase in reticulin fiber network (MF1).

Immunophenotype

-Flow cytometry study did not detect abnormal phenotype of hematopoietic cells and CD34+ myeloid precursor cells account for 0.35%.

-By immunohistochemistry, approximately 15% of CD34+/CD117+/MPO+/CD123- blasts were identified. In addition, a focal population of cells with round nuclei and elongated cytoplasm was observed, expressing CD123, SPIB, and BCL2, and lacking expression of CD34 and MPO. CD61 staining highlighted the increased number of hypolobated megakaryocytes.

Cytogenetics

-An insufficient number and quality of metaphases were obtained to perform the cytogenetic study.

Molecular Studies

-Next generation study was performed (Haematology OncoKit Dx) and pathogenic variants were identified in *TET2* (p.Tyr1661Ter; VAF 11.21%), *DDX41* (p.Arg525His; VAF 5.01% and p.Gly173Arg; VAF 44.55%), *JAK2* (p.Val617Phe; VAF 3.07%), and *ASXL1* genes (p.Gly646TrpfsTer12; VAF 6.65%).

-The *DDX41* p.Gly173Arg variant was also detected in hair follicle analysis, confirming its germline origin.

Proposed Diagnosis

-The case was reviewed by a multidisciplinary committee, and a definitive diagnosis of MDS/AML was proposed according to the ICC 2022; and MDS with increased blasts according to WHO 5th edition.

-Both classifications incorporate considerations of the patient's prior therapy-related exposure and underlying germline predisposition.

-The presence of a second possible blastic plasmacytoid dendritic cell neoplasm (BPDCN) component was suggested but could not be confirmed.

Interesting Feature(s)

-We present a case of myeloid neoplasm associated with germline *DDX41* mutation. The case displays the typical late-onset presentation and carries the recurrent somatic p.R525H *DDX41* mutation.

-The patient also had a previous history of colorectal adenocarcinoma. Notably, solid tumors occur in approximately 10% of individuals with germline *DDX41* mutations, the majority of which precede the hematologic neoplasm, which also aligns with our case.

-Approximately 20% of BPDCN cases are associated with a concomitant myeloid neoplasm of shared clonal origin. Although a recent work linked a mature plasmacytoid dendritic cell neoplasm to a myeloid neoplasm within a germline *DDX41* mutation, no previous cases of BPDCN in this context has been reported. We would greatly appreciate the expert committee's opinion regarding the possible presence of this second component.

Panel Diagnosis: MDS-IB1, post cytotoxic therapy / MDS-EB, therapy-related with germline *DDX41*

EA4HP26-BMWS-368

Hypoplastic MDS with monosomy 7, presented acutely with severe pancytopenia

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Case Description

A 29-year-old Asian man with no chronic illness presented with 10 days of dizziness, exertional shortness of breath, and palpitations, along with intermittent gum bleeding over 6 months. CBC showed severe pancytopenia (WBC $3.1 \times 10^3/\mu\text{L}$, ANC $0.8 \times 10^3/\mu\text{L}$, Hgb 4.9 g/dL, Platelets $26 \times 10^3/\mu\text{L}$, Retics $40.9 \times 10^3/\mu\text{L}$). Bone marrow examination was performed to rule out marrow pathology.

Biopsy Fixation Details

The trephine biopsy was formalin-fixed, paraffin-embedded, and decalcified using standard laboratory protocols.

Frozen Tissue Available

No frozen tissue was available

Details of Microscopic Findings

The peripheral smear reveals severe pancytopenia along with marked dysgranulopoiesis, most notably prominent hyposegmented neutrophils. The bone marrow aspirate is hemodilute, but a few small hypolobated cells are observed. Erythropoiesis is moderately increased overall and displays significant dyserythropoiesis, (nuclear karyorrhexis, cytoplasmic irregularities, internuclear bridging, and cytoplasmic inclusions). Granulopoiesis is moderately reduced and demonstrates dysgranulopoiesis, including hyposegmentation or abnormal segmentation and hypogranulation. Blasts are prominent, accounting for approximately 4%. The BM biopsy is hypocellular for age, estimated roughly at 20-30% with relatively increased erythropoiesis, decreased granulopoiesis and markedly suppressed megakaryopoiesis. No significant increase in reticulin fibers.

Immunophenotype

Flow cytometry on bone marrow sample shows approximately 3% CD34-positive blasts, 32% T-cells and 5% B-cells with no definitive immunophenotypic evidence of a monocytic B-cell population.

IHC: CD34 immunostain highlighted increased CD34 positive cells, roughly estimated at 7-10%

No phenotypic evidence of paroxysmal nocturnal hemoglobinuria (PNH).

Cytogenetics

Conventional karyotyping revealed a mosaic clone:

Karyotype: 45,XY,-7[33]/46,XY[2]

Molecular Studies

Pending

Proposed Diagnosis

Hypoplastic MDS with monosomy 7

Interesting Feature(s)

Hypoplastic MDS is defined by reduced bone marrow cellularity (i.e., <25% or an inappropriately reduced cellularity for their age in young patients). Their diagnosis is still an object of debate.

Hypoplastic MDS (MDS-h) that is more recently being proposed as a distinct entity in the World Health Organization (WHO) 2022 proposal for myeloid neoplasms. MDS-h particularly those associated with monosomy 7, shares several features with aplastic anemia (AA) and paroxysmal nocturnal hemoglobinuria (PNH), thus, it needs a careful diagnostic approach. Two different hMDS phenotypes may be encountered: one featured by inflammation and immune activation, with increased cytotoxic T cells, increased T and B regulatory cells, and better response to immunosuppression; and the other, resembling MDS, where T and B regulatory/suppressor cells prevail, leading to genetic clonal selection and an increased risk of leukemic evolution. The case presented here showed acute presentation with severe anemia and bleeding episodes.

Panel Diagnosis: MDS-LB /MDS, NOS

EA4HP26-BMWS-392

Aleukemic granulocytic sarcoma associated with a germline ITPKB mutation

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Case Description

-A 20-year-old male was diagnosed in 2024 with primary mediastinal B-cell lymphoma (PMBCL) and achieved complete remission following R-DA-EPOCH therapy.

-One year later, he presented asymptomatic nodular lesions involving the trunk, scalp, and upper extremities. The clinical differential diagnosis included insect bite reaction, lymphoma relapse, and blastic plasmacytoid dendritic cell neoplasm. An excisional cutaneous biopsy was performed, and the diagnosis of granulocytic sarcoma was made.

-Subsequent bone marrow and staging studies showed no alterations. The patient initiated FLAG-Ida therapy and is currently awaiting hematopoietic stem cell transplantation.

Biopsy Fixation Details

-Paraffin embedded tissue. Formol 10%.

- Bone marrow tissue sample was fixed in 10% neutral buffered formalin and embedded in paraffin. Fixation time was 24-28 hours.

Frozen Tissue Available

-There is frozen available tissue from skin sample.

Details of Microscopic Findings

-Supraclavicular lymph node biopsy (2024) showed effaced architecture by a pseudonodular proliferation of large atypical cells surrounding germinal centers, with scattered leucocytes. These cells were large, showed clear cytoplasm and round nuclei with small central nucleoli.

-Skin biopsy (2025) revealed a Grenz zone and a diffuse dermal infiltrate that dissected collagen bundles and extended into the subcutis. It consisted of intermediate/large-sized, monomorphic cells with eosinophilic cytoplasm and irregular nuclei with a monocytoïd appearance.

Immunophenotype

-By immunohistochemistry, neoplastic cells of the lymph node were positive for CD20/CD30/CD15/PAX5, and MUM1; and negative for CD10/CD23/BCL6/BCL2 and EBV (EBER).

-By immunohistochemistry, neoplastic cells of the skin biopsy were diffusely positive for MNDA/lysozyme/CD68, and focal/weak for myeloperoxidase (MPO); and negative for CD34/CD117/CD56/CD163/CD123/CD3/CD20/PAX5/CD30/TIA1/ LEF1 and EBV (EBER).

-Flow cytometric analysis of the skin biopsy showed that 97% of the cells were large CD45-bright cells expressing CD33/CD13/CD11b/CD64/CD36/CD14 /MPO, with absence of HLA-DR and CD16.

Cytogenetics

-Cytogenetic studies were done on bone marrow biopsy. No alterations were found.

Molecular Studies

-Initial IGH clonality assessment by PCR in the skin biopsy was non-informative.

-Targeted next-generation sequencing (NGS, SureSelect XT HS, Agilent Technologies) was performed on both lymph node and skin samples. In both samples, a truncating ITPKB mutation (c.1648C>T; p.Gln550Ter, VAF: 47% and 49% in lymph node and skin, respectively) was detected.

-Subsequent NGS analysis of hair and peripheral blood detected the same *ITPKB* mutation, confirming its germline origin.

-Family studies were further done identifying the same mutation in his mother

Proposed Diagnosis

- Aleukemic granulocytic sarcoma after PMBCL

Interesting Feature(s)

-We report a case of aleukemic cutaneous granulocytic sarcoma in a patient with a germline *ITPKB* mutation.

-*ITPKB* mutations have been reported in lymphomas, including PMBCL, but germline mutations associated with myeloid neoplasms have not been reported so far.

-The present case raises the diagnostic dilemma of whether it should be considered a therapy-related aleukemic granulocytic sarcoma or as two independent neoplasms arising in the context of genetic susceptibility. In our view, this distinction is of particular interest given the poor prognosis described for the former scenario and the young age of the patient.

Panel Diagnosis: Myeloid sarcoma, post cytotoxic therapy/therapy-related, with germline *ITPKB* variant of unknown significance

EA4HP26-BMWS-441

Hypoplastic MDS versus Constitutional Trisomy 8

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Case Description

64-year-old male with pancytopenia.

Biopsy Fixation Details

Biopsy fixation involves 10% neutral buffered formalin (NBF).

Frozen Tissue Available

None

Details of Microscopic Findings

- Hypocellular marrow with left-shifted myelopoiesis and megakaryocytic hypoplasia
- Dysplasia was not identified in the peripheral blood smear or the bone marrow

Immunophenotype

- Concurrent flow cytometry showed no abnormal immunophenotype or increase in blasts.

Cytogenetics

46,X,-Y,+8[10]/46,XY[10]

Abnormal male karyotype with trisomy 8 and loss of Y

In the setting of unexplained cytopenias, trisomy 8 can be seen in myelodysplastic neoplasms

Molecular Studies

- MDS FISH detected a trisomy 8 in 44% of the nuclei
- No abnormalities in the other probes involving chromosomes 5, 7, and 20 were detected.
- Myeloid NGS panel was negative.
- PIG-A gene mutation was not detected

Proposed Diagnosis

Hypocellular marrow with trisomy 8

The differential diagnosis includes:

Constitutional Trisomy 8

Hypoplastic myelodysplastic neoplasm (MDS)

Aplastic anemia

Interesting Feature(s)

- Hypoplastic myelodysplastic neoplasms (hMDS) are a diagnostic conundrum as they share morphologic and clinical features with constitutional trisomy 8, aplastic anemia (AA), and paroxysmal nocturnal hemoglobinuria (PNH). Clinical and morphological overlaps with MDS and AA include cytopenias, hypocellularity, and dysplasia, along with cytogenetic and molecular alterations.
- When trisomy 8 is present, but standard microscopic examination doesn't show the typical cellular abnormalities of dysplasia in the blood or bone marrow, it becomes a diagnostic challenge.
- It is a complex situation where it's crucial to differentiate between a constitutional finding (like mosaic Trisomy 8), a rare presentation of MDS, or an inflammatory condition; advanced genetic testing such as next genome sequencing (NGS) often needed to clarify prognosis and guide treatment.
- Further NGS testing is vital to find mutations that define high-risk MDS, even without visible dysplasia; in this patient, the NGS testing was negative.
- Constitutional Trisomy 8 is a rare genetic condition where some but not all of the cells have an extra copy of chromosome 8, leading to a wide range of symptoms, including developmental delays, distinctive facial features, intellectual disability, and organ abnormalities, with males affected more often.

Panel Diagnosis: CCUS

Bone Marrow Workshop Part II | Myeloid neoplasms with significant fibrosis (excluding classic MPN) [e.g. cases that challenge the differential of fibrotic MDS (with and without excess blasts) and MDS/MPN]

EA4HP26-BMWS-30	AML or MDS-EB WITH <i>NPM1</i> MUTATION AND SIGNIFICANT MARROW FIBROSIS
EA4HP26-BMWS-35	Myelodysplastic neoplasm with marked myelofibrosis in a young female with SAVI syndrome
EA4HP26-BMWS-44	ET with acquired BCR-ABL1 rearrangement
EA4HP26-BMWS-46	Myeloid neoplasm with mild to moderate reticulin fibrosis in a hypocellular marrow with superimposed acute insult
EA4HP26-BMWS-48	Rapid onset bone marrow fibrosis: MPN or MDS?
EA4HP26-BMWS-53	Myeloid overlap syndrome presenting with a myeloproliferative phenotype and borderline blastemia
EA4HP26-BMWS-64	Myelodysplastic/myeloproliferative neoplasm with neutrophilia (MDS/MPN-N).
EA4HP26-BMWS-73	Chronic Myelomonocytic Leukemia with Marked Fibrosis and Megakaryoblastic Differentiation
EA4HP26-BMWS-75	Autoimmune Myelofibrosis vs Primary Myelofibrosis
EA4HP26-BMWS-81	Complex Karyotype TP53 Mutated MDS Presenting with Severe Bone Marrow Fibrosis and Clonal T-cell Proliferation
EA4HP26-BMWS-92	Myeloid neoplasm with significant fibrosis in a patient with Schwachman-Diamond syndrome and congenital Pelger Huet anomaly
EA4HP26-BMWS-94	MDS with low blast count and fibrosis. Is there such an entity in The WHO 5th edition?
EA4HP26-BMWS-95	Chronic myeloid neoplasm with atypical morphologic and genetic features suggestive of MDS/MPN- <i>SF3B1</i> -T
EA4HP26-BMWS-105	<i>Chronic Myeloid leukemia with secondary myelofibrosis and extramedullary T-lymphoblastic lymphoma.</i>
EA4HP26-BMWS-109	Blast Phase Chronic Myeloid Leukemia with Extensive Marrow Fibrosis and Striking Trilineage Dysplasia: A Diagnostic Challenge Case-Based Workshop
EA4HP26-BMWS-110	Myeloid Neoplasm with <i>JAK2</i> V617F, Fibrosis, and Monocytosis
EA4HP26-BMWS-113	Myelodysplastic neoplasm (MDS) with increased blasts and fibrosis (MDS-F)
EA4HP26-BMWS-117	Myelodysplastic syndromes/neoplasms (MDS) with accompanying fibrosis: is it a matter of fibrosis or of covariates such as amounts of blast and/or mutational composition?
EA4HP26-BMWS-119	Myelodysplastic / myeloproliferative neoplasm with fibrosis and features overlapping between atypical CML and CMML
EA4HP26-BMWS-121	MDS-bi <i>TP53</i> , MDS-F, or both? WHO and why has it been decided what counts?
EA4HP26-BMWS-149	A difficult-to-classify myeloid neoplasm at the PMF–CMML interface
EA4HP26-BMWS-163	Case submission

EA4HP26-BMWS-165	MDS/AML with mutated <i>TP53</i> , significant fibrosis, and striking proliferation of dysplastic CD34-p53 co-expressing megakaryocytes
EA4HP26-BMWS-168	Case report
EA4HP26-BMWS-170	<i>SETBP1</i> -mutated myelodysplastic/myeloproliferative neoplasm with fibrosis and unusually pronounced thrombocytosis, arising from underlying CCUS
EA4HP26-BMWS-184	Myelodysplastic syndrome (MDS) with marked fibrosis, low blasts, and myeloid predominance associated with profound erythroid and megakaryocytic hypoplasia
EA4HP26-BMWS-203	Atypical CML with progression versus evolving MPN ?
EA4HP26-BMWS-205	Myelodysplastic Syndrome with Fibrosis, Increased Blasts and Biallelic <i>TP53</i> Mutation
EA4HP26-BMWS-210	Fibrotic MDS/MPN with Ring Sideroblasts, MPL Mutation and Monosomy 7: A Case Illustrating Diagnostic and Monitoring Challenges
EA4HP26-BMWS-211	Fibrotic Myeloid Neoplasm with Persistent Monocytosis and JAK2/TET2/SRSF2 Mutations: Diagnostic Challenges at the Interface of Primary Myelofibrosis and CMML
EA4HP26-BMWS-223	Myelodysplastic/Myeloproliferative Neoplasm, Unclassifiable with fibrosis
EA4HP26-BMWS-232	Myeloid neoplasm with marked fibrosis: a diagnostic challenge
EA4HP26-BMWS-233	Myelofibrosis, JAK2 mutation and del(5q): unexpected friends?
EA4HP26-BMWS-235	Ghosal Syndrome: A New Etiology of Medullary Fibrosis
EA4HP26-BMWS-241	Unusual myelodysplastic syndrome with <i>SF3B1</i> and <i>CALR</i> mutations
EA4HP26-BMWS-249	Chronic eosinophilic leukaemia with marked megakaryocytic proliferation and marrow fibrosis.
EA4HP26-BMWS-257	Patient with Lowe syndrome, pancytopenia, and marrow fibrosis found to have <i>ASXL1</i> mutations
EA4HP26-BMWS-279	Chronic myelomonocytic leukemia with fibrosis acquiring <i>NPM1</i> mutation at progression to acute myeloid leukemia
EA4HP26-BMWS-301	Acute myeloid leukemia with MECOM rearrangement and moderate reticulin fibrosis (MF-2)
EA4HP26-BMWS-331	Are these Monoblasts or Megakaryoblasts?
EA4HP26-BMWS-332	Acute megakaryoblastic leukaemia in the differential diagnosis with leukemic transformation of MDS-IB2 with fibrosis or PMF
EA4HP26-BMWS-336	Unusual myeloid neoplasm with bone marrow fibrosis
EA4HP26-BMWS-344	When del(5q) Meets JAK2: A Fibrotic Myeloid Neoplasm At the MDS/MPN interface
EA4HP26-BMWS-347	VEXAS-Associated Myelofibrosis
EA4HP26-BMWS-352	Myeloid Neoplasm with Fibrosis and Progressive Monocytosis
EA4HP26-BMWS-357	Atypical, fibrotic progression of a TKI resistant CML patient with compound BCR::ABL1 mutation.
EA4HP26-BMWS-376	Myelodysplastic syndrome with increased blasts-2 and fibrosis
EA4HP26-BMWS-377	Myeloid Neoplasm with Del(5q), Increased Blasts and Fibrosis.
EA4HP26-BMWS-387	Acute myeloid leukaemia with a novel <i>PRDM16::GATA2</i> fusion detected with optical genome mapping and associated bone marrow fibrosis

EA4HP26-BMWS-389	Myelodysplastic syndrome with progressive molecular and cytogenetic evolution to a proliferative/fibrotic neoplasm
EA4HP26-BMWS-400	A dry tap with a myeloid neoplasm with fibrosis
EA4HP26-BMWS-406	Myelodysplastic/myeloproliferative neoplasm with <i>SF3B1</i> mutation and thrombocytosis (MDS/MPN- <i>SF3B1</i> -T) with progression to myelofibrosis.
EA4HP26-BMWS-407	Acute Myeloid Leukemia, NPM1-mutated, with Fibrosis
EA4HP26-BMWS-421	Fibrotic Transformation in MDS-to-AML Progression Mimicking Primary Myelofibrosis
EA4HP26-BMWS-425	Myeloid neoplasm with fibrosis and concurrent chronic lymphocytic leukemia (CLL)
EA4HP26-BMWS-432	A Confusing Case of Systemic Mastocytosis with Associated Hematologic Neoplasm
EA4HP26-BMWS-435	MDS/MPN with myelofibrosis MF-2 versus MDS with fibrosis
EA4HP26-BMWS-453	Chronic myeloid leukemia with a concurrent CALR+ mutated myeloproliferative neoplasm.

AML or MDS-EB WITH *NPM1* MUTATION AND SIGNIFICANT MARROW FIBROSIS

Dr. Anna Ruskova

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Case Description

A 66-year old man presented to his GP with a one week history of fatigue and shortness of breath. Previously very fit and well. Past medical history – AF and hypertension, generally fit and well. No family history of malignancies. FBC showed pancytopenia and circulating blasts (fluctuating 2%-6%). The bone marrow aspirate was a dry tap. The trephine biopsy showed marked fibrosis and osteosclerosis (MF-3), granulocytic proliferation and significant reduction of erythropoiesis and megakaryopoiesis. IHC for CD34 and CD117 showed <1% positive cells. Flow cytometry analysis on a peripheral blood detected 5% myeloid blasts, positive for CD34, CD117, HLA-DR, CD13, CD33, CD11c and MPO. Further analyses on a BM sample showed normal karyotype and *NPM1* (type A) mutation at 25% VAF. *FLT3* ITD and TKD wild type. NGS on a PB sample in addition to the *NPM1* showed mutations in *DNMT3A* and *TET2*.

Biopsy Fixation Details

Fixation formalin for 24 hrs. Decalcification 10% formic acid for 6 hrs

Frozen Tissue Available

not available

Details of Microscopic Findings

FBC: Hb 97 g/L, WCC $2.2 \times 10^9/L$, Blasts $0.06 \times 10^9/L$ (3%), Neu $0.53 \times 10^9/L$, Ly $1.26 \times 10^9/L$, Mon $0.21 \times 10^9/L$, Plt $44 \times 10^9/L$

BM aspirate: dry tap. Biopsy imprints hypocellular, many smeared cells and a few osteoblasts

Trephine biopsy

Trabecular bone thickened with evidence of new bone formation

Variable cellularity with areas of increased cellularity and areas where normal haematopoietic elements are replaced by fibrotic tissue. Intertrabecular architecture grossly abnormal and disorganised with marked background fibrosis and streaming

Erythropoiesis: markedly reduced. Granulopoiesis: predominant with full maturation Megakaryocytes: not seen

Immunohistochemistry and other stains:

CD34, CD117 and CD123 – rare positive cells <1%; WVF – no megakaryocytes seen; PanCK and S100 negative; Reticulin – markedly increased; Van Gieson – bundles of collagen fibres

Immunophenotype

On PB sample: 5% blasts positive for HLA-DR, CD34, CD117, CD11c, CD13, CD33 and myeloperoxidase. A small proportion of the blasts show aberrant expression of CD7.

Cytogenetics

46, XY (PB sample)

Molecular Studies

BM sample

NPM1 mutational analysis - POSITIVE (type A) VAF 25%

FLT3 (ITD and D835) - negative

PB sample

Myeloid targeted gene panel:

NPM1 c860_863dup (2%), *DNMT3A* c.2645G>C (36%), *TET2* c.973C>T (27%)

Proposed Diagnosis

5th WHO: AML with *NPM1* mutation

ICC: MDS with excess blasts (MDS-EB)

Interesting Feature(s)

Diagnostically challenging case with marked marrow fibrosis and low blast count at 5%-6% in the peripheral blood but no demonstrable increase in blasts on the trephine biopsy. While morphology was inconclusive the molecular analysis was instrumental in reaching the diagnosis. Fulfills 5th WHO criteria for AML with *NPM1* mutation, but not the ICC criteria as blasts are <10%. The acute presentation argues in favour of AML.

AML with mutated *NPM1* has been associated with increased marrow fibrosis, however this case represents an extreme example of this.

Panel Diagnosis: AML with *NPM1* mutation / AML with mutated *NPM1*

EA4HP26-BMWS-35

Myelodysplastic neoplasm with marked myelofibrosis in a young female with SAVI syndrome

Jinjun Cheng, Jiehao Zhou, Sarah Gibson, Ryan Robetorye, Ktalin Kelemen

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Case Description

- A 26-year-old female with a past medical history of Behcet's disease at childhood, now presented with worsening symptoms including oral/vaginal/anal ulcers, fever, low back and abdominal pain.
- Serology: Anti-nuclear antibody, extractable nuclear antigen, ANCA, C3, C4, protein electrophoresis, cryoglobulin, HLA B51 genetic panel testing were negative.
- Imaging: Sacroiliac joint x-rays showed mild degenerative changes but no frank sacroiliitis. CT scan on chest abdomen pelvis, and echocardiogram were unremarkable.
- Genetic tests on blood (Autoimmunity/Autoinflammatory Panel) revealed a mosaic *TMEM173* (also known as *STING1*) c.758G>A variant with uncertain significance, which led to a clinical diagnosis of SAVI syndrome (STING-associated vasculopathy with onset in infancy). Patient received treatment with tofacitinib.
- Laboratory tests: DAT+, reticulocytes and LDH elevated. UBA1 mutation negative.
- During last few years, patient started to develop progressing cytopenia.
- A bone marrow biopsy was performed.

Biopsy Fixation Details

Formalin-fixed, paraffin embedded tissue, stained with hematoxylin and eosin as well as additional immunohistochemical stains

Frozen Tissue Available

None

Details of Microscopic Findings

Bone marrow biopsy shows variable cellularity (focally 5% up to 90%), erythroid hyperplasia, megakaryocytic dysplasia, and moderate myelofibrosis (MF1-2).

The blasts are not increased based on CD34 and CD117 immunostaining. No significant increase in lymphoid cells based on CD79a and CD3 immunostaining.

Immunophenotype

Bone marrow aspirate, flow cytometric immunophenotyping: 1. A small aberrant myeloblast population identified. 2. No immunophenotypic evidence of a monocytic B cell or aberrant T cell population identified.

Flow cytometric immunophenotypic analysis identified a small population of aberrant myeloid blast that represents about 2% of the total analyzed white blood cells and shows the following immunophenotype: CD34+, CD117+,

HLADR+, CD13+, CD33 dim+, CD7 partial+, CD123 dim+. The specimen also contains granulocytes, rare monocytes, heterogeneous T cells, NK cells, and polytypic B cells

Cytogenetics

47,XX,add(7)(q22),+8,add(19)(p13.3)[10]

The result is abnormal. Each of 10 metaphases had trisomy 8 and additional chromatin of undetermined origin attached to 7q and 19p, resulting in 7q and 19p deletions.

Molecular Studies

Pathogenic Mutations Detected:

1) ASXL1: Chr20(GRCh37):g.31022839del; NM_015338.5(ASXL1):c.2324del; p.Leu775* (29%)

2) RUNX1: Chr21(GRCh37):g.36252851_36252868dup; M_001754.4(RUNX1):c.494_508+3dup; p.Gly170_Lys171insSerArgSerGlyArgGly (35%)

Variants of Unknown Significance (VUS): None

TMEM173 gene was NOT listed in the panel. However, a custom sequencing study of TMEM173 gene was negative in the patient's bone marrow specimen.

Proposed Diagnosis

- Myelodysplastic neoplasm with low blasts (WHO 5th).
- Myelodysplastic syndrome, not otherwise specified (ICC).

Interesting Feature(s)

- The natural history of hematopoietic neoplasm risk in patients with SAVI syndrome remains unclear. In this case, our patient, diagnosed clinically with SAVI syndrome, subsequently developed myelodysplastic syndrome (MDS) characterized by significant marrow fibrosis.
- Diagnosing and managing cytopenic myelofibrosis in young individuals presents considerable challenges, given the broad range of clinical presentations, diverse underlying causes, and unpredictable treatment responses.
- For many young patients with MDS accompanied by pronounced fibrosis, hematopoietic stem cell transplantation is often required, either alone or preceded by bridging chemotherapy.

Panel Diagnosis: MDS-LB / MDS-NOS with progression to MDS-IB2 / MDS-EB

EA4HP26-BMWS-44

ET with acquired BCR-ABL1 rearrangement

Dr. Sheren Younes, Prof. Dita Grattinger

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Case Description

78-year-old female with prior history of post-essential thrombocythosis myelofibrosis, JAK2 V617F and TET2 mutations, on hydroxyurea. Two years later she presents with leukocytosis with 15% circulating blasts and undergoes bone marrow biopsy.

Biopsy Fixation Details

The bone marrow core biopsy was placed in Bouin's solution for 1 hour, rinsed with water, covered with 10% formalin, and followed by Immunocal decalcification.

Frozen Tissue Available

n/a

Details of Microscopic Findings

Prior bone marrow biopsy

Leukoerythroblastic smear, macrocytic anemia, polychromasia, and hypogranular and giant platelet forms; Bone marrow biopsy: Hypercellular for age (40%). Myeloid predominance with M/E ratio 5:1. Full spectrum of maturation

for myeloid and erythroid precursors. Megakaryocytes are increased in number and include megakaryocytic clustering with atypical pleomorphic forms; Karyotype:

46,XX,dup(1)(q42q12),inv(9)(p11q13)c[16]/46,XX,inv(9)(p11q13)c[4]; NGS: JAK2 V617F (VAF 76%), TET2 C1271fs (VAF 20%)

At presentation with circulating blasts:

Peripheral blood: Leukocytes show myelomonocytic left-shift with 15% circulating blasts/blast equivalents. Granulocytes show abnormal segmentation and hypogranularity. Erythrocytes appear macrocytic with polychromasia and occasional circulating nucleated erythroid cells. Occasional giant and hypogranular platelets seen.

Bone marrow biopsy: Hypercellular bone marrow (80%) with overall ~15% blasts (with focal areas reaching ~20%) in scattered isolates and loose clusters, positive for CD34 and CD117, within areas of maturing myelopoiesis.. There is MF-3 of 3 by reticulin and trichrome, and markedly atypical clustered megakaryocytes have a range of morphologies including small hypolobated and large pleomorphic forms.

After TKI inhibitor therapy

7% circulating blasts; Bone marrow fibrosis (MF-3 of 3); scattered hyperchromatic megakaryocytes, no apparent blast population; karyotype: 46,XX,dup(1)(q42q12),inv(9)(p11q13)c[18]/46,idem,t(9;22)(q34;q11.2)[2] ; NGS: JAK2 V617F (VAF 77%) , TET2 C1271fs (VAF 53%) , TET2 Q1389X (VAF 5%)

Immunophenotype

CD34-positive blasts (~7%) expressing CD34, CD117, MPO, CD13 (dim partial), CD33 (partial), CD64 (minor subset, dim partial), CD123 (dim partial), CD61 (subset), CD71 (dim partial), CD38 (variable), and HLA-DR.

Cytogenetics

46,XX,dup(1)(q42q12),inv(9)(p11q13)c[1]/46,idem,t(9;22)(q34;q11.2)[19]

Molecular Studies

NGS: JAK2 V617F (VAF 81%) , NRAS G12D (VAF 41%), TET2 C1271fs (VAF 3%)

Proposed Diagnosis

Secondary BCR::ABL1 rearranged myeloproliferative neoplasm arising in the setting of JAK2-mutated post essential thrombocythemia myelofibrosis.

Interesting Feature(s)

Reports of myeloproliferative neoplasms (MPN) carrying both JAK2 and BCR-ABL mutations are uncommon, and true transformation of essential thrombocythemia (ET) into chronic myeloid leukemia (CML) is even rarer. This case highlights several intriguing features of clonal dynamics in disease evolution. Cytogenetic findings suggest the presence of two biologically distinct and competing clones, although the high VAF of JAK2 V617F at the timepoint demonstrating BCR-ABL1 acquisition suggests the mutation may be present in both clones. Following tyrosine kinase inhibitor therapy with marked morphologic improvement in bone-marrow blasts and a decline in t(9;22)-positive cells, contrasted with persistent JAK2-mutated disease. In this patient the stable JAK2 variant allele frequency parallels the continued presence of myelofibrosis and atypical megakaryocytes despite blast reduction, underscoring the complexity of clonal coexistence and therapeutic implications.

Panel Diagnosis: CML arising in post-ET-MF

Myeloid neoplasm with mild to moderate reticulin fibrosis in a hypocellular marrow with superimposed acute insult

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Case Description

A 67-year-old gentleman presented with severe pancytopenia, massive splenomegaly and unintended weight loss with past medical history of B12 and folate deficiencies, splenic infarct and Crohn's Disease on treatment with Remicade. Mild splenomegaly was noted since October 2022 and considered to be a sequela of previous splenic infarction in 2014. Historic CBC data from March 2022 to May 2023 showed fluctuating normal to mildly increased platelet counts (up to 576 K/uL) and WBC counts (up to 13.6 K/uL). From August 2023-March 2024, CBC data was unremarkable. However, severe pancytopenia, elevated LDH and massive splenomegaly were noted since November 2024. Two bone marrow (BM) biopsies showed hypocellularity with patchy hematopoiesis and some megakaryocytic dyspoiesis with considerations of aplastic anemia and hypoplastic myelodysplastic syndrome (MDS). Additional BM biopsy was performed.

Biopsy Fixation Details

Formalin fixed and Decalcification.

Frozen Tissue Available

NA

Details of Microscopic Findings

Peripheral blood (PB) smear: Thrombocytopenia; normocytic anemia with polychromatic forms and marked anisopoikilocytosis; frequent nucleated RBCs with irregular nuclei; absolute neutropenia with occasional hyposegmented/pelgeroid forms and 2% circulating blasts.

Biopsy: Hypocellular marrow with largely mild to moderate fibrosis, and gelatinous transformation. The residual patchy hematopoiesis with overall 5-10% cellularity was composed of predominant clusters of erythroid precursors with some atypical features, atypical megakaryocytes with small hypolobated and hyperchromatic forms, and a diminishing myeloid component.

Immunophenotype

CD34 showed rare blasts (<1%); CD71 showed clusters of erythroid precursors; MPO highlighted a few scattered myeloid cells; CD61 showed increased scattered and clusters of megakaryocytes with frequent small hypolobated forms; Alcian blue showed diffuse staining in the extracellular matrix; Reticulin showed mild to moderate increase in fibrosis (grade 1-2 out of 3); Trichrome showed mildly increased collagen deposit.

Cytogenetics

Failed.

Molecular Studies

NGS on PB: mutations on *JAK2 p.V617F* (VAF 48%), *TET2p.Q461** (VAF 26%), *U2AF1 p.Q157P* (VAF 25%), and *ASXL1p.G646Wfs*12* (VAF 16%).

Proposed Diagnosis

Myeloid neoplasm with mild to moderate reticulin fibrosis and extensive gelatinous transformation, with differential considerations of primary myelofibrosis with dysplastic changes, MDS with myelofibrosis, and Myelodysplastic/myeloproliferative neoplasm (MDS/MPN), NOS.

Interesting Feature(s)

The patient presented with subacute pancytopenia and massive splenomegaly. All BM biopsies showed hypocellularity with residual patchy hematopoiesis and extensive gelatinous transformation concerning for an acute BM attack/insult due to possible malnutrition, weight loss, drug/toxin effect and/ or malignancy with secondary aplastic

anemia. History of Crohn's disease on recent treatment with Remicade may contribute to BM suppression and morphologic atypia and can be confounding for the earlier diagnosis of a myeloid neoplasm.

Although there were historically fluctuating mild increases in WBC and platelet count, and largely mild to moderate reticulin fibrosis with mild collagen deposition in the current biopsy, the overall morphologic findings were not typical for primary myelofibrosis (PMF). However, multiple gene mutations (*JAK2*, *TET2*, *U2AF1*, and *ASXL1*) on PB suggested a myeloid neoplasm. Further subclassification is challenging. Differential diagnoses include PMF with dysplastic changes, MDS with myelofibrosis, and MDS/MPN, NOS.

Panel Diagnosis: PMF with MDS progression

EA4HP26-BMWS-48

Rapid onset bone marrow fibrosis: MPN or MDS?

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Case Description

Male, 72 yrs. No relevant history.

January 2025: mild, unexplained temporary normocytic anemia (Hb 7,8 mmol/l),

October 2025: rapid onset symptomatic anemia and thrombocytopenia, requiring transfusions. No hepato/splenomegaly.

Hemoglobine 4.9 mmol/l; Leucocytes 4.6 x10E9/l; Trombocytes 70 x10E9/l; MCV 86 fl

Differential PB: Blasts 5%; Blasts (abs) 0.2 x10E9/l

BM: dry tap

Biopsy Fixation Details

Burckhardt fixative, overnight microwave enhanced fixation and EDTA decalcification

Frozen Tissue Available

no

Details of Microscopic Findings

Diffuse fibrotic marrow with streaming of collagen (MF3) and a moderate cellularity with massive increase of small hypolobular megakaryocytes. Sparse left shifted myelopoiesis, no increase of eosinophils. Few erythrons. Scattered hemosiderophages and some lymphoplasmacytic infiltrates. No enlarged megakaryocytes, bone remodeling or extramedullary hematopoiesis.

Immunophenotype

BM biopsy: CD61 highlights the many, predominantly small, loosely clustering megakaryocytes, including many micromegakaryocytes. Focal increase of CD34/CD117 positive blasts, overall <5%.

IFT on PB: 9% abnormal myeloid blasts (CD34+CD117+CD33+CD13+) with expression: CD45+/-SS+/- CD34+ CD117+ CD133+ CD33+ CD13+ HLADR+ CD11b- CD15- CD64- CD16- CD38+ CD14- CD36+(40%) CD19- CD4+- CD56+(75%) CD5+60%) CD7+(39%) CD2- CD123- CD71+- CD235a- CD135- CD22- TdT- MPO- NG2-

Cytogenetics

Karyotyping: 46,XY,del(16)(q12q22)[10]

Array: arr[GRCh37] 16q12.1q22.1(48516320_68764563)x1[0.75] [deletion 20 Mb; includes *CBFB* gene]

Molecular Studies

ASXL1 (exon 13) VAF 41%

MPL c.1544G>T, p.(Trp515Leu) VAF 32%

U2AF1 c. Q157 (or Y158/E159) VAF 39%

Proposed Diagnosis

Preferred: **MDS with fibrosis** (WHO5/ICC)

Rejected differential diagnoses:

- progression of *MPL*+ MPN: no preceding MPN or morphological evidence
- “acute panmyelosis with myelofibrosis” : less than 20% blasts
- variant AML-M4eo (inv16): different biology

Interesting Feature(s)

1. The role of del16q in MDS/AML: in contrast to inv(16) no eosinophilia and worse prognosis [doi.org/10.1093/ajcp/aqx020]
2. The incidence of *MPL* mutation in MDS/AML: <2% in AML [doi.org/10.1080/16078454.2022.2066244]
3. The role of fibrosis in MDS: adverse risk factor, associated with *U2AF1* mutations as in our case [doi.org/10.1182/blood.V128.22.5524.5524, doi.org/10.1016/j.jtct.2021.05.006]

Panel Diagnosis: MDS-F / MDS-EB

EA4HP26-BMWS-53

Myeloid overlap syndrome presenting with a myeloproliferative phenotype and borderline blastemia

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Case Description

A 57-years-old previously healthy, asymptomatic male patient was urgently referred to our haematology department by his GP upon detection, in a routine complete blood count, of 24 % blasts, and splenomegaly in physical examination.

The leukocyte count was high, at 59.200/nL, with monocytosis of 14.80/nL (25 %); haemoglobin and platelet count were normal (13.4 g/dL and 304/nL).

Other relevant blood parameters at presentation: LDH 747 UI/L and vitamin B12 1670 pg/mL.

Biopsy Fixation Details

Processing according to the Hammersmith protocol.

Frozen Tissue Available

No.

Details of Microscopic Findings

The bone marrow smear showed 21.4 % blasts, Sudan Black B-negative, with neither myeloid nor lymphoid features.

The trephine biopsy had an increased cellularity with a very high myeloid/erythroid ratio. The myeloid/granulocytic lineage was fully maturing, although with seemingly abnormal morphology. There was a proliferation of megakaryocytes with hyperlobated and at times hyperchromatic nuclei, which formed many loose clusters.

In the centre of the marrow spaces there were variably sized clusters of atypical histiocytoid cells with elongated or irregular nuclei and moderately abundant, ill-defined cytoplasm.

There was diffuse grade 1 fibrosis.

Immunophenotype

By immunohistochemistry, there was atypical localisation of immature myeloid precursors (MPO). There were just over 5 % (less than 10 %) CD34⁺ blasts, with no clustering. The megakaryocytic lineage also expressed CD34. The atypical histiocytoid clusters mentioned in the previous section were CD117⁺, weakly MPO⁺, and partly CD68⁺ (PGM1); and they were negative for CD34, CD14, and CD123.

By flow cytometry, the bone marrow aspirate had 19.2 % blasts, positive for CD34, CD117, HLA-DR, CD13, CD33, and CD45 (weakly); and negative for cMPO, CD19, cCD79a, CD22, CD10, nuTdT, CD38, cyCD3, sCD3, CD7, CD56, CD4, CD36, CD64, CD14, CD15, and CD11b.

Cytogenetics

Trisomy 8 was the only alteration found in 17 out of 20 bone-marrow metaphases.

By FISH, none of the following rearrangements was found: *BCR::ABL1* (also by RT-PCR), *KMT2A* (break-apart probe), *RUNX1::RUNX1T1*, *CBFB::MYH11*, and *GATA2::MECOM*.

Molecular Studies

By NGS (using two panels, one for myeloid neoplasms and another for bone marrow failure syndromes), there were pathogenic variants in the following genes: *KRAS* (10.2 % VAF), *FLT3* (two variants, with 2.7 % and 32.9 % respective VAFs), *ASXL1* (45.6 % VAF), *SRSF2* (47.6 % VAF), and *RRAS* (43 % VAF). In addition, there was a variant of uncertain significance in *RUNX1* (46.1 % VAF).

Proposed Diagnosis

Myelodysplastic/myeloproliferative overlap syndrome, unclassifiable.

Interesting Feature(s)

The inconsistent blast count and phenotype among different techniques, and the otherwise borderline highest count, presented a therapeutic challenge in an asymptomatic patient.

The high VAFs of many of the variants identified, besides the presence of an *RRAS* mutation (also associated with RASopathies), precluded the swiftest allogeneic HSCT from the patient's sister. Also the high VAFs conflict with the relatively low blast counts, which in turn pose the question of a divergent evolution of subclones.

The histology does not neatly fit into one of the standard overlap syndromes, given also the peripheral-blood and bone-marrow monocytosis.

Panel Diagnosis: AML-MR / AML with MR gene mutations

EA4HP26-BMWS-64

Myelodysplastic/myeloproliferative neoplasm with neutrophilia (MDS/MPN-N).

Maysaa Abdulla

Region Uppsala, Uppsala, Sweden

Case Description

A 56-year-old woman with no significant past medical history. She was a blood donor until april 2023, when her hemoglobin level declined.

On july 2023, laboratory values were:

Hemoglobin: 119 g/L

White blood cell count: 12.4 ×10⁹/L

LDH: 5.7

Platelet count: 262 ×10⁹/L

On august 2024 laboratory findings:

Hemoglobin: 104 g/L

White blood cell count: rapid increase from 28 to 67 ×10⁹/L over the summer

Platelet count: 222 ×10⁹/L

LDH: 10

Biopsy Fixation Details

The bone marrow core biopsy and the aspirate specimen were fixed in buffered 4% formalin

Frozen Tissue Available

No frozen tissue available

Details of Microscopic Findings

Bone marrow aspirate and biopsy with high cellularity approaching 100%. Megakaryocytes are well represented in number, with the presence of megakaryocytes showing hyperchromatic and hypolobated nuclei, and megakaryocytes with separate nuclei are also noted. There is an increase in fibrosis corresponding to grade 2 on a 3-grade arbitrary scale.

Bone marrow smears were cellular with left-shifted granulopoiesis with full maturation. No increase in blasts. Erythropoiesis is present and shows dysplasia.

Peripheral blood smear with leukocytosis. Differential count shows 75% neutrophils, 3% monocytes, 7% eosinophils, 6% lymphocytes, 6% myelocytes, 2% metamyelocytes, and 1% blast-like cells.

Immunophenotype

Flow cytometry of the bone marrow: 2% CD3+ T cells with a CD4/CD8 ratio of 1.7 and 0.3% CD19+ B cells with a kappa/lambda ratio of 1.3. The proportion of cells within the granulopoiesis gate amounts to 88.5%, and monocytes constitute 4.5%. A subpopulation of granulocytes and monocytes shows aberrant positive expression of CD56. CD34+ stem cells constitute 1.3%. The Ogata score fulfills one out of a total of four criteria.

Cytogenetics

Normal karyotype.

No JAK2, MPL, or CALR mutations were detected.

BCR::ABL1 was negative.

No FIP1L1::PDGFRA fusion and no JAK2 rearrangement were identified.

Using an NGS myeloid mutation panel (TWIST), a pathogenic ASXL1 mutation (VAF 40%) was detected; this mutation was present in July 2023. On repeat NGS in August 2024, a newly acquired variant in the CSF3R gene (VAF 28%) was identified.

The results are consistent with clonal evolution.

Molecular Studies

None

Proposed Diagnosis

Myelodysplastic/myeloproliferative neoplasm with neutrophilia (MDS/MPN-N).

Interesting Feature(s)

Myelodysplastic/myeloproliferative neoplasm with neutrophilia (MDS/MPN-N) formerly known as atypical chronic myeloid leukemia.

Panel Diagnosis: CNL progressed out of CMUS

Chronic Myelomonocytic Leukemia with Marked Fibrosis and Megakaryoblastic Differentiation

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Case Description

79-year-old female initially diagnosed as CMML (chronic myelomonocytic leukemia)-0 in 2019 with minimal blasts, 2+ bone marrow fibrosis, and normal cytogenetics. Follow-up marrows from 2020-2023 showed a slight increase in blasts up to 2.8% with an additional finding of del(5)(q11.2p22). She was started on Inqovi (decitabine and cedazuridine). Bone marrow evaluations in late 2024 and early 2025 were remarkable for 8% circulating blasts, a hypercellular marrow with 5-10% myeloblasts exhibiting megakaryocytic differentiation, and marked reticulin (3+) and collagen (2+) fibrosis. Megakaryocytes included many with markedly dysplastic features. Within months, the patient developed hepatic and multiple skin lesions. Biopsy of skin lesions confirmed megakaryoblastic transformation of CMML. The patient deteriorated rapidly and passed away, highlighting the aggressive nature of the disease.

Biopsy Fixation Details

Bone marrow aspiration and core biopsy were performed on the right posterior iliac crest. The bone marrow core (2.6 cm) was fixed in 10% neutral buffered formalin.

Frozen Tissue Available

No

Details of Microscopic Findings Peripheral blood smear in 2024 showed hypogranular and hyposegmented neutrophils with left shift and 7% blasts, including few blasts with cytoplasmic blebs. Platelets were markedly decreased. Bone marrow aspirate smears were aspiculate with scant hematopoietic elements and occasional identifiable blasts similar to those in the peripheral blood. Bone marrow core biopsy appeared markedly hypercellular with dense (3+) reticulin and moderate (2+) collagen fibrosis with interspersed hematopoietic elements. Cellular elements included a marked increase in pleomorphic megakaryocytic cells, including small hypolobate and hyperchromatic forms, as well as many immature-appearing forms. These immature-appearing cells were positive by immunohistochemistry for CD117, CD42b, and CD61, consistent with megakaryoblastic lineage.

A skin biopsy from 2025 revealed dermis and underlying subcutis with scattered large atypical, multinucleated cells, accompanied by frequent mitotic figures, in a background of mixed inflammation. By immunohistochemistry, these cells were positive for CD61 and weakly positive for CD117 and CD33. The concurrent bone marrow biopsy also revealed a few foci of immature-looking cells with a similar phenotype.

Immunophenotype

Atypical cells were positive for CD117, CD33, CD42b, CD61

Cytogenetics

No abnormality in 2019, del(5) in 2021, trisomy 12 in 2025.

Molecular Studies

Myeloid NGS on a peripheral blood specimen in 2019 showed three tier 1 mutations: *SRSF2*, and two *TET2*.

Myeloid NGS and Copy number variation (CNV) analysis in 2025 showed additional mutations in *NOTCH1*, *ASXL1*, *CBL*, and *STAG2* in addition to the previously described *SRSF2* and two *TET2* variants. Trisomy 12 was also identified by CNV analysis.

Proposed Diagnosis

CMML with marked fibrosis and megakaryoblastic differentiation.

Interesting Feature(s)

Bone marrow fibrosis in CMML is rare and is associated with increased megakaryopoiesis and an aggressive course. Transformation to acute myeloid leukemia (AML) happens in approximately 20% of patients with CMML. However, megakaryoblastic transformation with marked fibrosis in this patient makes this case unique.

Panel Diagnosis: CMML-2 progressed from CMML-1

EA4HP26-BMWS-75

Autoimmune Myelofibrosis vs Primary Myelofibrosis

Dr. Cuiping Zhang, Dr. Linnea Banker, Dr. Soumya Mikkilineni, Dr. Richard Hammer

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Case Description

A 32-year-old woman developed autoimmune hemolytic anemia during her fourth pregnancy in 2024. She presented at approximately 8 weeks' gestation with vaginal spotting and a positive antibody screen which identified a warm autoantibody with associated cold autoantibody activity and no red blood cell alloantibodies. Her hemoglobin declined to 6.2 g/dL, requiring transfusions and prednisone therapy. The pregnancy was complicated by a fetal ventricular septal defect and placental abruption, resulting in neonatal demise during the second trimester. Postpartum hemolysis improved; however, she developed recurrent, refractory anemia treated with rituximab and subsequent immunosuppressive therapy. Infectious and autoimmune serologies were negative, while inflammatory markers were elevated. She later developed pancytopenia (WBC 0.83 x 10⁹ /L, PLT 135 x 10⁹ /L) with mild hepatosplenomegaly, prompting bone marrow evaluation. At follow-up in 2025, she remained on multiple immunosuppressive agents with persistent anemia.

Biopsy Fixation Details

Bone marrow biopsy specimens were fixed in B-Plus fixative for 2 hours, decalcified in 3–4% hydrochloric acid for 2 hours, briefly rinsed, neutralized in 5% lithium carbonate for 1 minute, and processed routinely.

Frozen Tissue Available

Not performed.

Details of Microscopic Findings

Bone marrow aspirate smears: Partial hemodilution with mild erythroid predominance and a decrease of other hematopoietic lineages. Bone marrow core biopsy: Marked hypercellularity (~80–90%) with moderate to severe fibrosis. A decrease in myeloid-to-erythroid ratio. Myeloid precursors decreased with normal maturation. Erythroid lineage predominance with left-shift and normal maturation. Megakaryocytes show hyperplasia with atypical features, including monolobation, nuclear pyknosis, separated lobes and frequent clustering. No blast clusters were identified. Bony trabeculae were unremarkable, and no additional focal lesions were present.

Immunophenotype

Immunohistochemistry: CD34 demonstrated a mild increase in blasts (~4–5%). E-cadherin highlighted increased early erythroid precursors. Factor VIII highlighted dysplastic megakaryocytes. Special Stains: Reticulin highlights increased fibrosis (grade 2-3). Trichrome highlights focal increase in collagen fibrosis. Flow cytometry identified a slight increase in myeloid blasts (5.2%) and a prominent $\gamma\delta$ T-cell population (55.1% of lymphocytes).

Cytogenetics

Chromosome analysis identified an abnormal female karyotype: 47-48,XX,del(6)(q21),-9,+2-3mar[cp17]/46,XX[3].

Molecular Studies

MDS FISH is normal. Next Generation Sequencing (NGS) detected alterations in JAK2 (4.6%) and ASXL1 (2.4%) along with multiple variants of unknown significance.

Proposed Diagnosis

Hypercellular bone marrow biopsy showing moderate to severe myelofibrosis, with increased erythropoiesis displaying megaloblastic changes, markedly decreased granulopoiesis, and increased megakaryopoiesis with dysmegakaryopoietic changes. Aspirate smear and touch prep are hemodiluted. No significant increase in blasts seen.

Interesting Feature(s)

Underlying autoimmune hemolytic anemia with prior immunosuppressive/cytotoxic therapy complicated interpretation of marrow findings. Hypercellular marrow with erythroid hyperplasia, dysplastic megakaryocytes, and low-level JAK2 V617F (4.6%) raised autoimmune myelofibrosis (AIMF) versus primary myelofibrosis (PMF). Lack of classic myeloproliferative neoplasm features favored AIMF; dysplasia may reflect methotrexate effect. Repeat marrow and molecular studies were recommended; outside consultation concurred.

Panel Diagnosis: PMF, fibrotic / Overt PMF (with MF-2)

EA4HP26-BMWS-81

Complex Karyotype TP53 Mutated MDS Presenting with Severe Bone Marrow Fibrosis and Clonal T-cell Proliferation

Prof. Eric Duncavage¹, Prof. John Frater¹, Prof. Anjum Hassan¹, Dr. Yi-Shan Lee¹, Dr. Lisa Yuen¹, Dr. Anne Chen¹, Dr. Drew Hughes¹, Dr. David Spencer², Prof. Mariana Ruzinova¹

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Case Description

The patient is a 64 year old man with a history of COPD and recent onset cytopenia with associated fever and chest pain. CBC showed WBC 2.8, Hgb 6.7, and Plts 29. Peripheral smear showed increased large granular lymphocytes (LGLs) and patient was originally suspected to have a T/LGL leukemia at an outside hospital based on blood smear review and TCR gamma clonality studies. The patient was ultimately treated with decitabine/venetoclax and died 6 months later.

Biopsy Fixation Details

EDTA decalcified, 10% formalin buffered formalin fixed bone marrow core biopsy.

Frozen Tissue Available

None

Details of Microscopic Findings

A bone marrow biopsy resulted in a dry tap with no evaluable aspirate. The bone marrow core showed MF-3 of 3 fibrosis with trichrome positivity. The bone marrow core was hypercellular with dysplastic hyperchromatic megakaryocytes, a partial myeloid maturational block and decreased erythroids. Stains for CD34 and CD117 showed ~5% blasts which were MPX positive. CD61 stained dysplastic megakaryocytes including a small subset that expressed CD34 and CD117. E-cad highlighted decreased erythroids. A p53 stain was positive in 40-50% of cells.

Immunophenotype

Blood flow cytometry showed 4-5% myeloid blasts and no T-cell phenotypic abnormalities or increased NK cells. Blasts expressed CD34, dim CD45, CD38, HLA-DR, CD13, CD123, CD117, and CD33. TCR gamma rearrangement studies showed a clonal T-cell population with the largest rearrangement in TCR Vg4-JgP1 representing 8% of reads by NGS.

Cytogenetics

Metaphase cytogenetics could not be performed due to the lack of a bone marrow aspirate. FISH showed del (5q), del (7q), tetrasomy 11q, and trisomy 11q. There was no evidence of TP53 deletion, CBFB rearrangement, KMT2A rearrangement, or RUNX1 rearrangement.

Molecular Studies

Molecular studies including high coverage panel-based NGS and whole genome sequencing were performed on the bone marrow core and identified a TP53 p.Y163D mutation with a VAF of 46-58% in addition to multiple deletions on chromosomes 5, 7, 11, 12, 13, 20 and duplications on chromosomes 11 and 20. Subsequent re-analysis of the WGS data using more advanced informatic identified an 18Mb TP53 CN-LOH event.

Proposed Diagnosis

MDS with mutated TP53 (ICC)

MDS with low blasts (WHO HEME5) based on original WGS data

MDS with with biallelic TP53 inactivation (WHO HEME5) based on re-analysis of WGS data showing CN-LOH of TP53

Interesting Feature(s)

This case is an unusual presentation of MDS with trichrome positive MF-3 fibrosis, complex karyotype, low blast count and TP53 mutation that proved rapidly fatal despite therapy. While most cases of MDS with TP53 mutation are erythroid predominant, this case presented with an increased M:E ratio. Further, the patient has no prior history of MPN and no mutations typically associated with fibrosis (JAK2, CALR). Similar findings have been reported (Loghavi et al, BJH; PMID 26123119). This case also highlights discrepancies between the ICC and WHO HAEM5 classifications as the latter requires the presence of a second TP53 mutation (including CN-LOH or deletion event) to be considered for the higher risk MDS-biTP53 category. Subsequent re-analysis of the WGS data identified an 18Mb TP53 CN-LOH event illustrating the importance of TP53CN-LOH detection and the clinical utility of WGS.

We also note that clonal T-cell populations are commonly seen in MDS patients. While the patient had dry tap and cytogenetic studies could not be performed, we demonstrate the utility of WGS run from the bone marrow core specimen to generate a karyotype.

Panel Diagnosis: MDS-biTP53 / MDS-TP53

EA4HP26-BMWS-92

Myeloid neoplasm with significant fibrosis in a patient with Schwachman-Diamond syndrome and congenital Pelger Huet anomaly

Dr. Meredith Nichols

Cleveland Clinic, Department of Pathology and Laboratory Medicine, Cleveland, USA

Case Description

A 41-year-old female with a history of learning disability, recurrent pregnancy loss, and a multiyear history of cytopenias was transferred to our institution with epistaxis, pancytopenia, and 2% circulating blasts. Imaging demonstrated advanced liver fibrosis and a jaw abscess. A bone marrow biopsy performed at the outside hospital reportedly demonstrated morphologic findings concerning for a myelodysplastic syndrome (MDS). A bone marrow biopsy was repeated at our institution. Given the patient's age, the patient underwent testing for hereditary myeloid malignancy predisposition syndromes. She had ongoing mucosal bleeding that was refractory to platelet transfusion and was started on a TPO agonist. She was not a candidate for chemotherapy due to the severity of the cytopenias. She was discharged and then promptly readmitted with concerns for intra-cranial bleeding. She declined bone

marrow transplant after lengthy discussion and was discharged to hospice care, where she subsequently passed away.

Biopsy Fixation Details

The specimens were fixed in 10% neutral buffered formalin.

Frozen Tissue Available

No.

Details of Microscopic Findings

The peripheral blood demonstrated numerous hypolobated and non-lobated granulocytes. Bone marrow aspirate smears were not received, as the specimen was a dry tap. The bone marrow trephine biopsy demonstrated hypercellular areas with dysmegakaryopoiesis and erythroid predominance alternating with areas of significant stromal fibrosis.

Immunophenotype

Blasts were not increased by immunohistochemistry for CD34. Reticulin fibrosis was increased. Collagen fibers were present on a trichrome stain (overall called MF-3).

Cytogenetics

FISH studies demonstrated monosomy 7 and deletion(5q).

Conventional cytogenetic studies performed on the peripheral blood demonstrated 45,XX,del(4)(p13),-5,-7,add(12)(p11.2),+mar[8]/46,XX[12].

Molecular Studies

Telomere length testing was normal.

Germline genome sequencing identified the following pathogenic variants:

SBDS, c.258+2T>C and c.184A>T (p.K62*) (associated with Schwachman-Diamond syndrome).

ATP1A2, c.2500C>T (p.R834*) (associated with familial hemiplegic migraine).

LBR, c.1430_1433del (p.S477Ifs*12) •(associated with congenital Pelger-Huet anomaly).

Molecular testing on peripheral blood and bone marrow demonstrated the following pathogenic variants:

TP53 p.C141Y (VAF 7%)

MPL p.Arg592Gln (VAF 19%)

Testing for RNA fusions was negative.

Proposed Diagnosis

Myeloid neoplasm with at least single-lineage dysplasia and increased stromal fibrosis (MF-3) arising in the setting of Schwachman-Diamond syndrome, favor a myelodysplastic syndrome.

Interesting Feature(s)

In the absence of germline information, it was difficult to classify the patient's myeloid neoplasm. Given the mutation in *MPL*, the clinical differential diagnosis briefly included a fibrotic stage myeloproliferative neoplasm (MPN) or an MDS/MPN. This case underscores the importance of germline genetic testing to help establish a more definitive diagnosis. Given that the patient also had congenital Pelger-Huet anomaly, this served as a confounding factor when evaluating for granulocytic dysplasia. The abundance of non-lobated granulocytes in the peripheral blood also serves as a potential pitfall, as these could easily (and probably were) overcalled as circulating blasts during the patient's clinical course.

Panel Diagnosis: MDS-LB / MDS-NOS in the setting of Schwachman-Diamond

MDS with low blast count and fibrosis. Is there such an entity in The WHO 5th edition?

Dr. Merit Hanna

North Shore Hospital- Waitemata, Haematology, Auckland, New Zealand

Case Description

A 60 years old male with a background history of Adenoid cystic carcinoma of left neck - treated with radical radiotherapy (60 Gy in 30 fractions) completed in September 2023. Presented to our institution in November 2025 with abdominal discomfort and was diagnosed with terminal ileitis. He was subsequently admitted to hospital on 22 Dec 2025 with collapse after severe hematemesis and melena. He also described episodes of vigorous spastic "shaking", 13 Kg weight loss over last 4 weeks and drenching night sweats. Examination showed no hepatosplenomegaly which was confirmed on body CT scan. He also had a petechial rash on examination and pallor. Full blood count showed severe bicytopenia with haemoglobin 55g/L, MCV 82fL, reticulocytes 54 x 10e9/L, platelets <10 x 10e9/L, white blood cells 4.8 x 10e9/L, neutrophils 4.1, monocytes 0.2 x 10e9/L. Normal coagulation screen and raised LDH raised.

Biopsy Fixation Details

Paraffin embedded

Frozen Tissue Available

N/A

Details of Microscopic Findings

PB smear:

- Red blood cells: nucleated red blood cells present with dysplasia, occasional tear drop cells and stress cells (dark small polychromatic cells)
- White blood cells: normal numbers of neutrophils, occasional patchy hypogranularity although <10%. Occasional blast cells present (1%).
- Platelets: essentially absent, the observed rare form is large, giant or hypogranular.

Bone marrow trephine imprints:

- Erythropoiesis: active with predominance of basophilic erythroblasts. Increased proerythroblasts at 8-10% (<30%). Significant dysplasia present in >10%; abnormal nuclear shapes, irregular nuclear outline, nuclear fragmentation and cytoplasmic stippling.
- Granulopoiesis: active with occasional hypogranulation or pseudo Pelger Huet nuclei although involves <10% of cells. Blast cells comprise 1% of all marrow nucleated cells.
- Megakaryopoiesis: rare observed giant megakaryocytes.

Bone marrow trephine:

- Bone architecture is abnormal including new bone formation and osteosclerosis.
- Intertrabecular architecture is disorganized due to diffuse fibrosis almost completely replacing haematopoietic elements causing effacement of fat spaces and increased dilated sinusoids.
- In the majority of the specimen there is complete obliteration of the marrow space by fibrous material with accompanying cellular depletion.
- Increased fibroblasts, cellular streaming, reticulin fibrosis, collagen deposition; WHO MF-3
- Erythroid precursors show dominance of immature proerythroblasts but they are < 30% of nucleated cells.
- Granulopoiesis: active with no increase in blast cells; aided by CD34 immune staining.
- Megakaryopoiesis: increased number with abnormal topography and high degree of dysplasia appreciable on immune staining for CD61.
- No secondary bone marrow infiltration by metastatic disease or other neoplastic conditions.

Immunophenotype

NA

Cytogenetics

NA

Molecular Studies

Two mutations in TET2 (VAF 54.7% and 41.9%) and PHF6 (VAF 9.1%, 8.8%), mutation in SRSF2 (10.4%)

Proposed Diagnosis

Myelodysplastic neoplasm, with low blast count and fibrosis, post cytotoxic therapy.

Interesting Feature(s)•WHO 5th edition only recognises myelodysplastic neoplasm with increased blasts and fibrosis. How about those with a low blast count?

- Bone marrow fibrosis explains the severe thrombocytopenia and likely accounts for the patient's poor outcome.
- WHO 5 does not recognise increased fibrosis as a poor prognostic marker unless associated with increased blasts.
- Our case highlights the prognostic impact of bone marrow fibrosis irrespective of the IPSS-R risk category.
- Bone marrow fibrosis should be included in the scoring system

Panel Diagnosis: 1.MDS-pCT (WHO-5)/MDS-NOS-MLD, post cytotoxic therapy (ICC); 2. T-CUS non-LGL phenotype

EA4HP26-BMWS-95

Chronic myeloid neoplasm with atypical morphologic and genetic features suggestive of MDS/MPN-SF3B1-T

Dr. Curtiss Johnson, Dr. Kaaren Reichard, Dr. April Chiu

Mayo Clinic, Department of Laboratory Medicine and Pathology, Rochester, USA

Case Description

65-year-old female with mild normocytic anemia and splenomegaly (3-6 months). No known history of autoimmune disease; denies any bleeding events or malnutrition.

CBC: HGB 11.0 g/dL; RBC 3.42 x10¹²/L; MCV 95.3 fL; RDW 20.2 %; WBC 4.7 x10⁹/L; PLT 367 x10⁹/L Diff (%): neut 77, lymphs 19, monos 3, eos 1. Other studies: serum ferritin >700 mcg/L, LDH 248 U/L. PET-CT: splenomegaly with homogenous activity; diffusely moderately FDG avid bone marrow.

Biopsy Fixation Details

B5 followed by formalin and decalcification

Frozen Tissue Available

NA

Details of Microscopic Findings

PB: Moderate anisopoikilocytosis with elliptocytes and occasional dacrocytes; occasional large platelets; no circulating blasts

BM aspirate / biopsy: Markedly hypercellular (90%); markedly increased megakaryocytes with large, hyperlobated and hyperchromatic forms and cluster formation. Erythoid precursors show slight nuclear-cytoplasmic asynchrony with coarse cytoplasmic granulation. No overt dysgranulopoiesis; <5% blasts.

Iron stain: Increased storage iron with ring sideroblasts (36%)

Reticulin stain: Moderate fibrosis (MF-2)

Immunophenotype

Flow cytometry: no monocytic B-cell population or increased blasts identified; normal myeloid maturation pattern

Cytogenetics

46, XX[20]

Molecular Studies

NGS:

1. JAK2: Chr9(GRCh37):g.5073770G>T; NM_004972.3(JAK2):c.1849G>T; p.Val617Phe (7%)
2. MPL: Chr1(GRCh37):g.43815009G>T; NM_005373.2(MPL):c.1544G>T; p.Trp515Leu (29%)
3. SF3B1: Chr2(GRCh37):g.198267360T>G; NM_012433.2(SF3B1):c.1997A>C; p.Lys666Thr (44%)

Proposed Diagnosis

Chronic myeloid neoplasm with MF-2 fibrosis and ring sideroblasts; genetic features suggestive of myelodysplastic/myeloproliferative neoplasm with *SF3B1* mutation and thrombocytosis (MDS/MPN-*SF3B1*-T)

Interesting Feature(s)

1. Despite the genetic features suggestive of MDS/MPN-*SF3B1*-T, the patient did not present with thrombocytosis or have a history of sustained thrombocytosis (although there were episodes of borderline thrombocytosis), which is one of the required criteria per WHO-5 and ICC. In addition, there was no documented history of cytosis, making a pure MPN unlikely. Patients with a previous diagnosis of MDS with *SF3B1* mutation may later develop thrombocytosis, which would be considered progression of MDS. Although the presence of driver mutations such as *JAK2* are in favor of MPN rather than MDS, it is important to consider the possibility of MDS with acquired *JAK2* mutation, in which thrombocytosis may occur later in the disease course. However, this would be unusual in this patient with concurrent *MPL* (at a high VAF) and low-level *JAK2* mutations.
2. MF-2/MF-3 myelofibrosis is a relatively uncommon feature of MPN/MDS-*SF3B1*-T (formerly RARS-T). Prior studies demonstrate a possible risk of fibrosis progression after initial diagnosis, although the clinical significance and prognostic implications remains unclear, as there are more salient prognostic features such as Hgb <10 g/dl and abnormal karyotype.
3. Concurrent *MPL* and low-level *JAK2* mutations. *SF3B1* mutation with concurrent *JAK-2 p.V617F* is seen in approximately 50% of cases of formerly named RARS-T, with concurrent *MPLW515* being much rarer (<10%). There is documented concurrence of these mutations, which is seen within the spectrum of MPN (especially primary fibrosis) but not in MDS/MPN. Previous studies have shown that a subset of patients may later acquire *JAK2* or equivalent mutations resulting in thrombocytosis, but it is difficult to classify this case, as *SF3B1*, *JAK2*, and *MPL* are all seen within the spectra of myelodysplastic and myeloproliferative neoplasms.

Panel Diagnosis: Myeloid neoplasm with myelodysplastic and proliferative features; could represent MDS with proliferative progression

EA4HP26-BMWS-105

Chronic Myeloid leukemia with secondary myelofibrosis and extramedullary T-lymphoblastic lymphoma.

Dr. Vanessa Santiago-Pacheco^{1,2}, Dr. Fabiola Vizcarra-Reyes³

¹ Universidad de Antioquia, Pathology Department, Medellín, Colombia; ² Fundación Colombiana de Cancerología - Clínica Vida, Pathology, Medellín, Colombia; ³ Fundación Colombiana de Cancerología - Clínica Vida, Hematology, Medellín, Colombia

Case Description

A 43 year-old man presented with 1 month of abdominal pain and distension. On assessment, he had enlarged bilateral cervical and submandibular lymph nodes. CBC showed anemia, increased white-cell count (predominantly neutrophils).

Clinically, the patient showed distinctive features: short stature, developmental delay, intellectual disability, and craniofacial dysmorphism (microcephaly, midfacial hypoplasia, hypertelorism, flat nasal bridge, low-set ears, and eccentric nipples; on questioning, he had also low birth weight.

Biopsy Fixation Details

The tissue was fixed in 4% buffered formaline. Bone marrow was decalcified in TBD-1.

Frozen Tissue Available

No.

Details of Microscopic Findings

Bone marrow with a 100% cellularity, myeloid predominance and eosinophilia, increase in intermediate precursors and blasts, small hypolobulated megakaryocytes with hyperchromatic nuclei in loose aggregates.

Reticulin stain shows diffuse and dense reticulin fibers; Masson's Trichrome shows central and paratrabecular collagen deposition.

Lymph node shows loss a diffuse proliferation of medium to large cells with a discohesive appearance, irregular nucleus, open chromatin, inconspicuous nucleoli, and poorly defined cytoplasm. Mitoses and apoptotic debris are seen.

Immunophenotype

Bone marrow:

CD34 mild increase in blasts (5-6%), CD61 showed megakaryocytes in loose aggregates, TdT was negative. Flow cytometry showed predominance of mature myeloid cells, basophilia and a left shift, in addition to an increased myeloid blast count, up to 7.61%.

Lymph node:

CD34 (-), TDT (+), CD117 (-), MPO (-), CD43 (+), CD68 (-), CD20 (-), CD3 (+), PAX5 (-), CD30 (-), CYCLIN-D1 (-), CD1a (-), CD4 (-), CD8 (-), CD7 (+), CD2 (+), CD5 (-), P53 (+), C-MYC (+), Cytokeratin (-), EMA (-), KI67 90%

Cytogenetics

46, XY, t(9; 22)(q34; q11. 2)[16]/47, sl, +der(22)t(9; 22)[6]/48, sdl1, +19[17]

Cytogenetic analysis of the tissue revealed a main clone and two subclones: 16 metaphases have t(9; 22)(q34; q11. 2) translocation, 6 metaphases have double Philadelphia chromosome and 17 metaphases, in addition to the above alterations, trisomy of chromosome 19.

*Chromosome 19 FISH studies are currently in process.

Molecular Studies

qPCR BCR/ABL p210 isotype positive in 97%

*Currently in process, results not yet available:

- NGS for myeloid neoplasia.

- High resolution microarray for Chromosome 19 abnormalities

Proposed Diagnosis

Chronic Myeloid leukemia presenting with secondary myelofibrosis, double Philadelphia chromosome and Trisomy 19, with extramedullary T-lymphoblastic lymphoma.

Interesting Feature(s)

We present a rare example of a progressing CML, presenting with medullary fibrosis and extramedullary blast proliferation, with a complex karyotype that includes Ph duplication and Trisomy 19, in a patient with some clinical features that are compatible with mosaic trisomy 19.

Trisomy 19 is frequently encountered in cases of chronic myeloid leukemia (CML) as a secondary abnormality. Ph duplication is usually associated with an overexpression of BCR-ABL1, and therefore associated with an aggressive clinical outcome, and regarded as a resistance factor to imatinib therapy. Full trisomy 19 has not been reported in live births and presumably leads to early pregnancy loss. Cases of mosaic trisomy 19 (where some cells are normal and some cells have trisomy 19) have been described in the literature. These cases are expected to have a wide range of medical complications, as well as physical and developmental sequelae.

Panel Diagnosis: CML and T-LBL

Blast Phase Chronic Myeloid Leukemia with Extensive Marrow Fibrosis and Striking Trilineage Dysplasia: A Diagnostic Challenge Case-Based Workshop

Prof. Samah Kohla^{1,2}, Dr. Ibrahim Ganow¹, Dr. Hassan Rizfi⁵, Dr. Amna Gameil^{3,2}, Dr. Aliaa Amer¹, Dr. Amal Aly⁴, Dr. Samah Kohla made the initial diagnosis based on peripheral blood smear and flow cytometry, conceptualized the case report, and led the abstract preparation. Dr. Ibrahim Ganow performed the bone marrow examination. Dr. Hasan Rizfi reviewed and confirmed the bone marrow findings. Dr. Amal Salem conducted and interpreted cytogenetic studies, including FISH and karyotyping. Dr. Amna Gameil and Dr. Aliaa Amer reviewed the abstract.

¹ Hamad Medical corporation, DLMP/Hematopathology Division/NCCCR, Doha, Qatar; ² Weill Cornell Medicine Qatar, Clinical Pathology Department, Doha, Qatar; ³ National Center for Cancer Care and Research, Hematology and Bone Marrow Transplant/ Hamad Medical corporation, Doha, Qatar; ⁴ Hamad Medical corporation, Cytogenetic and Molecular Genetic Division, Doha, Qatar; ⁵ Hamad Medical Corporation, Histopathology Division, Doha, Germany

Case Description

Blast phase chronic myeloid leukemia (BP-CML) remains a major diagnostic challenge, particularly when bone marrow aspiration is unsuccessful due to extensive fibrosis and when marked dysplastic features obscure the underlying disease. Integrated evaluation of morphology, immunophenotype, and cytogenetics is essential for accurate diagnosis. We present a 36-year-old man with weight loss, fever, abdominal pain, marked splenomegaly, and extreme leukocytosis. Initial complete blood count in April 2025 showed total leukocyte count $244.4 \times 10^9/L$, hemoglobin 6.2 g/dL, and platelets $381 \times 10^9/L$. Peripheral smear demonstrated marked left shift, eosinophilia, basophilia, and 21% circulating blasts, raising suspicion for BP-CML. The patient was started on hydroxyurea.

Biopsy Fixation Details

The trephine biopsy was formalin-fixed, paraffin-embedded, and decalcified using standard laboratory protocols.

Frozen Tissue Available

No frozen tissue was available.

Details of Microscopic Findings

Following hydroxyurea therapy, leukocytosis decreased to $154 \times 10^9/L$ & blasts to (13%) and relative basophilia (20%). Peripheral blood showed marked neutrophilic leukocytosis with leukoerythroblastic picture, eosinophilia (6%), basophilia (20%), and 13% blasts, some with cytoplasmic blebs (megakaryoblastic differentiation). Dysplastic erythroid and granulocytic precursors, dwarf megakaryocytes, and megakaryocytic fragments were noted. Bone marrow aspiration was dry tap. Trephine biopsy revealed a markedly hypercellular marrow (~100%) with granulocytic hyperplasia, prominent blast infiltration, markedly decreased erythropoiesis, significantly increased atypical and dwarf megakaryocytes in clusters, and striking trilineage dysplasia. Extensive reticulin fibrosis (MF-2-3).

Immunophenotype

Flow cytometry identified (~10%) blasts expressing CD34, CD33, cytoplasmic MPO, CD36, partial CD117, HLA-DR, CD13, and aberrant CD7. A small subpopulation (~3%) co-expressed CD41 and CD61, supporting megakaryoblastic differentiation. Basophilia was confirmed immunophenotypically. Immunohistochemistry showed CD34-positive cells (~5–10%), MPO-highlighted granulocytic hyperplasia, and CD61/VWF-positive increased megakaryocytes with dwarf and clustered forms; occasional blasts were CD61/VWF-positive.

Cytogenetics

Interphase FISH demonstrated BCR-ABL1 rearrangement in 88% of nuclei. Conventional karyotyping showed 46,XY,t(9;22)(q34;q11.2)[20], confirming ph positive.

Molecular Studies

Positive for e13a2 BCR:ABL1 gene fusion by RT-PCR. with major (p210) with ratio 99.9%. Negative for V617F missense mutation

Proposed Diagnosis

Chronic myeloid leukemia, BCR-ABL1–positive, in blast phase associated with extensive marrow fibrosis and striking trilineage dysplasia.

Interesting Feature(s)

This case highlights blast phase CML presenting with extensive marrow fibrosis and striking trilineage dysplasia, creating significant morphologic overlap with myelodysplastic/myeloproliferative neoplasms and primary myelofibrosis. Such features represent recognized manifestations of advanced disease biology in CML and are associated with disease progression and adverse prognosis. The presence of severe fibrosis resulted in a dry tap and limited conventional aspirate-based evaluation, while prominent dysplasia and atypical megakaryocytic proliferation risked misclassification as an alternative myeloid neoplasm. This case emphasizes the necessity of cytogenetic and molecular confirmation of BCR-ABL1 in any high-grade myeloid neoplasm with fibrosis and dysplasia, to avoid diagnostic error and ensure appropriate therapeutic stratification.

Panel Diagnosis: CML, myeloid blast phase

EA4HP26-BMWS-110

Myeloid Neoplasm with *JAK2 V617F*, Fibrosis, and Monocytosis

Dr. Anna Shadid, **Dr. Megan O. Nakashima**

Cleveland Clinic, Pathology and Laboratory Medicine, Cleveland, USA

Case Description

The patient is a 90-year-old man being evaluated for multiple peripheral blood count abnormalities including monocytosis, thrombocytosis, eosinophilia, and normocytic anemia. Per the electronic medical record, monocytosis (11/2021) preceded thrombocytosis (02/2024). In March 2025 lactate dehydrogenase was elevated (268 U/L, normal range 135-225 U/L) and an ultrasound was negative for splenomegaly. Peripheral blood counts at the time of the bone marrow biopsy (April 2025): WBC $9.54 \times 10^9/L$, HGB 10.9 g/dL, PLT $711 \times 10^9/L$, monocytes 29%, absolute monocyte count $2.77 \times 10^9/L$. The patient is being treated with hydroxyurea and most recently displayed worsening thrombocytosis and monocytosis (November 2025: WBC $15.28 \times 10^9/L$, HGB 9.8 g/dL, PLT $820 \times 10^9/L$, monocytes 32%, absolute monocyte count $4.89 \times 10^9/L$).

Biopsy Fixation Details

Trephine biopsy and clot section fixed in formalin. Trephine acid decalcified.

Frozen Tissue Available

No

Details of Microscopic Findings

Aspirate smears are aspicular. Touch imprints and aspirate smears show monocytosis and progressive maturation of erythrocytes and granulocytes, with occasional nuclear hypersegmentation in the latter. Megakaryocytes are rare. The trephine biopsy is hypercellular (50-70%) due to panhyperplasia. Megakaryocytes are increased with focal tight clusters and hyperlobulated megakaryocytes. Reticulin stain shows MF2 fibrosis with focal collagen fibrosis on trichrome.

Immunophenotype

Flow cytometric analysis performed on the bone marrow aspirate shows no evidence of a lymphoproliferative disorder or increased CD34-positive blasts. IHC not performed.

Cytogenetics

46,XY,i(21)(q10)[5]/46,XY[15]

Molecular Studies

NGS performed on peripheral blood positive for *JAK2* V617F (VAF 9.3%) and a variant of potential clinical significance in *TET2* (P1889R, VAF: 45.7%). An NGS fusion panel (RNAseq, bone marrow aspirate) was performed due to the eosinophilia and shows no clinically relevant fusions.

Proposed Diagnosis

JAK2-positive myeloproliferative neoplasm with monocytosis versus a chronic myelomonocytic leukemia-1 with a *JAK2* mutation

Interesting Feature(s)

Myeloproliferative neoplasms with monocytosis and chronic myelomonocytic leukemia with a *JAK2* mutation and fibrosis present a difficulty in diagnosis based on overlapping characteristics. In this patient, monocytosis was the initial peripheral blood abnormality. Monocytosis is present in up to 20% of myeloproliferative neoplasms. Conversely, while most cases of CMML present with a mutation in *TET2*, *SRSF2*, *ASXL2*, or the RAS pathway, which have various indicators of prognosis, *JAK2* V617F is present in ~10% of CMML cases. This patient's *JAK2* mutation is a well-known pathogenic variant; however, the *TET2* variant is not well described and could represent a germline mutation.

Bone marrow megakaryocytic atypia and MF2 fibrosis, *JAK2* V617F with minor criteria anemia and elevated LD fulfill a diagnosis of PMF overt fibrotic stage if other myeloid neoplasms are excluded. The megakaryocyte morphology and clustering also favors MPN. However, the case also fulfills diagnostic criteria for CMML with monocytosis, anemia, clonality, hypercellularity, and lack of *BCR::ABL1* or other tyrosine kinase fusions. *JAK2* V617F VAF is relatively low.

Isoschromosome 21(q) is an uncommon cytogenetic abnormality most often reported in acute lymphoblastic leukemia, with only rare reports in myeloid neoplasms including one report of acute myeloid leukemia with eosinophilic differentiation (PMCID: PMC3777464). It is also most often accompanied by other cytogenetic abnormalities, instead of being an isolated abnormality, as in this case.

Panel Diagnosis: Myeloproliferative CMML-1 / CMML-1, myeloproliferative type

EA4HP26-BMWS-113

Myelodysplastic neoplasm (MDS) with increased blasts and fibrosis (MDS-F)

Dr. Kedar V. Inamdar¹, Dr. Madhu Menon²

¹ Henry Ford Health, Pathology, Detroit, USA; ² University of Utah, ARUP Laboratories, Pathology, Salt Lake City, USA

Case Description

69-year-old male with no significant past history, presented with 3 months of fatigue. Laboratory studies at presentation showed WBC 2.9 K/uL, Hemoglobin 10.8 g/dL, Platelets 58K/uL, MCV of 93.4 fL, normal Vitamin B12/folate and LDH. His last CBC 4 years ago was normal. CT chest, abdomen and pelvis at admission was negative for hepatosplenomegaly.

Biopsy Fixation Details

10% formalin.

Frozen Tissue Available

No

Details of Microscopic Findings

Peripheral Smear: Macrocytic normochromic anemia, mild dysgranulopoiesis. No blasts or monocytosis.

Bone marrow:

Core biopsy – Hypercellular for age (90-95% cellularity) with erythroid predominant trilineage hematopoiesis, and a marked atypical megakaryocytic hyperplasia. The latter show small, hypo- or monolobated, bulbous hyperchromatic or bizarre nuclei, micromegakaryocytes and loose clusters.

Bone marrow aspirates: Hemodilute and non-representative

Touch Preparation:

A 500-cell differential count reveals - Blasts 5%, Promyelocyte 1%, Monocytes 2%, Myelocyte 9%, Metamyelocyte 6%, Bands 11%, PMN:12 %, Erythroid precursors 39%, Eosinophils 1%, Plasma Cells 1% and Lymphocytes 13%. Significant (>10%) dysplasia is noted in granulocytes and erythroid precursors, the latter with increased (44%) ring sideroblasts on iron-stained touch preparation.

Immunophenotype**Flow cytometry:**

Blasts account for 6-7% and are mix of hematogones (approx. 5%) and myeloblasts (approx. 1.5-2%). The myeloblasts are :

Positive for: CD45 (dim), CD34 (bright), CD117, HLA-DR, MPO, CD13 and 33 (subset)

Negative for: CD2, CD3, CD5, CD7, CD10, CD11c, CD14, CD15, CD16, CD19, CD20, CD56, CD61, CD235, TdT, CD79a, cyto CD3

The maturing myeloid precursors demonstrate a moderate loss of SSc on the CD45 vs SSc profile and maturation abnormalities of CD13 vs CD16, CD13 vs CD11b and CD11b vs CD16 profiles.

Immunohistochemistry:

CD34, CD117: Increased blasts, estimated 5%

TdT: Increased hematogones

Factor 8: Abnormal megakaryocytic population with frequent monolobated forms and micromegakaryocytes

Special Stains:

Reticulin and Masson Trichrome reveal predominantly grade 1-2 and focally grade 3 fibrosis

Cytogenetics

Karyotype – Complex. -5,-7,dic(14;20)(p12;q11.2),der(15)t(15;17)(p11.2;q21),-17,-18,-21,+2mar[4]/41,sl,-der(15)t(15;17)(p11.2;q21),der(15)t(11;15)(q13;p11.2), +17,der(17)t(8;17)(q13;p11.2), 2mar[2]/40,sl,- 5,-dic(14;20)(p12;q11.2),-16, add(20)(q11.2)[2]/46, XY[12]

FISH: -5q, -7, -20q and endoreduplication

Molecular Studies

Targeted NGS- negative for *JAK2*, *CALR* and *MPL* targeted regions

Proposed Diagnosis

Myelodysplastic neoplasm with increased blasts (MDS-IB1) and fibrosis (MDS-F)

Interesting Feature(s)

Some of the megakaryocytes exhibit cloudy bulbous and hyperchromatic nuclei and form clusters, raising the possibility of MDS/MPN. Patient, however, never had cytoses and targeted NGS was negative for driver mutations, thus arguing against MDS/MPN.

Progressive pancytopenia, trilineage dysplasia and complex cytogenetics are consistent with myelodysplastic syndrome (MDS) - MDS with excess blasts (MDS-IB-1). Significant degree of myelofibrosis corresponding to WHO grade 2-3 is consistent with MDS with fibrosis (MDS-F).

An interesting feature in this case is the mild increase in hematogones. Hematogones are typically decreased/absent in MDS, a helpful feature to distinguish MDS from treatable conditions like copper deficiency or B12 deficiency, which can mimic MDS. Some MDS subtypes, particularly MDS with ring sideroblasts (MDS-RS) or MDS with no detectable mutations, show preserved levels of hematogones, likely suggesting better prognosis in these cases (PMID: 31211490).

Panel Diagnosis: MDS-biTP53 / MDS-TP53 (presumptive by IHC)

Myelodysplastic syndromes/neoplasms (MDS) with accompanying fibrosis: is it a matter of fibrosis or of covariates such as amounts of blast and/or mutational composition?

Dr. Alexandar Tzankov, Dr. Maria Tapken

University Hospital Basel, Pathology, Basel, Switzerland

Case Description

M 66

pancytopenia

vitamin B12 deficiency

bilateral hilar lymphadenopathy

due to a high clinical suspicion of MDS, a diagnostic bone marrow biopsy (BMB) was obtained prior to vitamin supplementation

Biopsy Fixation Details

4% buffered formalin/EDTA-based decalcification utilizing DecalMATE

Frozen Tissue Available

no

Details of Microscopic Findings

Hhypercellular BMB (cellularity approx. 90%). Increased megakaryopoiesis, occasionally grouped, with hypolobated and alobated forms in the sense of dysmegakaryopoiesis. Increased erythropoiesis with megaloblastic changes. Slightly impaired maturation of the myelopoiesis. No blast increase. Diffuse myelofibrosis (MF) grade 2.

Immunophenotype

Immunohistochemistry:

CD34: massive increase of the microvessel density, positivity in atypical megakaryocytes, the proportion of CD34-positive blasts is 2%, in some areas 4%

CD61: highly atypical megakaryopoiesis with at least 50% dysplastic forms

TP53: stains approximately 3% of cells and is completely negative in the remaining cells (both patterns considered pathologic)

Flow cytometry:

not performed because of a dry tap.

Cytogenetics

not performed because of a dry tap

Molecular Studies

Next Generation Sequencing:

CEBPA H195_P196del with a VAF of 47% (germline)

TP53 R213* with a VAF of 10% (pathogenic)

Proposed Diagnosis

MDS, NOS with multilineage dysplasia (ICC 2022) and accompanying MF grade 2 / borderline to MDS with mutated TP53

MDS with low blast content (WHO-5) and accompanying MF grade 2

Interesting Feature(s)

The case clearly illustrates

1. the arbitrariness of introducing a threshold value of 5% blasts for the diagnosis of MDS with fibrosis (our case shows focally up to 4% blasts in the trephine biopsy, and a further reliable determination was not possible on cytology due to a dry tap, which will generally apply to the majority of myeloid neoplasms with associated fibrosis)

2. the difficulties of accurately classifying genetically defined diseases, in which the VAF of mutations plays a decisive role in the definition, if the results of the molecular tests are at the threshold
 3. the interconnection between the genetic background of the myeloid neoplasm (in this specific case, *TP53* mutated, but not meeting the criteria for a multihit/biallelic *TP53* mutation according to WHO-5, admittedly being borderline according to ICC, as the VAF of the *TP53* mutation was exactly 10%) and MF
 - In general, high-risk mutations, including monoallelic *TP53* mutations, are present in such cases at an above-average frequency (Zhao et al., Cancers 2022;14:2984).
 4. the arbitrariness of introducing MF $\geq$ 2 as the threshold value for this diagnosis and the lack of robust evidence that MF (either 2 or 3) is an independent prognostic factor in MDS
 - In the original publications, particularly MF $>$ 2 in MDS seemed to be a blast-independent prognosticator (MF $>$ 2 worsens the prognosis of both MDS with increased blasts and with low blast counts) but it was anyway tightly linked to covariates of high risk such as age (MDS patients with MF $>$ 2 were on average 3-5 years older than those without MF or with MF1-2), blast counts (MDS patients with MF $>$ 2 had on average 2-5% more blasts than those without), high-risk MDS types according to WHO-4 (e.g., cumulation of therapy-associated MDS and MDS-EB), and high-risk mutations, mostly *TP53*, *U2AF1* and *SETBP1* (Della Porta et al., J Clin Oncol 2009; 27:754, Melody et al., Clin Lymphoma Myeloma Leuk 2020;20:324; Zhao et al., Cancers 2022;14:2984).
- Together with other cases in this workshop, our case questions the rationale for retaining the category of MDS-F in any future classifications of myeloid neoplasms.

Panel Diagnosis: MDS-LB / MDS-NOS

EA4HP26-BMWS-119

Myelodysplastic / myeloproliferative neoplasm with fibrosis and features overlapping between atypical CML and CMML

Dr. Elizaveta Flerova, Dr. Kaaren Reichard, Dr. April Chiu

Mayo Clinic, Department of Laboratory Medicine and Pathology, Rochester, USA

Case Description

78-year-old female with history of unprovoked pulmonary emboli presented with splenomegaly, neutrophilic leukocytosis (WBC $39.3 \times 10^9/L$, ANC $23.2 \times 10^9/L$), borderline monocytosis (intermittently $>1.0 \times 10^9/L$, not sustained for ≥ 3 months), anemia (Hgb 11.7 g/dL, MCV 88.8 fL), and thrombocytopenia (PLT $83 \times 10^9/L$). Three months after the diagnosis, she developed progressive leukocytosis (WBC $111.6 \times 10^9/L$) with neutrophilia, 11% granulocytic precursors (metamyelocytes 1%, myelocytes 10%), worsening monocytosis [21%, AMC $23.4 \times 10^9/L$], 3% circulating blasts, and 5-9% bone marrow blasts, concerning for progression. The patient passed away due to stroke.

Biopsy Fixation Details

10% buffered formalin, decalcified

Frozen Tissue Available

N/A

Details of Microscopic Findings

Peripheral blood: Neutrophilia with granulocytic left shift [neutrophils 59%, metamyelocytes 3%, myelocytes 9%, promyelocytes 1%, monocytes 10% [AMC ($3.9 \times 10^9/L$)]. Hypogranular and hypolobated neutrophils. Large hypogranular platelets.

Bone marrow:

- Hypercellular (90%). Increased M:E ratio.

- Granulocytic precursors: Markedly increased. Left-shifted maturation. Blasts <5%. Hypogranular and hypolobated forms.
- Megakaryocytes: Markedly increased with clustering. Small forms with monolobated and separated nuclei.
- Erythropoiesis: Decreased. Normal morphology.
- Moderately increased reticulin fibrosis (MF-2).

ImmunophenotypeFlow cytometry:

Bone marrow: abnormal granulocyte maturation by CD13/CD16. Myeloblasts with abnormal HLA-DR/CD13 expression pattern and aberrant CD7 expression.

Peripheral blood (monocyte repartition assay): increased classic monocytes (MO1, 97.8%) by CD14/CD16.Immunohistochemistry:

CD61: increased megakaryocytes with dysplastic morphology and clustering. CD14: increased monocytes. CD123: no nodules of plasmacytoid dendritic cells

Cytogenetics

46,XX[20]

Molecular Studies

RT-PCR, *BCR/ABL*: Negative

PCR, *JAK2 V617F*: Positive (VAF 0.3%)

NGS: pathogenic mutations in *ASXL1* (46%), *EZH2* (93%), *NF1* (56%), *RAD21* (42%), *STAG2* (10%), *TET2* (23%).

At progression: increase in *NF1* VAF (87%).

Proposed Diagnosis

Chronic myeloid neoplasm; with features overlapping:

1. Atypical chronic myeloid leukemia (aCML; ICC) / myelodysplastic/myeloproliferative neoplasm with neutrophilia (MDS/MPN-N, WHO5)
2. Chronic myelomonocytic leukemia-1 (CMML-1)

Interesting Feature(s)

1. Features overlapping between MDS (anemia, thrombocytopenia, granulocytic and megakaryocyte dysplasia) and MPN (splenomegaly, leukocytosis with neutrophilia, megakaryocyte clustering, monocytosis).

2. Features favoring aCML: neutrophilic leukocytosis with >10% circulating immature myeloid cells, dysgranulopoiesis, *EZH2/ASXL1* co-mutation. Features favoring CMML: progressive monocytosis, increased MO1 fraction, *NF1* mutation.

3. Molecular profile

- *EZH2/ASXL1* co-mutated subgroup was proposed in molecular taxonomy of MDS (*PMID: 38958467*). It was enriched in MDS/MPN, specifically aCML, and characterized by high mutational complexity and poor prognosis, despite low blast count.
 - *NF1* VAF increased at progression, illustrating its role in MDS/MPN evolution. RAS pathway mutations, including *NF1*, have worse prognosis in MDS/MPN (*PMID: 32513965*).*NF1* mutation was also more frequent in CMML than in other MDS/MPNs in one study (*PMID: 40151938*).
 - *ASXL1* and *TET2* mutations are seen in both CMML and aCML.
 - *JAK2* mutation has low VAF (0.3%) and less likely to contribute to the findings.
4. Fibrosis may contribute to disease aggressiveness. It is associated with shorter progression-free survival in CMML (*PMID: 29262560*). In aCML, it is seen in 20-30% of cases, but its effects have not been systematically studied.

Panel Diagnosis: Myeloproliferative CMML-1 / CMML-1 myeloproliferative type

MDS-biTP53, MDS-F, or both? WHO and why has it been decided what counts?

Dr. Maria J. Tapken, Prof. Alexandar Tzankov

Universitätsspital Basel, Institut für medizinische Genetik und Pathologie, Basel, Switzerland

Case Description

68 yrs old female, presents with pancytopenia. CT-scan revealed an old fracture of the thoracic vertebral body XII but no lymphadenopathy, hepatosplenomegaly, osteodestruction or tumor manifestation. A bone marrow biopsy was performed.

Biopsy Fixation Details

4% buffered formalin/EDTA-based decalcification utilizing DecalMATE

Frozen Tissue Available

No

Details of Microscopic Findings

Pre-allo: Hypercellular BM (approx. 85 %). Increased megakaryopoiesis, relevant amount of micromegacaryocytes. Left-shifted erythropoiesis. Slightly impaired maturation of the myelopoiesis. Blast increase (18.8%). Myelofibrosis grade 1.

Relapse: Hypercellular BM (irregular distribution, focally up to 90 %). Dysmegakaryopoiesis with relevant amounts of hypolobated forms. Slightly reduced erythropoiesis. Impaired maturation of the myelopoiesis. Blast increase (15%). Myelofibrosis grade 2.

Immunophenotype

Blast phenotype by FCM pre-allo:

CD34+ CD117+ HLA-DR+ CD13+ cMPO part+ (31%) CD123+ CD105- CD71-

Blast phenotype by FCM relapse post-allo

P1: CD34+ CD117+ HLA-DR+ CD13+ CD105dim/+ CD71+ cMPO part (19%) CD123 part + (70%)

P2: CD34dim/+ CD117+ HLA-DR- CD13- CD105- CD71+ cMPO- CD123 part + (22%) CD42a/CD61 part + (57% +) CD42b part + (57%) CD41 part+ (58%)

Cytogenetics

Pre-allo (microarray):

Complex karyotype with several imbalances:

Monosomy 7, trisomy 8, deletions of 3p, 5p, 5q (incl. *EGR1*, *PDGFRB*, *RPS14*), 12p (incl. *ETV6*), 18q and 19p, gains of 19q and 22q

Relapse (DNA quality impaired; FISH):

Deletion of 5q, monosomy 7

Molecular Studies

Pre-allo:

1) *TP53*; chr17:7578271; T>C; H193R; VAF 41%

2) *TP53*; chr17:7578406; C>T; R175H; VAF 45%

Relapse:

1) VAF 26 %

2) VAF 31 %

Proposed Diagnosis

Myelodysplastic neoplasm with biallelic *TP53* inactivation (WHO 5)/

myelodysplastic syndrome / acute myeloid leukemia with *TP53* mutation (ICC 2022)

Interesting Feature(s)

Another case of MDS, myelofibrosis and *TP53*-mutation, here biallelic, with reference to BMWS #117 (A. Tzankov) - there monoallelic.

Interconnection of the genetic background and myelofibrosis:

MF is tightly linked to high-risk mutations (e.g. *TP53*, but also *SETBP1*; Della Porta MG, J Clin Oncol 2009;27:754, Zhao et al., Cancers 2022;14:2984), questioning the rationale of promoting MDS-F as a stand-alone subentity.

MDS with MF seems to run a poor clinical course irrespective of the blast counts (Della Porta MG, J Clin Oncol 2009;27:754), questioning the rationale of a blast count requirement $\geq 5\%$ to define (high(er) risk) MDS with MF, as suggested by the WHO-5 for MDS-F.

The case finally iterates the poor prognosis of MDS with biallelic *TP53*-mutation, which is shown to be independent of the blast count (Bernard E et al., Nat Med 2020 Oct;26(10):1549-1556), as our patient died of progressive disease shortly after her relapse, and underscoring the rationale of introducing the category of MDS-(bi)*TP53*.

Panel Diagnosis: MDS-bi*TP53* / MDS-*TP53*

EA4HP26-BMWS-149

A difficult-to-classify myeloid neoplasm at the PMF–CMML interface

Dr. Claire Royer¹, Dr. Natacha Dewarrat², Dr. Noemy Friedrich², Dr. Julien Dereme², Prof. Sabine Blum², Prof. Laurence de LEVAL¹

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Case Description

A 75-year-old man with untreated hypertension presented in 12.2024 with a progressive asthenia, dyspnea, and diffuse muscle spasms. Laboratory findings showed leukocytosis ($43 \times 10^9/L$) with 37% neutrophils, 165 lymphocytes, 25% monocytes and 13% circulating blasts, thrombocytopenia (80G/L), anemia (89 g/L), hypocalcemia with hyperphosphatemia, and elevated urate and LDH levels, suggestive of tumor lysis syndrome in the context of suspected chronic myelomonocytic leukemia. Blood smear revealed left shifted dysplastic granulopoiesis, circulating erythroblasts, increased blasts, and true thrombocytopenia. CT imaging showed marked splenomegaly (17 cm) with heterogeneous bone marrow, consistent with an underlying myeloproliferative neoplasm.

Biopsy Fixation Details

Zinc-Acetic-Formalin

Surface decalcification of bone marrow biopsy

Frozen Tissue Available

No

Details of Microscopic Findings

The patient underwent two biopsies eight months apart, showing similar microscopic findings dominated by marked osteomyelosclerosis with irregularly thickened trabeculae and extensive reticulin and collagen fibrosis (MF-2 to MF-3), largely replacing the marrow spaces. Adipocytes accounted for less than 25% of marrow spaces but overall hematopoietic cellularity was reduced ($<30\%$) and unevenly distributed.

It comprised an atypical megakaryocytic hyperplasia, composed of large hyperchromatic forms, naked nuclei, and small hypolobated megakaryocytes arranged in loose clusters with paratrabecular and intrasinusoidal distribution. Granulocytic proliferation predominated with left-shifted maturation, while the erythroid lineage was markedly decreased. An increased monocytoid population was present.

Immunophenotype

Megakaryocytes highlighted by factor VIII, CD42b, and CALR, included intrasinusoidal forms, without evidence of micromegakaryocytes. CD34 highlighted increased vessels and an excess of blasts with heterogeneous distribution, accounting for approximately 6–7% of cellularity (focally >10%). CD68 and CD14 showed an increased monocytic/histiocytic population (15–20%).

Cytogenetics

Normal karyotype : 46,XY[20]

Molecular Studies

DNA microarray : *TET2* : loss of 4q24q24 ; 90%.

NGS : *CALR*- type 2 like : mutation c.1138_1144del, p.(Glu380GlnfsTer48) ; 38% VAF; *CALR* : mutation c.1227_1228delinsGT, p.(Pro410Ser) ; 38% VAF; *TET2* : mutation c.3812dup, p.(Cys1271TrpfsTer29) ; 62% VAF

Proposed Diagnosis

Myeloid neoplasm that is difficult to categorize, exhibiting characteristics shared between primary myelofibrosis (PMF) in the osteosclerosis stage, with monocytosis and excess blasts, and chronic myelomonocytic leukemia (CMML) with fibrosis.

Interesting Feature(s)

This 75-year-old patient presents a rare overlap of clinical, morphological, and molecular features bridging PMF and CMML. CMML characteristics include advanced age at presentation, coexisting cytopenias (anemia, thrombocytopenia) and leukocytosis with monocytosis, bone marrow myelomonocytic predominance with reduced erythropoiesis, normal karyotype, and a *TET2* mutation. Concurrently, *CALR* mutations plus features associated with PMF are present, such as marked leukocytosis, splenomegaly, megakaryocytic proliferation with atypia, and grade 2–3 reticulin/collagen fibrosis in a marrow with reduced age-adjusted cellularity. The coexistence of monocytosis with a *CALR*-mutated fibrotic marrow highlights an uncommon CMML/MPN overlap phenotype.

Panel Diagnosis: PMF, fibrotic / Overt PMF, accelerated phase vs Myeloproliferative CMML-2; presenting with features of both

EA4HP26-BMWS-163

Case submission

Kärt Tomberg

North Estonia Medical Centre, Pathology, Tallinn, Estonia

Case Description

Clinical history: 53 y old man with arthralgias in different joints for some months, fatigue, no weight loss, no regular medication use. Former alcohol abuse, now nondrinker for several years.

GP performed blood analyses; moderate thrombocytopenia was detected:

WBC 7,35x10⁹/L RBC 4,18x10¹²/l Hgb 132g/L MCV 90,0fL Hct 37,6%, Plt 70x10⁹/L. Microscopic evaluation showed 1% blasts and left shift proMc 2% Mc21% MMc 4% band 18% Segm 36% Eo 2% Mono 2% Ly14%, normoblasts 1/100. LDH 259 U/L (N:<250).

CT scan showed splenomegaly 24cm.

Biopsy Fixation Details

fixed with 10% neutral buffered formaldehyde and decalcified with 5% formic acid.

Frozen Tissue Available

No

Details of Microscopic Findings

Morphologic findings: BM aspirate „dry tap “. Current myeloid cells showed dysplastic features – hypogranulation, pelgeroid nuclei. Severely decreased cellularity with moderate reticulin fibrosis. Reduced granulopoiesis and erythropoiesis and single dysplastic megakaryocytes with one bigger cluster. He reached to the hospital one and a half months later with severe anaemia and thrombocytopenia: WBC 13,29x10⁹/L RBC 2,33x10¹²/l Hgb 73g/L MCV 90,1fL Hct 21,0%, Plt 20x10⁹/L. For some unknown reason second biopsy was performed before therapy was started. Biopsy was more informative. Moderate to severe reticulin fibrosis (grade 2-3), and patchy collagen fibrosis. Cellularity mostly severely decreased with some hypercellular regions. Hypervascularized, with increased numbers of dilated sinuses. In cellular region MPO/CD33 myeloid proliferation, single CD34 blasts and CD117 early precursors, scattered mastocytes. CD71 erythropoiesis severely reduced, single cells. Small hypolobulated CD61 megakaryocytes.

Immunophenotype

FC detected CD34+CD117+ blasts 1,8%.

Cytogenetics

Karyotype: 46,XY,i(17)(q10)[20].

Molecular Studies

NGS myeloid panel *OGT SureSeq Pan-Myeloid panel* (70 genes).

NM_015338.6(ASXL1):c.1934dup, p.(Gly646Trpfs*12), coverage 1157x, VAF 34,47%.

NM_004343.4(CALR):c.1099_1150del, p.(Leu367Thrfs*46), coverage 637x, VAF 15,9%.

Proposed Diagnosis

MDS/MPN-U with isolated isochromosome 17

Interesting Feature(s)

CALR positive along with i17q is very rare. No concomitant SRSF2 or SETBP1 mutations, usually detected with i17q. Is it progression of prior underlying CALR positive MPN not diagnosed before? Dysplasia not consistent with MPN. Blood analyses in that case were normal in 2022. According to the study published: Rashmi K.-S. et al: Modern Pathology (2022) 35:470 – 479 Proposed criteria for MDS/MPN with isolated isochromosome (17q) suggests to exclude cases with MPN associated mutations from MDS/MPN with isolated isochromosome (17q).

Panel Diagnosis: MDS-LB / MDS-NOS

EA4HP26-BMWS-165

MDS/AML with mutated *TP53*, significant fibrosis, and striking proliferation of dysplastic CD34-p53 co-expressing megakaryocytes

Dr. Joshua Lewis, Dr. Sam Sadigh

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

Case Description

The patient is a 65-year-old male with a history of multiple deep venous thromboses, on rivaroxaban, who presented with acute-on-chronic fatigue and shortness of breath. He was found to have severe pancytopenia (WBC 0.7 K/ μ L, Hgb 8.1 g/dL, PLT 10 K/ μ L). Additional laboratory findings included elevated LDH and ferritin levels, as well as positive parvovirus B19 and EBV IgG serologies, with negative IgM serologies. A bone marrow biopsy was performed.

Biopsy Fixation Details

Bouin's solution

Frozen Tissue Available

No

Details of Microscopic Findings

The bone marrow aspirate is aspicular and hemodilute. The touch preparation is hypocellular/limited but shows increased blasts (9%) and dysplastic megakaryocytes, including small forms with hypolobated nuclei. The core biopsy is moderately hypercellular for age, with areas of severe architectural distortion consistent with fibrosis, left-shifted erythroid lineage and reduced myeloid maturation. There is a pronounced proliferation of megakaryocytes, with small clusters associated with areas of fibrosis, and with marked dysplasia including numerous micromegakaryocytes. Binucleate erythroid forms are also noted. Clusters of immature cells with irregular nuclei are present, consistent with an increased blast population.

Immunophenotype

CD34+ blasts comprise 15% of the cellularity. Strong CD34 expression is observed among both the myeloblast and dysplastic megakaryocyte populations, the latter also express CD61 and GATA1. A dual immunostain for CD34/GATA1 further highlights the two populations. A mutant p53 overexpression pattern is present, with highly expressing cells comprising 30% of cellularity. Notably, p53-CD34 co-expression is observed in both the myeloblast and megakaryocytic lineages. Moderate (MF-2) reticulin fibrosis is present, with focal areas of marked (MF-3) fibrosis and focal collagen deposition.

Cytogenetics

Karyotype: No metaphases obtained

FISH: 5q deletion (9% of nuclei), 7q deletion (19% of nuclei)

Molecular Studies

NGS studies showed:

- *TP53* p.V272G 18.6% VAF
- *TP53* p.R273P 8.5% VAF
- *PPM1D* p.L482Rfs*3 3.7% VAF

TP53 mutations are noted to be in trans. No RNA fusion transcripts detected.

Proposed Diagnosis

Myelodysplastic neoplasm with biallelic *TP53* inactivation (WHO)

MDS/AML with mutated *TP53* (ICC)

Interesting Feature(s)

A minority of cases of MDS with increased blasts (~15%) demonstrate moderate to marked (MF-2-3) fibrosis; these cases are associated with more prominent megakaryocytic dysplasia and a higher frequency of biallelic *TP53* inactivation. This case shows a particularly striking degree of megakaryocytic proliferation and dysplasia with numerous micromegakaryocytes, associated with areas of fibrosis. While the degree of megakaryocytic proliferation with occasional clustering may raise consideration for a fibrotic-phase myeloproliferative neoplasm, the absence of large MPN-like megakaryocytes precludes this diagnosis. In addition, strong co-expression of CD34 (unusually strong for megakaryocytes) and p53 is observed among both the myeloblast and megakaryocyte populations; this co-expression has previously been associated with shorter overall survival amongst patients with MDS with fibrosis.

Panel Diagnosis: MDS-biTP53 / MDS-TP53

EA4HP26-BMWS-168

Case report

Kärt Tomberg

North Estonia Medical Centre, Pathology, Tallinn, Estonia

Case Description

74 y old, previously athletic man with cough. No fever. Complains with night sweats, fatigue, reduced physical activity level, shortness of breath on exertion. Weight loss of 6 kg over a few months.

Was in hospital two months ago with elevation of inflammatory markers and anaemia. SR >140 mm/h, CRV 100 mg/L, WBC 4,41x10⁹/L RBC 2,81x10¹²/L Hgb 77g/L MCH 27,4pg MCV 84,7fL Hct 23,8% Plt 373x10⁹/L S-Ferritin 359 g/L S-Fe 3,61 µmol/L S-Transferrin 1,71g/L Tra sat 8,8% FH, B12 N. Treated with AB and 2 units blood transfusion for anaemia with iron deficiency.

Approximately six months ago his blood test revealed mild anaemia only: WBC 4,59x10⁹/L RBC 4,7x10¹²/L Hgb 129 g/L MCH 27,4pg MCV 85,7fL Hct 40,3% Plt 274x10⁹/L.

During this hospital visit: WBC 5,0x10⁹/L RBC 3,55x10¹²/L Hgb 90g/L MCH 25,4 pg MCV 83,1fL Hct 29,5% Plt 420x10⁹/L. Microscopic evaluation: Blasts 20%, Mc 5%, MMc 1%, Band 4%, Segm 37%, Baso 1%, Mono 5%, Ly 27% Nbl 8/100, severe aniso-, poikilocytosis. LDH 456 U/L (N: <250U/L).

CT: no lymphadenopathy, no splenomegaly 2 mo ago, now spleen 12.9 x 14.8 x 6.3 cm, index 1202

Biopsy Fixation Details

fixed with 10% neutral buffered formaldehyde and decalcified with 5% formic acid.

Frozen Tissue Available

No

Details of Microscopic Findings Bone marrow aspirate dry tap. Moderate dysplastic features in granulocytes.

In BM biopsy cellularity severely decreased. Moderate reticulin fibrosis (grade 2), with slight collagen fibrosis. Hypervascularization, with increased numbers of dilated sinuses. MPO+ granulopoiesis severely reduced, single CD34 blasts and some clusters of CD117 early precursors. Scattered CD117+ mastocytes. CD71 erythropoiesis moderately reduced, unevenly distributed single cells. CD61 megakaryocytes moderately increased, with dysplastic features, unevenly distributed, loose clusters and atypical peritrabecular localization.

Immunophenotype

FC of bone marrow aspirate examination, with presumed additional peripheral blood, detected 16% myeloid blasts CD34+, CD117+, CD13+, HLADR+, CD38+(dim), MPO-, CD33-, CD11b-, CD15-, cyto79a-, CD19-, cyto3-, CD3-, CD7-, CD2-, CD56-.

Cytogenetics

karyotype performed from peripheral blood: 46,XY,+14,der(14;14)/q10;q10[12]/46,XY[1].

Molecular Studies

NGS myeloid panel *OGT SureSeq Pan-Myeloid panel* (70 genes).

NM_015338.6(ASXL1):c.4233G>A, p.(Trp1411*), coverage 2200x, VAF 36,32%.

NM_006758.3(U2AF1):c.470A>C, p.(Gln157Pro), coverage 1566x, VAF 33,87%.

NM_015559.3(SETBP1):c.2612T>C, p.(Ile871Thr), coverage 2652x, VAF 33,7%.

NM_015559.3(SETBP1):c.2602G>A, p.(Asp868Asn), coverage 2729x, VAF 1,14%.

NM_004972.4(JAK2):c.1849G>T, p.(Val617Phe), coverage 2422x, VAF 14,5%.

NM_005896.4(IDH1):c.395G>A, p.(Arg132His), coverage 2163x, VAF 3,84%.

Proposed Diagnosis

MDS-MPN-U with fibrosis and extramedullary hematopoiesis with transformation to acute myeloid leukemia.

Interesting Feature(s)

Patient has splenomegaly, extramedullary blastosis, BM fibrosis, JAK2 positivity – consistent with JAK positive MPN, but reportedly no former changes in blood values half a year ago, nor clinical or radiological features of MPN, and considering dysplastic morphology of bone marrow and molecular studies, it preferably supports transformation of MDS-MPN-U.

Panel Diagnosis: MDS-F / MDS/AML

SETBP1-mutated myelodysplastic/myeloproliferative neoplasm with fibrosis and unusually pronounced thrombocytosis, arising from underlying CCUS

Dr. Joshua Lewis, Dr. Sam Sadigh

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

Case Description

The patient is an 84-year-old male with a history of stage I colon cancer, chronic kidney disease, and type 2 diabetes mellitus. He was noted to have mild anemia (Hgb 11-12 g/dL) since 2016, with otherwise normal white blood cell and platelet counts. A bone marrow biopsy performed at an outside institution in 2019 showed a mildly hypercellular marrow with maturing trilineage hematopoiesis, without significant dysplasia, increased blasts, or reticulin fibrosis. At the time, diagnostic morphologic features of a myeloid neoplasm were not identified, and low-VAF mutations in *DNMT3A* (8.4%, 4.5%), *SF3B1* (7.2%), and *ZRSR2* (7.5%) were interpreted as consistent with clonal cytopenia of undetermined significance (CCUS).

In 2025, routine laboratory evaluation demonstrated marked thrombocytosis (PLT 2,020 K/ μ L), and three months later, the patient had developed transfusion-dependent anemia (Hgb 6.7 g/dL) and intermittent leukopenia (WBC 2.63 K/ μ L) with 6.1% circulating blasts, prompting a repeat bone marrow biopsy.

Biopsy Fixation Details

Bouin's solution

Frozen Tissue Available

No

Details of Microscopic Findings

The bone marrow aspirate and touch preparation show myeloid-predominant maturing trilineage hematopoiesis with 4% blasts. Neutrophilic dysplasia is present, including forms with pale cytoplasm or abnormal nuclear segmentation and chromatin patterns. Megakaryocytic dysplasia, including micromegakaryocytes, is also identified. No ring sideroblasts are seen on the iron stain.

The core biopsy is markedly hypercellular for age, with maturing trilineage hematopoiesis and areas of severe architectural distortion consistent with fibrosis. Large clusters of highly dysplastic megakaryocytes are present, predominantly composed of forms with small hypolobated nuclei and micromegakaryocytes, which are seen interspersed within areas of fibrosis.

Immunophenotype

CD34+ blasts are focally increased, overall comprising 5% of the cellularity. Moderate to marked (MF-2-3) reticulin fibrosis is present, with increased deposition of loose collagen fibers.

Cytogenetics

Karyotype: 46,XY[cp20]

Molecular Studies

NGS studies showed:

- *DNMT3A* p.A269Gfs*12 3.9% VAF
- *DNMT3A* p.G118* 0.2% VAF
- *EZH2* p.P351Hfs*73 81.7% VAF
- *SETBP1* p.G870S 31.9% VAF
- *SF3B1* p.K666N 0.3% VAF
- *ZRSR2* p.R169* 77.1% VAF

BCR::ABL1 p210 is negative. No RNA fusion transcripts detected.

Proposed Diagnosis

Myelodysplastic/myeloproliferative neoplasm, NOS (WHO, ICC)

Interesting Feature(s)

This case represents evolution of a prior anemia-associated myeloid clone into a myelodysplastic/myeloproliferative neoplasm characterized by marked thrombocytosis and fibrosis, with acquisition of new *EZH2* and *SETBP1* mutations, posing a challenging classification. The very low VAF of the identified *SF3B1* mutation and the absence of ring sideroblasts preclude a diagnosis of MDS/MPN with *SF3B1* mutation and thrombocytosis. Additionally, although this neoplasm is associated with a myeloid-predominant marrow and *SETBP1* mutation, diagnostic thresholds of leukocytosis or neutrophilia are not met for classification as MDS/MPN with neutrophilia (WHO) / atypical chronic myeloid leukemia (ICC). While fibrotic-phase MPN could be considered, thrombocytosis developed after longstanding history of anemia, there is lack of larger MPN-type megakaryocytes, and no canonical MPN driver was identified, arguing against this consideration. Given the absence of defining features for a more specific myeloid neoplasm, this case is best classified as myelodysplastic/myeloproliferative neoplasm, NOS.

Panel Diagnosis: MDS/MPN, NOS (potentially from CCUS)

EA4HP26-BMWS-184

Myelodysplastic syndrome (MDS) with marked fibrosis, low blasts, and myeloid predominance associated with profound erythroid and megakaryocytic hypoplasia

Dr. Haluk Kavus, Dr. Jon Aster

Brigham and Womens Hospital, Hematopathology, Boston, USA

Case Description

- 74 year-old very active, robust person with decreased exercise tolerance, went to the emergency department where he was found to be quite anemic with a hemoglobin of 9.
- Previous CBC from a year ago was normal.
- His platelet count was 82,000 and he had a normal white count.
- Bone marrow biopsy was performed.

Biopsy Fixation Details

- Bouin's solution; specimen is briefly decalcified in RapidCal-Immuno for 30 minutes

Frozen Tissue Available

Unavailable

Details of Microscopic Findings

- H&E sections of the bone marrow core biopsy reveal a markedly hypercellular marrow (>90% cellularity) with an unusual architectural pattern characterized by a proliferation of myeloid elements and near-complete absence of erythroid precursors and megakaryocytes.
- The myeloid elements demonstrate complete maturation.
- The myeloid-to-erythroid ratio is markedly elevated.
- The aspirate smears attempted but resulted as dry-tap.
- The touch preparation is hypocellular and demonstrates a marked predominance of maturing myeloid forms (92%), with only rare erythroid precursors (1%) and occasional blasts (2%).
- Peripheral blood evaluation reveals cytopenias with anemia, leukopenia, and thrombocytopenia, along with circulating nucleated red blood cells and occasional hypogranular and hyposegmented neutrophils, consistent with dysplastic features.

Immunophenotype

- Erythroid precursors are profoundly decreased, and megakaryocytes are essentially absent, as demonstrated by CD61 immunostaining.
- Blasts comprise less than 5% of the cellularity by CD34 immunohistochemistry.
- Notably, there is marked reticulin fibrosis (WHO grade MF-3) on reticulin staining, with trichrome stain confirming the presence of marked collagen deposition.

Cytogenetics

- Cannot be done due to dry-tap, a bone marrow biopsy 5 months prior showed normal karyotype.

Molecular Studies

- Cannot be done due to dry-tap on this marrow but a bone marrow biopsy 5 months prior showed
- Molecular myeloid panel:
- TET2 M695 fs*5 VAF 42.9%
- U2AF1 mutation S34F VAF 42.7%.

Proposed Diagnosis

- Myelodysplastic neoplasm with fibrosis (WHO 5th ed.); however blasts are not increased
- Myelodysplastic syndrome, not otherwise specified, with single lineage dysplasia (ICC)

Interesting Feature(s)

- Marked hypercellularity (>90%), driven almost entirely by myeloid expansion.
- Near-complete absence of erythroid precursors and megakaryocytes.
- The presence of WHO grade MF-3 reticulin fibrosis with marked collagen deposition is atypical for MDS with low blasts and raises important diagnostic and prognostic considerations.
- Presence of nucleated red blood cells in peripheral blood (8.4 nRBC/100 WBC) despite profound marrow erythroid hypoplasia, consistent with a leukoerythroblastic reaction pattern.
- These findings support a diagnosis of myelodysplastic neoplasm with multilineage dysplasia, although the profound hypoplasia of erythroid and megakaryocytic lineages coupled with overt marrow fibrosis represents an unusual and atypical presentation for this entity.

Panel Diagnosis: MDS-LB / MDS-NOS

EA4HP26-BMWS-203

Atypical CML with progression versus evolving MPN ?

Dr. Filiz Sen, Dr. Mikhail Roshal, Dr. Kseniya Petrova

Memorial Sloan Kettering Cancer Center, Department of Pathology, New York, USA

Case Description

- 72 yr old man presented at MSKCC on 5/1/2024
- History of rheumatoid arthritis, treated with Celebrex for 50 yrs, and an outside diagnosis of myeloid neoplasm, seeking transfer of care

Outside BM biopsy•Myeloid neoplasm

- Per report, ASXL1/TET2 mutations by NGS
- Per report, FISH BCRABL 0.5% positive

US abdomen•Spleen borderline size: 15.2 x 6.1 x 7.0 cm, calculated volume 336 mL

Biopsy Fixation Details

Formalin fixed biopsy

Frozen Tissue Available

N/A

Details of Microscopic Findings

Please refer to powerpoint presentation. Sample below.

Markedly hypercellular marrow with myeloid hyperplasia with left shifted maturation, relatively reduced erythroid elements and increased megakaryocytes with increased pleomorphism and atypia.

Immunophenotype

N/A

Cytogenetics

Normal karyotype

Molecular Studies

Complex mutational profile. Please refer to powerpoint presentation.

Proposed Diagnosis

Fibrotic progression of atypical CML converging with morphologic features of PMF with accelerated phase

Interesting Feature(s)

- Initial presentation of the case was in the form of atypical CML (aCML) with a complex mutational profile including mutations of SRSF2, ASXL1, TET2 (x2), NF1 and PRDM1 with high VAF (over 40%)
- Both the 2022 WHO and ICC classifications suggest that documenting the **absence of JAK2, CALR, and MPL mutations** is desirable to support an aCML diagnosis and rule out other MPNs
- At presentation, there was granulocytic dysplasia. Megakaryocytes showed a hybrid (MDS/MPN) morphology which converged into an MPN-like morphology.
- The patient developed myelofibrosis, osteosclerosis and progressed into an accelerated phase rapidly.
- Expansion of JAK2 V617F clone drives the phenotype of rapid progression converging with morphologic features of PMF with accelerated phase

Panel Diagnosis: MDS/MPN with neutrophilia / aCML with progression to borderline AML

EA4HP26-BMWS-205

Myelodysplastic Syndrome with Fibrosis, Increased Blasts and Biallelic TP53 Mutation

Dr. Matthew Zunitch, Dr. Karthik Ganapathi, Dr. Yi Xie, **Dr. Sonam Prakash**

University of California San Francisco, Laboratory Medicine, San Francisco, USA

Case Description

68-year-old patient with rectal bleeding and no other hematologic/oncologic history, presented to ED after a syncopal event. CBC showed pancytopenia. No hepatosplenomegaly was identified on physical exam. A bone marrow biopsy was performed with the following concurrent CBC:

WBC 1.7 x10E9/L, HGB 8.3 g/dl, MCV 83.0 fl, PLTS 133 x10E9/L (Neutrophils 0.4 x10E9/L, Lymphocytes 1.0 x10E9/L, Monocytes 0.1 x10E9/L, Eosinophils 0.0 x10E9/L, Basophils 0.1 x10E9/L)

Biopsy Fixation Details

Marrow core and clot were fixed in B-plus; core was decalcified in Immunocal solution.

Frozen Tissue Available

None

Details of Microscopic Findings

Peripheral blood smear: RBCs showed anisopoikilocytosis and nucleated RBCs. Platelets showed hypogranularity. WBCs were notable for hypogranular neutrophils and 5% circulating blasts.

Marrow aspirate smears and clot section showed no marrow particles.

Core biopsy: Markedly hypercellular (>95% cellularity) with maturing myeloid and erythroid precursors and slight erythroid predominance. Megakaryocytes were increased in number, scattered in distribution, and predominantly small with hypolobated nuclei. Reticulin stain showed moderate fibrosis (MF-2).

Immunophenotype

CD34 immunostain on the core biopsy showed increased blasts, ~10% of marrow cellularity.

Flow cytometric immunophenotyping on a hemodilute marrow showed increased atypical myeloid blasts (12.9%) and relative atypical monocytosis (14%) with partial CD56 expression.

Cytogenetics

Complex karyotype with deletion 5q and monosomy 7 (17/20 metaphases).

45,XX,del(5)(q31q35),-7,t(10;12)(q24;q13)[7]/45,XX,der(2)t(2;14)(q22;q11.2),del(5q),-7,der(12)add(12)(p13)dup(12)(q15q22),der(14)del(14)(q13q32)t(2;14)[cp6]/45,XX,del(5q),-7,del(12)(p13p11.2),t(13;19)(q13;p13.3)[2]45,XX,del(5q),-7,t(18;19)(q11.2;p13.3)[2]/46,XX[3]

Molecular Studies

Hybrid capture-based NGS identified the following pathogenic mutations:

TP53 splice site 375+5G>T (VAF 65.5%)

DNMT3A N519fs*27 (VAF 34.4%)

TET2 N801fs*10 (VAF 32.6%)

U2AF1 Q157P (VAF 31.6%)

SETBP1 G870S (VAF 0.97 %)

Proposed Diagnosis

Myelodysplastic syndrome/acute myeloid leukemia (MDS/AML) with *TP53* mutation (ICC)

Myelodysplastic neoplasm with biallelic *TP53* inactivation (WHO-HAEM5).

Interesting Feature(s)

1. Findings characteristic of MDS with fibrosis and distinction from myeloproliferative neoplasm (MPN):

Features that support a diagnosis of MDS over MPN include:

-Dysplastic scattered megakaryocytes (small megakaryocytes with hypolobated nuclei)

-Lack of splenomegaly

-Mutational profile with *TP53* mutation and lacking driver mutations of MPN (i.e. *JAK2*, *CALR* and *MPL*)

2. Discrepancy in classification between ICC and WHO-HAEM5:

The presence of increased blasts ($\geq 10\%$) and significant fibrosis (MF-2) could be compatible with MDS with increased blasts and fibrosis (WHO-HAEM5). However, a pathogenic *TP53* mutation with a VAF of 65.5% was identified. The presence $\geq 10\%$ blasts qualifies as MDS/AML by ICC. Therefore, this MDS is classified as:

-MDS/AML with *TP53* mutation, (ICC)

-MDS with biallelic *TP53* inactivation, (WHO-HAEM5)

3. MDS versus chronic myelomonocytic leukemia (CMML)

There was relative monocytosis in the marrow (by flow cytometry); however, peripheral monocytes were below the threshold for absolute and relative monocytosis ($0.5 \times 10^9/L$, $\geq 10\%$ of WBC), a required criterion for diagnosis of CMML. The marrow morphology with relatively increased erythroid precursors is more in keeping with MDS. *TP53* mutations can be seen in MDS with fibrosis and are uncommon in CMML.

Panel Diagnosis: MDS-bi*TP53* / MDS-*TP53*

Fibrotic MDS/MPN with Ring Sideroblasts, MPL Mutation and Monosomy 7: A Case Illustrating Diagnostic and Monitoring Challenges

Prof. Samah Kohla^{1,2}, Dr. Ibrahim Ganow¹, Dr. Amna Gamie^{3,2}, Dr. Dina Soliman^{1,2}, Dr. Ama S. Aly⁴, Dr. Ibrahim Ganow established the initial diagnosis. Dr. Samah Kohla performed and interpreted the follow-up bone marrow examinations, prepared the abstract, and submitted the case. Dr. Dina Soliman performed one of the follow-up bone marrow examinations. Dr. Amna Gameil managed the clinical care of the patient. Dr. Amal Salm reported the cytogenetic, molecular, and next-generation sequencing results.

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Case Description

A 67-year-old male was referred to the hematology clinic for evaluation of an abnormal peripheral blood smear showing mild anemia, leucoerythroblastic picture, 3% circulating blasts, and prominent tear-drop cells. Serial bone marrow (BM) examinations were performed over a 2-year period for diagnostic confirmation and disease monitoring while on azacitidine therapy.

Biopsy Fixation Details

Bone marrow trephine biopsies were fixed in buffered formalin, decalcified, processed routinely, and stained with H&E.

Frozen Tissue Available

Not available.

Details of Microscopic Findings

The BM aspirate was hemodilute with occasional dysplastic megakaryocytes and 5% blasts. Iron stain demonstrated 30% ring sideroblasts. The biopsy was markedly hypercellular (90–95%) with increased trilineage hematopoiesis. Megakaryocytes were markedly increased, showing numerous small mononuclear hyperchromatic forms with atypical features. CD34-positive cells were estimated at 2–5%. Reticulin staining revealed moderate to marked fibrosis with focal collagen deposition (MF 2–3).

Immunophenotype

Flow cytometry demonstrated ~5% CD34-positive blasts expressing CD45(dim), CD117, HLA-DR, partial CD13(dim), and negative cytoplasmic MPO. Immunohistochemistry confirmed mildly increased CD34-positive cells.

Cytogenetics

Conventional karyotyping revealed monosomy 7: 45,XY,-7 in the majority of metaphases. FISH confirmed loss of chromosome 7 in ~50% of interphase nuclei.

Molecular Studies

JAK2 V617F and CALR mutations were negative. Next-generation sequencing identified MPL W515L (VAF ~45%) and IDH2 R140Q (VAF ~29%) mutations. No SF3B1 mutation was detected.

Proposed Diagnosis

Subsequent bone marrow examinations showed persistent marrow fibrosis (MF 2–3), sustained megakaryocytic hyperplasia, and ongoing ring sideroblasts. Over time, peripheral blood demonstrated progressive cytopenias with increasing circulating blasts (up to 11%), while bone marrow blasts rose to 7%, indicating disease progression despite azacitidine therapy. Monosomy 7 remained present in all follow-up studies, confirming clonal persistence. The proposed diagnosis is Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis, fibrotic variant (MDS/MPN-RS-T), with MPL mutation and monosomy 7, showing progressive disease.

Interesting Feature(s)

This case demonstrates an unusual overlap myeloid neoplasm characterized by prominent marrow fibrosis, persistent ring sideroblasts, and marked megakaryocytic proliferation, evolving over time from a myelodysplastic neoplasm with fibrosis to a myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis. The presence of MPL W515L and IDH2 R140Q mutations together with persistent monosomy 7 represents a rare molecular combination in fibrotic MDS/MPN and likely contributes to both myeloproliferative features and adverse disease biology. Notably, classical morphologic features of MDS/MPN with ring sideroblasts and thrombocytosis were present despite absence of SF3B1 mutation, illustrating the diagnostic flexibility introduced in WHO 2022 when characteristic morphology and MPL mutation coexist. Serial bone marrow examinations documented stable high-grade fibrosis but progressive blast accumulation despite hypomethylating therapy, providing a valuable example of the role of integrated morphologic, cytogenetic, and molecular monitoring in assessing disease evolution and therapeutic response in complex fibrotic myeloid neoplasms.

Panel Diagnosis: MDS-F / MDS-EB with subsequent progression to MDS/AML

EA4HP26-BMWS-211

Fibrotic Myeloid Neoplasm with Persistent Monocytosis and JAK2/TET2/SRSF2 Mutations: Diagnostic Challenges at the Interface of Primary Myelofibrosis and CMML

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Case Description

- A 79-year-old patient presented in 2020 for treatment of intravascular large B-cell lymphoma, with a prior history of anemia, thrombocytopenia, and monocytosis dating back to 2016.
- BM biopsies revealed a fibrotic myeloid process with overlapping features of myelodysplastic/myeloproliferative neoplasm (MDS/MPN) and myeloproliferative neoplasm (MPN).
- The diagnostic dilemma centered on whether this represented a fibrotic MDS/MPN (CMML-like) or an MPN with secondary monocytosis.

Biopsy Fixation Details

Posterior iliac crest core biopsies fixed in formalin and decalcified.

Frozen Tissue Available

Not available.

Details of Microscopic Findings

- The initial 2020 biopsy showed variable (5-60%) cellularity with myeloid predominance, mild reticulin fibrosis (MF-1), and no excess blasts. Dysplasia was mild and limited to megakaryocytes. CBC showed anemia, thrombocytopenia, and monocytosis.
- In 2021 and 2022, the marrow turned hypercellular (90%) with progression to MF-2–3 fibrosis and prominent megakaryocytic atypia (with tight clusters). CBC showed striking leukocytosis (79.9 K/mcL) with monocytosis (5.6 K/mcL). Moreover, RBC reached high levels, 6.9 K/mcL (with Hgb 15.2 g/dL).
- In 2024, fibrosis and megakaryocytic atypia persisted together with clustering. Anemia and thrombocytopenia were noted (Hb 10.7 g/dL; platelets 81 K/mcL). Monocytes remained increased (3.8 K/mcL). Blasts remained <5% throughout.
- Overall, sequential biopsies demonstrated progressive fibrosis and megakaryocytic atypia with persistent monocytosis, raising concern for MPN and CMML.

Immunophenotype

- CD61 immunohistochemistry is used to demonstrate megakaryocytes and clustering.
- Flow cytometry persistently showed abnormal blast population (<1%): CD5 (partial), CD7 (partial), CD38 (variable, negative to intermediate), CD45RA (negative), CD117 (bimodal, negative to bright), HLA-DR (variable, negative to bright).

Cytogenetics

Normal karyotype. FISH for *PDGFRB*, *BCR-ABL1*, *EVI1*, *MLL*, and *TP53* were negative.

Molecular Studies

Targeted sequencing (MSK-IMPACT Heme, matched) identified stable pathogenic mutations with evolving allele frequencies:

- 2021: *ASXL1* (32%), *JAK2* (44%), *TET2* (49%), *TET2* (14%), *SRSF2* (49%)
- 2022: *ASXL1* (33%), *JAK2* (91%), *TET2* (50%), *TET2* (16%), *SRSF2* (50%)
- 2024: *ASXL1* (39%), *JAK2* (72%), *TET2* (47%), *TET2* (15%), *SRSF2* (50%)

Proposed Diagnosis

- Myeloid neoplasm with significant marrow fibrosis and persistent monocytosis (harboring *JAK2* and *TET2/SRSF2* mutations), best classified as primary myelofibrosis with monocytosis.
- Differential diagnosis: Chronic myelomonocytic leukemia-1 with fibrosis

Interesting Feature(s)

- The coexistence of *JAK2* & spliceosome/chromatin modifier (*ASXL1*, *TET2*, *SRSF2*) mutations from the earlier biopsies underscores early molecular complexity.
- 2021 NGS data implied *JAK2/TET2/SRSF2* as a founding clone, and later samples showed *JAK2* LOH.
- This case exemplifies the diagnostic gray zone between fibrotic MDS/MPN (CMML-like) and MPN with monocytosis. Despite morphologic progression and increasing fibrosis, the coexistence of *JAK2/TET2/SRSF2*-mutations in the same clone blur classification.
- Persistent monocytosis and fibrosis from the outset highlight how clonal evolution can culminate in an unclassifiable fibrotic myeloid neoplasm that challenges current disease boundaries.
- The presence of high VAF in *JAK2* and tight clusters of megakaryocytes favor primary myelofibrosis and monocytosis.

Panel Diagnosis: PMF, fibrotic / Overt PMF, with monocytosis

EA4HP26-BMWS-223

Myelodysplastic/Myeloproliferative Neoplasm, Unclassifiable with fibrosis

Dr. Beenu Thakral¹, Dr. Andres E. Quesada¹, Dr. Wei Wang¹, Dr. Karan Saluja², Prof. L. Jeffrey Medeiros¹, Prof. Sa A. Wang¹

¹ MD Anderson Cancer Center, Department of Hematopathology, Houston, USA; ² UT Health Science Center at Houston, Department of Pathology and Laboratory Medicine, Houston, USA

Case Description

A 74-year-old man with no prior history of malignancy presented with moderate anemia, mild thrombocytopenia and increased serum LDH. On examination, massive splenomegaly was noted. CBC showed: WBC 8.0 K/uL, Hgb 8.0 g/dL, MCV 103 fL, 130 K/uL, basophils 5% and neutrophils 60%, ANC 4.86

Biopsy Fixation Details

Fixation: 3 hours in 10% neutral buffered formalin

Frozen Tissue Available

N/A

Details of Microscopic Findings

Peripheral blood smear showed leukoerythroblastosis, dysplastic neutrophils and 9% blasts. A bone marrow biopsy showed myelofibrosis (MF-3), atypical/ hyperchromatic megakaryocytes, multilineage dysplasia, 35% ring sideroblasts and 9% blasts on aspirate smears.

Immunophenotype

Flow cytometry immunophenotyping identified 9% aberrant myeloblasts.

Cytogenetics

Chromosome analysis showed abnormal male karyotype:47,XY,+der(1;9)(q10;p10),t(1;9)(p10;q10)[14]/46,XY[6].

Molecular Studies

A targeted NGS study using an 81-gene panel detected mutations in variants in *JAK2* V617F (VAF 18.8%), *SF3B1* (VAF 43.4%) and *KRAS* (VAF <5%).

Proposed Diagnosis

MDS/MPN-unclassifiable with fibrosis (MF-3).

Interesting Feature(s)

- Classifying myeloid neoplasm with fibrosis is challenging because MDS with fibrosis, MDS/MPN with fibrosis, and PMF are in the differential diagnosis
 - These disease entities can cross boundaries due to overlapping features.
- In the current definition, a diagnosis of MDS/MPN requires the presence of cytosis (not seen in our case) without considering other “MPN” features such as:
 - The presence of MPN driver mutations (*JAK2*, *MPL* and *CALR*).
 - Organomegaly, thrombotic events, high serum LDH.
 - MPN-type megakaryocytic proliferation, peripheral blood leukoerythroblastic changes.
- We propose a diagnosis of MDS/MPN, NOS with fibrosis (MF-3) for these reasons:
 - Both proliferative and dysplastic morphologic features were present at initial diagnosis.
 - Proliferative features: Although no leukocytosis/thrombocytopenia/increased Hb/Hct was seen, features for MPN (mild basophilia with leukoerythroblastosis in PB, BM with atypical/hyperchromatic megakaryocytes and advanced myelofibrosis, splenomegaly, increased LDH) and the presence of MPN driver mutation *JAK2* V617F was noted.
 - MDS (multilineage dysplasia and ring sideroblasts): Dysplastic features can be seen in advanced/accelerated phase of MPN, but this patient had no prior history of MPN. *JAK2* VAF was less when compared to *SF3B1* VAF.
 - CBC features did not support chronic myelomonocytic syndrome or atypical CML.
 - Presence of ring sideroblasts but lack of thrombocytosis excludes MDS/MPN with ring sideroblasts with thrombocytosis.
 - Patient received Roxulotinib and Azacitidine therapy - covers both myeloproliferative and myelodysplastic neoplasm.
 - He underwent allogeneic stem cell transplant in Sept,2019
 - Last follow up (3/2023) showed:
 - CT abdomen: Normal spleen
 - Normal CBC: WBC 4.5 K/uL, Hgb 12.9 gm/dL, MCV 93.6 fL, Platelets 180 K/uL

Panel Diagnosis: MDS-F / MDS-EB

Myeloid neoplasm with marked fibrosis: a diagnostic challenge

PhD/MD Maria Rozman, PhD/MD **Leonor Arenillas**, Dr. Jose Ramon Alamo, Dr. Dolors Colomer, PhD/MD Marta Garrote, Dr. Monica Lopez-Guerra, Dr. Dolors Costa

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Case Description

A 58 year-old male with aortic atheromatosis with left iliac artery thrombosis, resection of prostatic adenoma and renal lithiasis consulted in June 2020 for gluteus hematoma with functional limitation following accidental trauma. Constitutional symptoms were absent. Physical exam was unremarkable.

The hemogram showed severe pancytopenia: Hb 56 g/L, MCV 112 fL, platelets $26 \times 10^9/L$, leukocytes $0.9 \times 10^9/L$ (neutrophils 4%, lymphocytes 91%, monocytes 3%, blasts 2%). LDH 316U/L (N<234). Coagulation tests were normal.

Biopsy Fixation Details

B20-014493 A: fixation formalin 10%, decalcification formic acid 10%; B20-014493 B: fixation Bouin solution, decalcification hydrochloric acid 1-5% decalcifier

Frozen Tissue Available

No

Details of Microscopic Findings

The bone marrow aspirate was unsuccessful, obtaining a suboptimal specimen but where 22% blasts were noted. The bone marrow biopsy was hypercellular, within a diffusely fibrotic stroma, and showed megakaryocytic hyperplasia with mostly small dysplastic forms and micromegakaryocytes, together with erythroid hyperplasia with megaloblastic changes. There were some granulocytic precursors, reactive lymphoid aggregates and about 20% blasts. Marked increase in reticulin fibers without collagenous fibrosis was noted.

Immunophenotype

Flow cytometry (BM aspirate): 12% blasts positive for CD34, CD117, HLA-DR, CD13 partial, CD33 partial, negative for MPO, monocytic and lymphoid markers. Megakaryocytic markers not tested (insufficient sample). By immunohistochemistry part of the blasts were myeloid (MPO-/CD33+) and part megakaryocytic (CD61+).

Cytogenetics

BM karyotype: no metaphases

FISH: monosomy 5 (21%).

Normal *MECOM* (3q26.2), 7q22/7q31, and *TP53* (17p13.1)

Molecular Studies

Mutational NGS study (myeloid panel): *NF1* variant: c.1246C>T; p.Arg416Ter (8.20%)

PCR for *FLT3* ITD and TKD negative

Proposed Diagnosis

Acute myeloid leukemia with myelodysplasia-related cytogenetic abnormality (ICC 2022), AML, myelodysplasia related (WHO 2022)

Interesting Feature(s)

The present case is characterized by severe pancytopenia, and bone marrow showing panmyelosis with increased dysplastic megakaryocytes, 20% blasts and markedly increased reticulin fibrosis. These features resemble those of acute panmyelosis with myelofibrosis (APMF). Nevertheless, according to the present ICC /ELN classification¹ due to the deletion of chromosome 5 the case must be classified as AML with myelodysplasia-related cytogenetic abnormality.

The role of bone marrow fibrosis in AML has not extensively described, but some studies have found marrow fibrosis (MF) to be a poor prognostic factor. In a recent study², patients with MF 2–3 had a higher proportion of complex cytogenetics, *JAK2* mutations and lower proportion of *IDH2* and *CEBPA* mutations. In this study 16% cases were classified as ELN unfavorable based on the presence of abnormalities in chromosomes 5 or 7, and grade 2–3 MF

in AML was associated with worse outcomes. In summary, there has been no formal definition or characterization of AML with fibrosis, and up to this point this feature is not included in modern morphologic or risk classifications.

1. Arber et al, The international Consensus Classification of Myeloid and Lymphoid Neoplasms. Wolters Kluwer 2025.
2. Urrutia S et al, J Hematol Oncol 2024 Nov 15;17(1):112.

Panel Diagnosis: AML-MR / AML with MR cytogenetic abnormalities

EA4HP26-BMWS-233

Myelofibrosis, JAK2 mutation and del(5q): unexpected friends?

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Hospital Clinic Barcelona, Hematopathology Section, Barcelona, Spain

Case Description

An 89-year-old man with a history of pulmonary thromboembolism secondary to deep vein thrombosis in June 2023 and urothelial carcinoma surgically treated in 2024 was referred for evaluation of leukocytosis, anemia, and thrombocytopenia. He had lost 10 kg in weight in recent months and had generalised bone pain. The blood counts revealed Hb 10.7 g/L, MCV 92.9 fL, platelets 85x10⁹/L, leukocytes 20.45 x10⁹/L (%: neut 35, bands 15, eos 1, bas 1, lymph 18, mono 4, myelo 1, metamy 15, promy 7, blasts 3) and 3 erythroblasts per 100 leukocytes. Biochemistry was normal except for elevated LDH 1082 U/L (N: 135-225). Physical examination revealed the presence of splenomegaly, which was confirmed by CT-scan.

Biopsy Fixation Details

B25-032788: fixation formalin 10%, decalcification formic acid 10%.

Frozen Tissue Available

No.

Details of Microscopic Findings

Bone marrow aspiration was performed with little material obtained without clots. Bone marrow biopsy was hypercellular. Increased M/E ratio. The erythroid series shows megaloblastic changes, and the megakaryocytes are dysplastic with small hypolobulated forms and other large forms. Myeloid blasts (CD34 and CD117+) were less than 5%. Grade 3 fibrosis was observed.

Immunophenotype

Flow cytometry (BM aspirate): hemodiluted sample with 0.5% myeloblasts.

Cytogenetics

BM karyotype: 45,X,-Y[11]/45,X,-Y,del(5)(q22q33)[11].

Molecular Studies

Mutational NGS study showed the following pathogenic/likely pathogenic variants: TP53 p.Arg273His (VAF: 46.7%), JAK2 p.Val617Phe (VAF: 16.19%) and SH2B3 p.Glu395Lys (VAF: 96.5%).

Proposed Diagnosis

Primary myelofibrosis with JAK2 mutation and 5q deletion.

Interesting Feature(s)

We present an 89-year-old man with cytopenias, leukocytosis, splenomegaly, and constitutional symptoms. Bone marrow showed hypercellularity, dysplastic megakaryocytes, and grade 3 fibrosis with <5% blasts. Cytogenetics revealed del(5q), and molecular studies identified JAK2, TP53, and SH2B3 mutations.

Primary myelofibrosis (PMF) is a myeloproliferative neoplasm marked by cytopenias, extramedullary hematopoiesis leading to splenic and/or hepatic enlargement, increased megakaryocyte proliferation, reactive fibrosis of the bone

marrow, and a range of systemic manifestations. The JAK2 p.V617F mutation is detected in nearly 60% of patients. Isolated del(5q) in PMF is extremely rare.

Myelodysplastic syndrome with isolated del(5q) is a subtype of MDS characterized by a deletion of the long arm of chromosome 5, either alone or with one additional cytogenetic abnormality other than chromosome 7 alterations. It usually presents with macrocytic anemia, normal or increased platelet counts, and normal leukocyte levels. Bone marrow findings include normal to mildly increased cellularity and characteristic hypolobated megakaryocytes. Concurrent JAK2 mutation has been described in 5% of cases, but significant MF (≥ 2) was not reported.

In our patient, the findings support a diagnosis of primary myelofibrosis with an uncommon del(5q) abnormality and high-risk molecular features. The coexistence of dysplastic and proliferative features highlights the biological overlap between MPN and MDS and emphasizes the importance of integrated morphologic, cytogenetic, and molecular evaluation.

Panel Diagnosis: MDS/MPN, NOS

EA4HP26-BMWS-235

Ghosal Syndrome: A New Etiology of Medullary Fibrosis

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Case Description

In 2019, a 26-year-old patient with no previous medical history was admitted for bicytopenia with aregenerative normocytic anemia (hemoglobin 5.4 g/dL) and thrombocytopenia ($90 \times 10^9/L$). An inflammatory syndrome was also present (CRP 56 mg/L). Clinical examination was unremarkable. Infectious and autoimmune investigations were negative. No paroxysmal nocturnal hemoglobinuria (PNH) clone was detected. Lymphocyte count and immunophenotyping in peripheral blood were normal. Bone marrow aspiration was a failure and bone marrow biopsy showed a hypocellular bone marrow without tumoral infiltration. Radiological evaluation revealed increased cortical density of the diaphyses of long bones without skeletal deformities. The patient required monthly red blood cell transfusions for one year. Because of the persistence of the bicytopenia, the patient was referred, in 2020, to Saint-Louis Hospital. A new bone marrow biopsy was performed.

Biopsy Fixation Details

The bone marrow biopsy was fixed in neutral buffered formalin and decalcified in EDTA for 48 hours at 37°C.

Frozen Tissue Available

Not available

Details of Microscopic Findings

The bone marrow was hypocellular (30%). Megakaryocytes were decreased, mostly small to medium-sized with hypolobulated nuclei. The erythroid lineage was proportionally increased with dyserythropoiesis, whereas the granulocytic lineage was decreased with dysgranulopoiesis. No excess of blasts was observed. A strong fibroedema was present with reticulin fibrosis grade 1, focal grade 2 on Gordon Sweet staining, without collagen fibrosis on trichrome or osteosclerosis. An important non tumoral predominantly T-cell lymphocytic and plasmacytic infiltration was present within this fibroedema. Perls staining showed iron deposits.

Immunophenotype

CD34 immunostaining did not show any blast increase.

Cytogenetics

Cytogenetic analysis failed.

Molecular Studies

Next-generation sequencing revealed a germline *TBXAS1* mutation: c.1417G>T (p.Gly473Trp).

Proposed Diagnosis

Ghosal syndrome

Interesting Feature(s)

We report a case of Ghosal syndrome with characteristic bone marrow biopsy findings, including fibroedema and dysmyelopoiesis without excess blasts. This rare syndrome is clinically characterized by anemia, thrombocytopenia, and skeletal abnormalities, and is caused by mutations in the thromboxane synthase gene (*TBXAS1*) (*Isidor et al Hum Genet* 2007). It is treated with non-steroidal anti-inflammatory drugs or aspirin, leading to resolution of both hematological abnormalities and bone changes. We are currently working on the histopathological characteristics of bone marrow biopsies on a series of Ghosal Syndrome. Recognition of the typical bone marrow features of Ghosal syndrome is essential for pathologists, as it may be misdiagnosed as myelodysplastic neoplasia or autoimmune myelofibrosis, whereas Ghosal Syndrome allows for the prescription of a simple and effective treatment.

Panel Diagnosis: Non-neoplastic myelofibrosis in the setting of Ghosal syndrome

EA4HP26-BMWS-241

Unusual myelodysplastic syndrome with *SF3B1* and *CALR* mutations

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Case Description

A 88-year old male, with history of prostatic adenoma, presented with a macrocytic anemia (8.3g/dl, VGM =107), mild and fluctuant leuconeutropenia (leukocytes = 2.2G/L, neutrophils =1.4 G/L) and normal platelets (315G/L). LDH were elevated. Anterior blood cell counts were normal. The patient was initially asymptomatic. Bone marrow cytology showed a poor cellularity with dyserythropoiesis, dysgranulopoiesis and few ring sideroblasts. Megakaryocytes were not seen. Blasts represented 2%.

Biopsy Fixation Details

We received a bone marrow biopsy measuring 12mm, fixed in formalin 4%. After 12 hours of decalcification in an EDTA-based solution (ready to use), the biopsy was embedded in one paraffin block.

Frozen Tissue Available

No frozen tissue available.

Details of Microscopic Findings

Microscopic analysis showed an increased cellularity (70%), predominantly due to the proliferation of the megakaryocyte and myeloblast lineages. Megakaryocytes were often large, with hypersegmented or hyperchromatic nuclei, less frequently hypolobated. The megakaryocyte clusters were numerous and some elements were closed to the trabeculae. Atypical mitosis were also observed. Increased clusters of myeloblast were associated with matures neutrophils. The erythroblastic lineage was decreased, with few mature erythroblast and atypical proerythroblasts

or basophilic erythroblasts. There was an extensive reticuline and collagenic fibrosis. Numerous hemosiderin deposits were observed.

Immunophenotype

Blasts remained inferior to 5% on the CD34 immunostaining. Scarce mastocytes were observed with CD117 antibody.

Cytogenetics

Bone marrow karyotype found anomalies of the 9q and 20q (46,XY,add(9)(q21),add(20)(q12)[20]).

Molecular Studies

The targeted sequencing performed on the DNA extracted from the fresh bone marrow (Qiaseq technology, NextSeq) detected single nucleotide variants of *SF3B1* (VAF 31%) and *MPL* (VAF 70%).

Proposed Diagnosis

A diagnosis of myelodysplastic syndrome with *SF3B1* mutation was proposed, regarding the cytopenia and the absence of cytolysis.

Interesting Feature(s)

This case illustrates the difficulties to interpret a bone marrow biopsy without the biological and molecular data, and the impact of molecular abnormalities on the morphology. The shape of the megakaryocytes have initially raised the possibility of a myeloproliferative neoplasm or an overlap syndrome, but there was no past or present cytolysis, notably no thrombocytosis. The mutation of *SF3B1* is a defined genetic feature according to the WHO and ICC classifications. The association with *CALR* is observed in less than 10% of the overlap syndrome, and has also been rarely described in myelodysplastic syndrome. For this case, the presence of a *CALR* mutation may be responsible for the megakaryocytes morphology.

Panel Diagnosis: MDS-SF3B1

EA4HP26-BMWS-249

Chronic eosinophilic leukaemia with marked megakaryocytic proliferation and marrow fibrosis.

Dr. Leonie Saft, Dr. Anna Kwiecinska

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Case Description

A 84 y o female patient with DM type 2 and hypothyroidism, presenting with eosinophilia (>3 months) and asthma-like symptoms (ANCA-negative). PMH: breast cancer (1993), relapse 2008; op and status post chemotherapy. Laboratory tests at initial presentation showed mild leukocytosis (WBC 13x10⁹/L ; ANC 3,8x10⁹/L , AEC 5,6x10⁹/L; hemoglobin 89 g/L, MCV 105 fl; Platelets 146x10⁹/L. Serum tryptase normal.

The initial BM examination showed a myeloid neoplasm with significant eosinophilia, blast increase and MF grade 2, diagnosed as CEL based on current (WHO/ICC) classification criteria. The case was remarkable for significant megakaryocytic proliferation with MDS-like small megakaryocytes. Repeated BM examinations were performed after treatment with Hydrea. The treatment was interrupted due to worsening PB values. The follow-up BM examinations (10 months after initial presentation) showed progression with transformation to AML.

Biopsy Fixation Details

Formalin-fixed and paraffin-embedded bone marrow trephines (buffered 4% formalin).

Frozen Tissue Available

Not available.

Details of Microscopic Findings

PB smear: eosinophilia with mature eosinophils; no granulocytic dysplasia. No blasts.

BMA smears: hemodiluted; no particles; mostly mature granulocytes with increase of eosinophils. No significant dysplasia, <5% blasts.

BM trephine biopsy (initial diagnosis): cellularity 40-70% with significant eosinophilia and paratrabecular fibrosis with scattered (paratrabecular) mast cells, but no aggregates. Marked megakaryocytic proliferation with many små (MDS-like) megakaryocytes. Reticular stain shows MF grade 2.

Follow-up biopsies were taken 10 and 11 months later showing a similar morphology with increasing myeloid blasts (leukemic transformation).

Immunophenotype

IHC (at initial diagnosis) showed an increase in CD34+/CD117+ blast cells (<5%, but in some areas 5-<10%).

Scattered (isolated) tryptase-positive mastcells (CD2+, CD25+, CD30-). Small reactive T-cell infiltrates (CD3+). No p53 overexpression. CD71 shows well represented erythropoiesis.

Flow cytometric immunophenotyping at initial diagnosis showed <1% myeloid blasts with normal immunophenotype; no MDS-related immunophenotypic aberrancies. No aberrant (clonal) lymphoid population detected.

Cytogenetics

Normal karyotype.

FISH normal findings. No PDGFRA, PDGFRB, FGFR1, JAK2 rearrangement. No bcr:abl1 fusion.

Molecular Studies

NGS (TruSigh Myeloid Panel, 64 genes): ASXL1 VAF 45%, SETBP1 VAF 46%, SRSF2 VAF 45%, STAT5B 36%, EZ2 46%.

No c-KIT D816 mutation.

Negative for MPN-related mutations.

Proposed Diagnosis

Chronic eosinophilic leukaemia (CEL) (WHO/ICC)

Interesting Feature(s)

Myeloid neoplasm with significant PB and marrow eosinophilia, blast increase and MF2, tranforming to AML during follow-up. This case shows a combination of cytopenia, dysplasia and proliferative features and a mutational profile seen in myeloid neoplasms, excluding classical MPN (lack of MPN-related mutations). The case is best classified as CEL (according to WHO-5/ICC), but challenges the current concept of classifying CEL as MPN rather than MDS/MPN. Additionally, the STAT5 mutation is frequently seen in such cases and appears to drive the eosinophilia and probably marrow fibrosis.

Ref.:

Yin et al, "STAT5B mutations in myeloid neoplasms differ by disease subtypes but characterize a subset of chronic myeloid neoplasm with eosinophilia and/or basophilia." Haematologica, 2024, 109: 1825-1835, PMID 37981812

Cross et al, "Recurrent activating STAT5B N642 H mutation in myeloid neoplasms with eosinophilia." Leukemia 2019; 33: 415-425, PMID 30573779

Panel Diagnosis: CEL / CEL, NOS with progression to AML

Patient with Lowe syndrome, pancytopenia, and marrow fibrosis found to have *ASXL1* mutations

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Case Description

An 18 year-old man with Lowe syndrome (X-linked, *OCRL1* mutation) causing epilepsy, glaucoma, and chronic kidney disease, presented with two months of fatigue, bruising, and fevers. CBC demonstrated cytopenias [WBC 2.4 K/uL (ANC 1.7 K/uL), Hb 6.5 g/dL, MCV 111 fL, Platelets 31 K/uL] and an elevated reticulocyte count of 2.1%. LDH was mildly elevated (702 IU/L). PNH by flow cytometry, viral serologies, DAT, hepatitis panel, and ANA were negative. The patient underwent three bone marrow biopsies within a year of presentation. Each demonstrated a hypercellular marrow with erythroid predominance and decreased megakaryocytes, without morphologic dysplasia. Progressive marrow fibrosis was identified (ranging from MF0-1 to MF2 of 3). Given the identification of anti-neutrophil and anti-platelet antibodies, the differential included autoimmune myelofibrosis; however, the patient's cytopenias and marrow findings did not resolve despite multiple immunosuppressive therapies. A fourth bone marrow evaluation showed similar morphologic features, but included a myeloid NGS panel that identified two mutations in *ASXL1*. The patient was initiated on luspatercept, with resolution of his transfusion dependence.

Biopsy Fixation Details

Core biopsies were submitted in 10% NBF and decalcified with formic acid.

Frozen Tissue Available

No

Details of Microscopic Findings

The first three biopsies showed a hypercellular marrow for age (>90%) with trilineage hematopoiesis, marked erythroid predominance, and myeloid left-shift. No dysplasia was identified, although megakaryocytes were not identifiable in aparticle aspirates. Mild reticulin fibrosis was present (grade MF0-1 up to 2 of 3). A patchy increase in foamy appearing histiocytes, without hemophagocytosis or granulomas, was noted in the biopsies. A fourth marrow, 1.3 years after presentation, showed similar features, without dysplasia, and with MF-1 reticulin fibrosis. Subsequent marrows (after initiating luspatercept) have shown similar findings, but with decreasing fibrosis (only focal MF-1).

Immunophenotype

Flow cytometry was negative for abnormal myeloid or lymphoid populations.

Cytogenetics

Normal karyotype, normal MDS FISH.

Molecular Studies

A myeloid NGS panel on the fourth bone marrow identified two mutations in *ASXL1* (NM_015338.5: p.Glu728Ter, c.2182G>T [VAF 13%] and p.Gly643ArgfsTer15, c.1926dup [VAF 11%]). Testing for *JAK2*, *CALR*, and *MPL* mutations was negative. Fanconi anemia breakage study and telomere length studies were normal. While the first myeloid NGS panel performed after initiating luspatercept showed no clinically significant variants, a subsequent NGS panel identified solely a new *ASXL1* variant (NM_015338.6:c.2885_2886del, p.Val962AlafsTer7 [VAF 3%]).

Proposed Diagnosis

Clonal Cytopenia of Undetermined Significance (CCUS)

Interesting Feature(s)

The case demonstrates cytopenias and progressing mild to moderate marrow fibrosis in a patient without overt morphologic dysplasia. Mutations in *ASXL1* were identified, and overall features were deemed compatible with CCUS. The clinicians trialed an erythropoiesis-stimulating agent, luspatercept, with favorable clinical response, but

with fluctuating mutations in *ASXL1*. It is unclear if the patient's myeloid disease may relate to his diagnosis of Lowe syndrome, an X-linked oculocerebrorenal syndrome due to mutations in *OCRL1*. It is also unclear if the fibrosis is related to CCUS, or to other aspects of the patient's medical history, such as medications. Mild thrombocytopenia and intrinsic platelet defects have been described in Lowe syndrome, which may be contributing to the patient's ongoing thrombocytopenia.

Panel Diagnosis: CCUS with myelofibrosis of unknown etiology in a patient with Lowes syndrome; ?germline predisposition

EA4HP26-BMWS-279

Chronic myelomonocytic leukemia with fibrosis acquiring *NPM1* mutation at progression to acute myeloid leukemia

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Case Description

A 70-year-old previously healthy female presented with leukocytosis, relative and absolute monocytosis, anemia and thrombocytopenia. Imaging showed hypermetabolic splenomegaly without lymphadenopathy and diffuse skeletal uptake. An initial bone marrow biopsy showed a markedly hypercellular, fibrotic marrow (MF1-2) with significant crush artifact precluding morphologic diagnosis but positive for *SRSF2* P95L, *TET2* Q847*, G1861V mutations, concerning for a myeloid neoplasm. The patient underwent a repeat biopsy.

Biopsy Fixation Details

Fixed in 10% neutral buffered formalin and decalcified for at least 60 minutes in Immunocal.

Frozen Tissue Available

No

Details of Microscopic Findings

The peripheral smear shows normocytic anemia with polychromasia and teardrop cells, relative and absolute monocytosis with atypical monocytes, hypogranular neutrophils, rare circulating blasts (<5%), and thrombocytopenia. The core biopsy is hypercellular (~ 95%) with granulocytic hyperplasia (> 90%), markedly reduced erythroid precursors, near-absent megakaryocytes, increased fibrosis, and osteosclerosis. Blasts are not overtly increased.

Immunophenotype

CD34 highlights rare blasts (< 5%); MPO/CD71 highlight increased granulocytic/erythroid ratio; CD61 does not highlight any megakaryocytes. Reticulin fibrosis is increased (MF 2-3) and trichrome shows increased collagen fibrosis (Grade 2).

Cytogenetics

Prior bone marrow: Normal female karyotype; FISH negative for common MDS/MPN abnormalities.

Molecular Studies

Prior BM NGS: Positive for *SRSF2* P95L, *TET2* Q847* G1861V mutations; negative for RNA fusions or CNVs.

Subsequent PB NGS: Positive for *FLT3* D835Y, *NPM1* W288Cfs*12, *NRAS* G12S, *SRSF2* P95L, *TET2* Q847* G1861V mutations.

Proposed Diagnosis

Chronic myelomonocytic leukemia - 1, with increased fibrosis.

Interesting Feature(s)

Definitive subclassification of myeloid neoplasms with increased fibrosis requires integration of blood counts, bone marrow morphology (especially megakaryocytes), and genomic findings. In this case, the near-absence of

megakaryocytes was particularly challenging, but we favored a diagnosis of CMML-1 with fibrosis based on peripheral monocytosis, marrow hypercellularity with granulocytic hyperplasia and mutational profile.

The presence of a markedly hypercellular marrow with granulocytic hyperplasia and fibrosis also raised the possibility of a myelofibrotic myeloproliferative neoplasm. Monocytosis can be seen in both MPN and CMML. However, the reduced megakaryocytes, mutational profile on a prior marrow biopsy with two *TET2* mutations and *SRSF2* mutations, and lack of MPN-associated driver mutations favored a diagnosis of CMML. The presence of persistent leukocytosis and monocytosis argued against a diagnosis of MDS with fibrosis.

Soon after this bone marrow biopsy, the patient developed marked leukocytosis ($> 40 \times 10^9/L$) with $> 20\%$ circulating myeloid blasts, meeting criteria for a diagnosis of acute myeloid leukemia. Peripheral blood NGS analysis identified the prior *SRSF2*, *TET2* and additional pathogenic *NPM1*, *FLT3* and *NRAS* mutations. The patient expired due to multiorgan failure before initiation of therapy.

This is an interesting case of CMML with fibrosis and progression to acute myeloid leukemia with acquisition of an *NPM1* mutation. Increased fibrosis and *NPM1* mutations are independent poor prognostic factors in CMML. It is important to distinguish this scenario from *de novo* AML with *NPM1* mutation, as the underlying disease biology, clonal evolution, and clinical implications differ.

Panel Diagnosis: Myeloproliferative CMML-1 / CMML-1, myeloproliferative type, with progression to AML with *NPM1* mutation / AML with mutated *NPM1*

EA4HP26-BMWS-301

Acute myeloid leukemia with MECOM rearrangement and moderate reticulin fibrosis (MF-2)

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Case Description

The patient is a 42-year-old woman with a past medical history of asthma, polycystic ovary syndrome, and hypertension who presented after one month of progressive severe fatigue, palpitations, and light-headedness. Complete blood counts revealed a white blood cell count of $2.1 \times 10^9/L$, hemoglobin of 8.4 g/dL, and platelet count of $180 \times 10^9/L$. She underwent a bone marrow biopsy. Following the final diagnosis, she received induction chemotherapy with FLAG-IDA plus venetoclax (fludarabine, cytarabine, granulocyte colony-stimulating factor, idarubicin, and venetoclax), followed by treatment with azacitidine plus venetoclax at relapse. At the most recent follow-up, she has residual acute myeloid leukemia with 5-10% blasts in a 20% cellular marrow, MF-3 reticulin fibrosis, and acquisition of a new TP53 mutation.

Biopsy Fixation Details

The bone marrow core biopsy was fixed in acetic zinc formalin, followed by HCl-based decalcification.

Frozen Tissue Available

Not available

Details of Microscopic Findings

The bone marrow is hypocellular (approximately 30%) with 40% blasts. Morphologically, the blasts are small with densely reticular chromatin and resemble lymphoblasts. Megakaryocytes demonstrate dysplastic features, including hypo- and hyperlobated nuclei with abnormal chromatin patterns. Reticulin staining shows moderate reticulin fibrosis (MF-2), while trichrome staining is negative for collagen fibrosis.

Immunophenotype

CD34 highlights increased blasts, which are also positive for CD117. CD61 stains dysplastic-appearing megakaryocytes. Blasts are negative for CD3, CD7, CD20, PAX-5, TdT, and Myeloperoxidase.

Flow cytometry: Demonstrates a 44% population of abnormal myeloblasts, which are CD3(-), CD4(-), CD10(-), CD11c(-), CD13(uniformly+), CD19(-), CD33(-), CD34(+), CD41(-), CD56(-), CD61(-), CD117(+), HLA-DR(dim+), MPO(-).

Cytogenetics

46,XX,inv(3)(q21q26.2)[2]

Molecular Studies

FLT3-ITD and FLT3-TKD (codon D835) alterations NOT detected.

IDH1 and IDH2 mutations not detected.

Myeloid NGS panel: Demonstrates variants in *SF3B1* (c.2098A>G, VAF 27%), *ASXL1* (c.1573A>T, VAF 23.3%), *GATA2* (c.8_12dupTGGCG, VAF 21.5%)

Proposed Diagnosis

Acute myeloid leukemia with MECOM rearrangement and moderate reticulin fibrosis (MF-2)

Interesting Feature(s)

- Reticulin staining demonstrates moderate reticulin fibrosis (MF-2) at initial diagnosis, with progression to MF-3 fibrosis at follow-up, in the absence of collagen fibrosis by trichrome staining.
- Bone marrow fibrosis is frequently associated with AML harboring MECOM rearrangements, particularly the common inv(3), and is thought to result from aberrant cytokine production by dysplastic megakaryocytes and leukemic blasts.
- The presence of marrow fibrosis contributes to hypocellularity, ineffective hematopoiesis, and therapeutic resistance, thereby complicating disease assessment and response evaluation.
- Progressive fibrosis and evidence of clonal evolution, including acquisition of a TP53 mutation, underscore the high-risk biology and aggressive clinical course characteristic of MECOM-rearranged AML.

Panel Diagnosis: AML with MECOM rearrangement / AML with inv(3)(q21;q26.2/GATA2; MECOM

EA4HP26-BMWS-331

Are these Monoblasts or Megakaryoblasts?

Dr. Oi-Lin Lee

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Case Description

A 68 year old woman, previously well with no previous haematological history.

- Presented with abdominal pain and fatigue.
- Blood counts revealed pancytopenia:
- Hb 100 MCV 101.4 WCC 1.65 ANC 0.45 Plt 32
- Poikilocytosis with occasional helmet and tear drop cells, rare blasts and leucoerythroblastic blood picture.

Biopsy Fixation Details

-

Frozen Tissue Available

-

Details of Microscopic Findings

Bone marrow aspirate

- Few hypocellular particles and blood diluted

- Increased in blasts- majority resembles monoblasts. Occasional ones with cytoplasmic blebs resembling megakaryoblasts

- Most cells seen are of monocytic lineage (80%).
- Granulocytes 10% and are hypogranular.
- Reduced erythroids and no megakaryocytes seen.

Bone marrow trephine

- Cellularity variable- <5% to approaching 100%.
- In cellular areas- marked architecture distortion. Infiltrated by sheets of large immature cells with prominent nucleoli.
- Normal haematopoiesis is reduced however, megakaryocytes are increased.

Bone marrow trephine-IHC

- CD45+ confirming they are haematopoietic in origin.
- CD34+ highlights large cells, overall 60% of cells.
- MPO negative. • CD61+ and FVIII+ strongly positive and highlights many cells including the CD34+ cells
- Reticulin shows increased coarse fibres-MF-2

Immunophenotype

Myeloid blasts (CD33+/CD(34, 117)+)- 4.5% of total cells.

Expanded monocytic population comprising 40% of total cells.

Myeloid cells comprise approximately 15% of total cells.

No megakaryoblastic markers.

No atypical expression of T cells or B cell monoclonality

Cytogenetics

46, XX, t(3;21)(q26;q22)[10}

Balanced translocation between long arms of chromosome 3 and 21 in all 10 metaphases examined.

FISH:

MECOM(EVI1)(3q26.2): rearrangement of the MECOM locus was detected.

Molecular Studies

Variants detected:

Gene	Protein	VAF
EZH2	p.(Asn693Lys)	12%
SF3B1	p.(Lys700Glu)	11%.

Proposed Diagnosis

Acute Myeloid Leukaemia with MECOM rearrangement.

Interesting Feature(s)

- Hypercellular bone marrow with increased blasts and fibrosis.
- Poor aspirate made differential count and subtyping challenging.
- Morphologically, most blasts resemble monoblasts but some also resemble megakaryoblasts.
- Other cells are mostly of monocytic lineage (>80%), we initially called it Acute monocytic leukaemia.
- However, there blasts show increased CD61 and FVIII expression by immunohistochemical stain which raises the question of Acute Megakaryoblastic Leukaemia.
- MECOM rearrangement was detected and therefore a diagnosis of Acute Myeloid Leukaemia with MECOM rearrangement was made.
- Acute Myeloid Leukaemia with MECOM rearrangement is often associated with increased fibrosis and blasts and can resemble AML without maturation, acute myelomonocytic leukaemia, acute monocytic leukaemia and acute megakaryoblastic leukaemia.

Panel Diagnosis: AML with *MECOM* rearrangement (WHO-5)/AML with other *MECOM* rearrangement*
(ICC) *(t(3;21)(q26;q22)/*RUNX1::MECOM*)

Acute megakaryoblastic leukaemia in the differential diagnosis with leukemic transformation of MDS-IB2 with fibrosis or PMF

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Case Description

62 yo male was evaluated for elevated acute-phase reactants (APR) and pancytopenia.

PET/CT, diffuse FDG uptake compatible with bone marrow involvement was observed throughout the skeletal system (SUVmax: 7.7). There was no hepatomegaly, while splenomegaly was present (12 cm).

WBC: 1.880/μL, Hb: 8.9 g/dL, MCV: 84.7 fL, Platelets: 29.000/μL, Neutrophils: 45.3%, Eosinophils: 0.5%, Lymphocytes: 35.1%, Basophils: 0.5%, LDH: 372 U/L.

Peripheral blood smear demonstrated atypical cells

Peripheral flow cytometry revealed 5–10% blasts.

A bone marrow biopsy was performed to investigate the possibility of acute myeloid leukemia (AML).

Biopsy Fixation Details

- Fixation with %10 formalin
- Decalcification with %10 Nitric acid for 12 hour

Frozen Tissue Available

no

Details of Microscopic Findings• 100% cellularity with a marked reduction of adipocytes

- The myeloid lineage was continuous but decreased in cellularity,
- The erythroid lineage was represented by small colonies identifiable only by immunohistochemistry.
- Megakaryocytes were markedly increased in number and showed small hypolobated/monolobated forms, loose clustering, and hypersegmentation; micromegakaryocytes were highlighted by immunohistochemistry.
- No hemosiderin was identified on iron staining.
- Reticulin fibrosis was graded as MF-3, with prominent increases in both reticulin fibers and myxoid bluish interstitial material.
- In addition, small immature-appearing cells were observed infiltrating between the fibers.

Immunophenotype• Scattered myeloperoxidase (MPO) positivity in myeloid precursors with evidence of disorganization.

- CD71 was positive in erythroid precursors and megakaryocytes and showed positivity in blasts,
- CD61 was positive in megakaryocytes and also exhibited equivocal staining in blasts.
- The blasts were positive for CD117 and CD34.

Cytogenetics

not done

Molecular Studies

FISH negative for: CBFB(t16;16), inv (16) break, del5q, del7q31, del20q, t(15;17), t(8;21), trizomi/monozomi 8, t(9;22), inv/t(3)(q21q26)(RPN1/MECOM)

NGS has been sent, and the result is pending.

Proposed Diagnosis

Acute megakaryoblastic leukemia

Interesting Feature(s)

The findings observed in the biopsy are compatible with acute megakaryoblastic leukemia.

However, the presence of marked fibrosis, dysplasia involving all three hematopoietic lineages, and clustered megakaryocytes raises suspicion for leukemic transformation of an underlying disorder, such as primary myelofibrosis or a myelodysplastic/myeloproliferative neoplasm–like disease.

The acute onset of symptoms and the absence of preceding or longstanding complaints favor an acute rather than a chronic disease process. Splenomegaly measuring 12 cm is insufficient to account for the degree of reticular fibrosis observed in the bone marrow. Moreover, the marrow fibrosis is characterized not by a predominance of reticulin and collagen fibers, but rather by a matrix rich in myxoid (mucoid) stroma. In this case, the status of JAK2 and other CMPN-associated mutations is currently unknown; this may be clarified by the results of next-generation sequencing (NGS).

In light of the aforementioned findings, acute megakaryoblastic leukemia has been considered the leading diagnosis, and leukemic transformation arising from myelodysplastic syndrome with fibrosis (MDS-F) or from a chronic myeloproliferative neoplasm (CMPN) such as PMF has been regarded as less likely. However, acute megakaryoblastic leukemia is exceedingly rare in adults, and the degree of fibrosis is not invariably as pronounced as observed in this case. Therefore, MDS-F and leukemic transformation of a CMPN must be carefully excluded in the differential diagnosis.

Panel Diagnosis: AML (cannot classify due to lack of genetic information)

EA4HP26-BMWS-336

Unusual myeloid neoplasm with bone marrow fibrosis

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Case Description

21-year-old Caucasian male with weight loss, neutropenia ($1.63 \times 10^9/L$), anemia (67 g/L) and thrombocytopenia ($62 \times 10^9/L$). Peripheral blood (PB) smear showed 4% blasts with anisopoikilocytosis of red blood cells, dysplastic neutrophils and atypical monocytes. Following the diagnosis, complete remission was achieved after several lines of therapy and two haploidentical allogeneic stem cell transplantations.

Biopsy Fixation Details

Bone marrow (BM) biopsy was fixed in formalin.

Frozen Tissue Available

n.a.

Details of Microscopic Findings

The BM biopsy was hypercellular with intertrabecular spaces almost completely occupied by immature-looking myeloid cells, with variably dispersed chromatin, irregular nuclei and scant cytoplasm. Reticulin stain showed severe BM fibrosis (MF-3) with thickened bone *trabeculae*. Cytological evaluation was not available due to dry tap.

Immunophenotype

- Flow cytometry on PB blasts: positivity for CD45, CD33, CD15, CD11b; negativity for CD34, CD117, sCD3/cCD3, CD19, TdT, CD56.

- Immunohistochemistry (IHC) on BM biopsy: positivity for CD33 and CD15; partial expression of MPO and CD68PGM1; negativity for CD34, CD117, CD3, CD19, TdT, CD303, CD123, TCL1, CD56, CD61, glycophorin, E-Cadherin, CD163.

Cytogenetics

Normal male karyotype (46,XY[40]) with cryptic *KMT2A* (11q23.3) rearrangement by interphase FISH with break apart probe (100/300 [33%] nuclei).

Molecular Studies

KMT2A::MLLT3 fusion by RT-PCR on PB. No other mutations by NGS analysis (30-gene Sophia MYS-v1 panel).

Proposed Diagnosis

Acute myeloid leukemia with *KMT2A::MLLT3* rearrangement

Interesting Feature(s)

Acute myeloid leukemia (AML) with t(9;11)(p21.3;q23.3)/*KMT2A::MLLT3* rearrangements accounts for 1-2% of adult and 5-10% of pediatric AMLs. It usually demonstrates myelomonocytic or monocytic/monoblastic differentiation, with blasts being often negative for CD34 and CD117. The 2022 International Consensus Classification requires ≥10% blasts in the BM and/or PB to make the diagnosis, whereas the 2022 WHO Classification does not set a minimum blast threshold.

This case presented several unusual features, which challenged the diagnosis of AML with t(9;11)(p21.3;q23.3)/*KMT2A::MLLT3* rearrangement. These included (i) extensive BM fibrosis (rarely, if ever, reported in this disease); (ii) the cryptic *KMT2A::MLLT3* fusion (undetectable by conventional karyotyping); (iii) the dry tap and IHC negativity for CD34 and CD117 of myeloid cells, which challenged blast counting. Despite this, morphological assessment of BM biopsy suggested an immature myeloid population (putatively blasts) well above 20% of BM cellularity and FISH/RT-PCR studies identified *KMT2A* rearrangement. This led to the final diagnosis of AML.

Panel Diagnosis: AML with *KMT2A* rearrangement / AML with t(9;11)(p21.3;q23.3)/*KMT2A::MLLT3*

EA4HP26-BMWS-344

When del(5q) Meets JAK2: A Fibrotic Myeloid Neoplasm At the MDS/MPN interface

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Case Description

A-65-year-old lady presented with long standing history of fatigue, presented with severe anemia (Hb: 3 A-65-year-old lady presented with exertional dyspnea and generalized fatigability and found to have bicytopenia with severe anemia (Hb: 3g/dL), mild thrombocytopenia (platelet $\times 10^3/\mu\text{L}$: 105), normal WBC $\times 10^3/\mu\text{L}$: 7.6, leukocytoclastic picture and few circulating blasts. Abdominal U/S showed mild splenomegaly (15 cm) with high LDH: 338 U/L.

Biopsy Fixation Details

The trephine biopsy was formalin-fixed, paraffin-embedded, and decalcified using standard laboratory protocols.

Frozen Tissue Available

No frozen tissue was available.

Details of Microscopic Findings

P.S shows severe normocytic normochromic anemia with anisopoikilocytosis, tear drop cells, few polychromatic cells, and NRBCs. There is a left shift with 2% circulating blasts, mild basophilia, and notable dysgranulopoiesis, including abnormal segmentation and hypogranular forms.

BM aspirate is partially hemodilute, revealing trilineage hematopoiesis. Megakaryocytes are increased and markedly atypical, with some hypolobated/monolobated forms. Erythropoiesis is active, displaying normoblastic maturation and

significant dyserythropoiesis (megaloblastoid chromatin, nuclear karyorrhexis, bi-nucleation, inter-nuclear bridging, cytoplasmic irregularities). Granulopoiesis is sufficient, with hypersegmented and hyposegmented forms. Basophils account for around 2%.

The core biopsy reveals pronounced hypercellularity (nearly 100%), featuring trilineage hematopoiesis with an increase in granulopoiesis and megakaryocytes. The megakaryocytes display significant atypia, including numerous monolobated forms, varied chromatin patterns some of which are hyperchromatic and appear as dense clusters associated with marked intrasinusoidal hematopoiesis. Abnormal paratrabecular localization is observed. CD34-positive cells are estimated at roughly 3–8%. Reticulin fibers show a moderate to marked increase (MF 2-3), with focal collagenization.

Immunophenotype

Flow cytometry shows 3% CD34-positive cells expressing CD117 (majority) with partial CD33 and CD13 expressions suggestive of myeloblasts. Monocytes comprise approximately 3% and express CD64 and CD14

Cytogenetics

Karyotype: 46,XX,del(5)(q31q35)[18]/92,idem[3]/46,XX[4]

Molecular Studies

NGS:

- NM_022552.5(DNMT3A):c.2645G>A (p.Arg882His) VAF 24.25%
- NM_0049724 (JAK2):c.1849G>T(p.Val617Phe) VAF 27.94% This G to T base substitution in the JAK2 gene resulting in substitution of the amino acid Valine for Phenylalanine at codon 617.

Proposed Diagnosis

Myelodysplastic/ Myeloproliferative neoplasm-Unclassifiable (MDS/MPN- U) (MDS/MPN-NOS) with JAK-2 mutation and 5q deletion and overt fibrosis.

Interesting Feature(s)

This is a complex case most appropriately classified as MDS/MPN-unclassifiable, given the significant overlap and nearly equal representation of myeloproliferative and myelodysplastic features. The findings suggest the presence of two concurrent or sequential myelodysplastic and myeloproliferative clones, supported by the comparable variant allele frequencies of driver mutations relevant to both phenotypes (DNMT3 and JAK2). An alternative consideration is the development of a 5q deletion in the context of established primary myelofibrosis, which typically indicates disease progression and is generally associated with a poorer prognosis. While the WHO 5th advised classifying these cases as “MDS with del(5q) associated with JAK2 p.V617F” instead of MDS/MPN, we have chosen to retain the classification as MDS/MPN-U due to the predominance of myeloproliferative features, particularly fibrosis and bone marrow stroma.

Panel Diagnosis: PMF, fibrotic / overt PMF"

EA4HP26-BMWS-347

VEXAS-Associated Myelofibrosis

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Case Description

The patient is a 43-year-old transgender female with a history of hemophilia A, transfusion-related iron overload with secondary hemochromatosis, polychondritis, and an episode of severe sepsis due to Penicillium-associated pneumonia. A CBC showed WBC $4.5 \times 10^3/\mu\text{L}$, Hgb 7.7 g/dL, platelet 110, and MCV 111.6 fL. A bone marrow biopsy was performed to evaluate persistent cytopenias, with clinical concern for myelodysplastic syndrome.

Biopsy Fixation Details

The bone marrow core biopsy is fixed with B+ fixative and decalcified with HCl.

Frozen Tissue Available

Not performed.

Details of Microscopic Findings

The core biopsy demonstrates markedly hypercellular marrow for age (>90% cellularity) with myeloid-predominant trilineage hematopoiesis (M:E ratio >5:1). The myeloid lineage is expanded with full maturation. The erythroid lineage is relatively decreased and left-shifted. Megakaryocytes are increased and show atypical features, including variably sized forms with peripherally placed nuclear lobes ("wreath-like" nuclei), occasional small nuclear fragments, and condensed eosinophilic cytoplasm. A reticulin stain shows grade 1-2 of 3 reticulin fibrosis, with progression to grade 2-3 of 3 on repeat biopsy one year later.

The aspirate smear demonstrates myeloid hyperplasia (M:E ratio ~10:1), with left-shifted granulopoiesis. The erythroid lineage is decreased and left-shifted, with occasional multinucleated basophilic normoblasts. Distinct cytoplasmic vacuolization is observed in both erythroid and granulocytic precursors. Megakaryocytes are increased and include forms with separated nuclear lobes. Blasts are not increased.

Immunophenotype

Flow cytometric and immunohistochemical studies do not identify overt immunophenotypic aberrancy.

Cytogenetics

Karyotype: 46,XY,inv(2)(p11.2q23)c. The pericentric inversion on chromosome 2 is a known constitutional chromosome abnormality. No other clonal chromosome abnormalities are detected.

Molecular Studies

Next generation sequencing studies identify a UBA1 mutation. No other mutations are identified using a 50 gene myeloid panel.

Proposed Diagnosis

VEXAS syndrome.

Interesting Feature(s)

VEXAS syndrome (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) is an adult-onset monogenic disorder caused by somatic UBA1 mutations in hematopoietic progenitors, leading to combined autoinflammatory and hematologic manifestations.

In this case, the marrow findings initially raised a differential of VEXAS syndrome, autoimmune myelofibrosis, and MDS; subsequent identification of a somatic UBA1 mutation confirmed VEXAS but left the increased reticulin fibrosis as a noteworthy feature. Moreover, it has been described that the association of VEXAS with MDS was characterized by more frequent multilineage dysplasia. Up to 50% of VEXAS meet WHO criteria for MDS, but the diagnostic and prognostic values of MDS-related morphological and genetic features in this setting are unclear.

Low grade reticulin fibrosis has been described in sporadic case reports in VEXAS; however, the overall prevalence and severity spectrum remain incompletely defined. The mechanism of the patient's fibrosis is unknown, however, it may represent a reactive stromal response to the VEXAS inflammatory phenotype: UBA1-driven innate immune dysregulation with cytokine excess (including IL-1/IL-6/TNF) provides a plausible pro-fibrotic marrow milieu analogous to autoimmune myelofibrosis.

Awareness of VEXAS-associated, immune-driven marrow fibrosis may prevent misclassification as a primary myeloid neoplasm and supports consideration of therapies directed at upstream inflammatory signaling rather than fibrosis itself.

Panel Diagnosis: BM fibrosis, MF2, of uncertain etiology in a patient with VEXAS

Myeloid Neoplasm with Fibrosis and Progressive Monocytosis

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¹ "Dr Josep Trueta" University Hospital, Pathology department, Girona, Spain; ² "Arnau de Vilanova" University Hospital, LLeida, Spain

Case Description

We present the case of a 33 y/o male original from Morocco with no significant past medical or surgical history. In August 2023, he was admitted with leucoerythroblastosis, dacrocytosis and 7-8% of circulating blasts in the peripheral blood (PB). Bone marrow aspirate was a "dry tap". The first trephine biopsy revealed severe fibrosis (MF3) and 7-8% blasts, consistent with MDS-EB with fibrosis. At that time, NGS results were still pending. CT scan showed 20 cm splenomegaly. The patient declined treatment at this stage.

In October 2023 he developed progressive monocytosis (up to $20.44 \times 10^3 / 33,6\%$) with pancytopenia and 20% PB blasts. A second bone marrow biopsy showed a remarkable spontaneous decrease in the fibrosis grade (MF2) and 12% blasts consistent with MDS/AML. Sequencing result were then available (see below), and the case was referred for expert consultation.

The patient entered a clinical trial, but succumbed to his disease in December 2024.

Biopsy Fixation Details

Following fixation during 24 hours in 10% neutral buffered formalin, the specimen was decalcified in pure unbuffered 10% EDTA solution for 12 hours at 39°C. Afterwards, tissue underwent standard automated processing (graded alcohol dehydration, xylene clearing, and paraffin infiltration) and was embedded in paraffin.

Frozen Tissue Available

Not available

Details of Microscopic Findings

First biopsy (August 2023)

The bone marrow was hypercellular (>90% cellularity) with reticulin and collagen fibrosis (MF3) with sinusoidal dilation and intrasinusoidal hematopoiesis. The myelo-erythroid ratio was increased (M:E=4-5:1) with erythroid dysplasia (architectural distortion and megaloblastoid change with nuclear irregularities) and somewhat left-shifted granulopoiesis. Megakaryocytes were predominantly small, with hypolobated nuclei and paratrabecular localization. Upon review, the presence of bigger cells with highly irregular, hyperchromatic and hyperlobulated nuclei, with occasional formation of more-or-less dense aggregates/clusters was noted. Blasts were increased: 7-8% approx.

Second biopsy (October 2023)

The bone marrow remained hypercellular (90% cellularity), with less degree of fibrosis (MF2) without collagen deposits, mild sinusoidal dilation and capillary proliferation. The myelo-erythroid ratio was increased (M:E=5-6:1) with some megaloblastoid changes in the red lineage. Megakaryocytes displayed a polymorphic appearance, including small hypolobated forms as well as larger atypical-looking cells, with some clustering. Blasts were up to about 12% focally.

Immunophenotype

Additional immunohistochemistry was performed in the consultation centre. CD14 demonstrated an increase in monocytes and monocyte precursors in both biopsies, reaching about 10-15% in the second biopsy. IRF8 failed to demonstrate an increase of monoblasts.

Cytogenetics

46,XY,der(15)t(1;15)(q21;p11.2),add(21)(p11.2),add(22)(p11.2)[20]

Molecular Studies

Pathogenic Variants: CALR (VAF 37,54%); IDH1 (VAF 11,56%); CALR (VAF 3,64%);

VUS: JAK2 (VAF 44,41%); GATA2 (VAF 35,29%);

Proposed Diagnosis

Myeloproliferative Neoplasm (MPN) in accelerated phase with acquired monocytosis.

Interesting Feature(s)

We find this case difficult to classify on the basis of morphology and immunophenotype alone and, in our opinion, the results of the molecular studies do not completely resolve the differential diagnosis between MDS, MDS/MPN and MPN, in particular, the possibility of de novo MPN with acquisition of IDH1 and additional cytogenetic abnormalities.

Panel Diagnosis: PMF, fibrotic / Overt PMF (MF3) with subsequent monocytosis and progression to blast phase

EA4HP26-BMWS-357

Atypical, fibrotic progression of a TKI resistant CML patient with compound BCR::ABL1 mutation.

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Case Description

60 years old male with known CML (diagnosis of CML was in 2022, chronic phase and grade 1 fibrosis). The best response was partial cytogenetic response, never reached MMR. BCR::ABL1 kinase domain mutation analysis was performed, in 2023 a compound mutation was found. (c.749G>A(p.G250E), c.1076T>G(p.F359C) és a c.1375G>A(p.E459K) next to imatinib treatment. Subsequent nilotinib and dasatinib treatment the patient developed progressing thrombocytopenia and anaemia, bone marrow showed extreme eosinophilia without blast increase (flowcytometry showed 0,8% myeloid blasts, morphology showed 1-2%). After 4th line asciminib and 5th line ponatinib the patient had severe pancytopenia, however the previous marked splenomegaly showed regression.

Biopsy Fixation Details

Jamshidi bone marrow biopsies fixed in Schaefer's fixative (4% neutral buffered formaldehyde containing methanol and glucose), decalcified overnight in 10% ethylenediaminetetraacetic acid disodium salt (EDTA-Na2) and embedded into paraffin wax

Frozen Tissue Available

no

Details of Microscopic Findings

Before ponatinib and asciminib:

The crista biopsy sample contains 5 complete bone marrow samples. The cellularity in the bone marrow is almost 100%, with pronounced granulopoietic hyperplasia and eosinophil differentiation. The eosinophil cells visible in the sample are slightly shifted to the left, with slightly hypo- or unilobulated nuclei. Both erythroids, and megakaryocytes are suppressed in the sample, however occasional groups of micromegakaryocytes are seen. Diffuse grade 2 reticular proliferation is visible with silver staining, no blast proliferation is detectable with the CD34 reaction, a few scattered erythroid cells are visible with the CD71 reaction, and a low number of micromegakaryocytes are visible with the CD61 reaction.

After asciminib and ponatinib:

Bone marrow biopsy. The 2 cm long bone marrow contains more than 15 complete bone marrow cavities. The bone marrow spaces are filled with connective tissue showing a cell-poor, swirling pattern. Minimal haematopoiesis can be observed between the connective tissue fibers. The hematopoietic cells are mostly mature, and no dysplastic features can be observed in the granulo- and erythroid cell lines in the sections. No blast proliferation is visible with CD34 or CD117. The CD61 reaction reveals micromegakaryocytes between the fibers. The megakaryocytes are scattered individually and loosely arranged, accounting for 10-15% of the haematopoietic cells. In addition, the bone trabeculae moderately widened and show a lamellar structure.

Immunophenotype

-

Cytogenetics

Interphase cytogenetics: t(9;22) (BCR::ABL1): 97% Metaphase cytogenetics: 46XY, t(9;22), iso (17

Molecular Studies

BCR-ABL1 major gene (M-bcr p120, b2a2 or b3a2e) expression level: 53%

BCR-ABL1 kinase domain mutation analysis: compound mutation

-ABL1 ENST00000318560.6: c.692 A>G (p.N231S) missense mutation.

-ABL1 ENST00000318560.6: c.730A>G (p.M244V) missense mutation.

-ABL1 ENST00000318560.6: c.951C>G (p.F317L) missense mutation.

-ABL1 ENST00000318560.6: c.982A>G (p.R328G) missense mutation.

-ABL1 ENST00000318560.6: c.1172A>G (p.D391G) missense mutation.

JAK2V617F mutation: not present,

Calreticulin mutation: not present,

MPL mutation not present.

NGS (myeloid NGS panel- custom panel analysing 59 genes): data pending

Proposed Diagnosis

Therapy refractory chronic myeloid leukemia with grade 3 fibrosis, osteosclerosis and micromegakaryocytes, without blast increase. With a possible myelotoxic effect of ponatinib and/or asciminib.

Interesting Feature(s)

-unusual progression pattern, presence of micromegakaryocytes.

Panel Diagnosis: CML, initially in chronic phase, with subsequent accelerated phase (with MF3)

EA4HP26-BMWS-376

Myelodysplastic syndrome with increased blasts-2 and fibrosis

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Case Description

•A 64-year-old male had been under follow-up at an external center for myelodysplastic syndrome because of prolonged anemia and was referred to our institution following the development of pancytopenia.

•MRI;The spleen is normal.

•WBC $0.73 \times 10^9/L$, hemoglobin 4.9 g/dL, hematocrit 15%, platelet count $87 \times 10^9/L$, MCV 89 fL, LDH 1839 U/L, serum iron 41 µg/dL, total iron-binding capacity 139 µg/dL, ferritin 2000 ng/mL, and vitamin B12 569 pg/mL.

Biopsy Fixation Details

•A bone marrow biopsy was performed, decalcified using formic acid prior to formalin fixation, and subsequently processed routinely for histopathological evaluation.

Frozen Tissue Available

Frozen tissue was not available.

Details of Microscopic Findings

•The bone marrow biopsy revealed a hypercellular marrow with an overall cellularity of approximately 75–85%.

•Marked erythroid hyperplasia with normoblastic maturation was present.

•The myeloid lineage was markedly decreased with rare neutrophils.

- Megakaryocytes showed focal increase and consisted predominantly of mature forms, including small megakaryocytes and micromegakaryocytes, consistent with dysmegakaryopoiesis.

- Reticulin fibrosis was graded as MF-2 to MF-3 on reticulin stain.

Immunophenotype

- MPO immunohistochemistry demonstrated a reduced number of myeloid precursors.

- CD71 immunostaining demonstrated erythroid hyperplasia.

- CD61 immunostaining demonstrated an increased number of megakaryocytes, including micromegakaryocytes.

- CD34-positive immature cells accounted for approximately 10% of the bone marrow cellularity.

- CD117 immunostaining showed positivity in blasts and erythroid precursors.

- Immunohistochemical studies showed scattered CD20-positive B lymphocytes and CD3-positive T lymphocytes

Cytogenetics

not done

Molecular Studies

The results (FISH) were negative for;

- t(16;16)(p13;q22),

- del5q31,

- t(3;3)(q26.2;q21.3),

- t(8;21)(q21.3;q22),

- Trisomy 8 and 17q21

Proposed Diagnosis

Myelodysplastic syndrome with increased blasts-2 and fibrosis (MDS-IB2, WHO 5th edition).

Interesting Feature(s)

- This entity may pose diagnostic challenges in the differential diagnosis with Myelodysplastic/Myeloproliferative neoplasms and myeloproliferative neoplasms, because there were hyperplasia with fibrosis in the bone marrow.

- However, presentation with pancytopenia, absence of splenomegaly, and the presence of small hypolobulated megakaryocytes and micromegakaryocytes together with prominent dysplastic features on histopathological examination favor a diagnosis of myelodysplastic syndrome.

- In this group of patients, marked myelofibrosis may result in a “dry tap” during aspiration, which can hinder reliable assessment of bone marrow cellularity and particularly the blast percentage.

- In fibrotic myelodysplastic syndromes(MDS-F), genetic alterations that are also observed in patients with myelofibrosis may be encountered; however, mutations characteristic of myeloproliferative neoplasms, such as JAK2, CALR, and MPL, are rarely detected in MDS. Also multi-hit TP53 and SF3B1 should be excluded for MDS-F diagnosis.

- Reporting the presence of fibrosis accompanying myelodysplastic syndrome (MDS) is important, as there are differing views regarding the prognostic significance of myelofibrosis in MDS. The prevailing opinion is that MDS with associated fibrosis is associated with a poorer prognosis.

- The patient received azacitidine in combination with venetoclax, followed by autologous transplantation. Four months later, disease progression was noted with an increase in blast count, and the patient ultimately died from HHV-6 encephalitis.

Panel Diagnosis: MDS with increased blasts and fibrosis, lacking genetic studies

Myeloid Neoplasm with Del(5q), Increased Blasts and Fibrosis.

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Case Description

50 year-old female.

Presented with fatigue and menorrhagia.

Full blood count showed pancytopenia.

No significant past medical history.

No lymphadenopathy.

No splenomegaly.

Biopsy Fixation Details

5% buffered formalin

EDTA de-calcification

Frozen Tissue Available

No

Details of Microscopic Findings

Blood Film: 4% blasts

Bone Marrow Aspirate:

Aparticulate, haemodilute sample, therefore trephine roll analysed.

Granulopoiesis: Markedly reduced, prominent dysplastic changes including hypolobation, and hypogranularity

Erythropoiesis: Reduced, prominent dysplastic changes including frequent nuclear outline irregularity and N/C asynchrony.

Megakaryopoiesis: Occasional megakaryocytes present, displaying normal morphology.

Blasts: 8% of TNCs. Blasts are medium-sized with fine chromatin pattern, indistinct nucleoli, high NC ratio, and weakly basophilic cytoplasm.

Bone Marrow Trephine:

Hypercellular, cellularity around 90%.

Stromal reticulin fibres increased, with cellular streaming. WHO grade 2 fibrosis.

Interstitial collagen fibre formation confirmed on Masson Trichrome stain.

Myeloid:erythroid ratio at upper limit of normal.

Erythropoiesis: singly dispersed erythroid cells with prominent dyserythropoiesis. Expanded, left-shifted erythroid islands.

Granulopoiesis: Left shifted, disorderly and reduced maturation to neutrophils.

Megakaryocytes: Increased in number, loose clustering, prominent platelet shedding. Pleomorphic morphology, including medium to occasionally large forms with hypersegmented nuclei, medium to small forms with separated nuclear lobes or hypolobated nuclei and scattered micro-megakaryocytes.

3-4% CD34(+) blasts.

Immunophenotype

Bone Marrow Aspirate Immunophenotyping:

3% CD34+ myeloid progenitors Clotted sample, therefore interpreted with caution

CytogeneticsKaryotyping:

46,XX,del(5)(q12q33)[9]/46,XX[1]

FISH:

Deletion of 5q detected

Molecular Studies**Myeloid Gene Panel:**

DNMT3A (VAF 37%), STAG2 (VAF 29%), TET2 (VAF 34%), and KMT2A-PTD

Molecular:

FLT3/NPM1/IDH1 negative.

Proposed Diagnosis

Myelodysplastic neoplasm with increased blasts and fibrosis (MDS-F) (WHO 5th Ed)

Myelodysplastic syndrome with excess blasts (MDS-EB) (ICC 2022)

Interesting Feature(s)

- Does not fulfil diagnostic criteria for MDS with low blasts and 5q deletion (MDS with del (5q)) due to increased blasts on blood film (4%)
- Fibrosis is not typically seen in MDS with low blasts and 5q deletion at presentation.
- Typical presentation and bone marrow morphology of MDS with increased blasts and fibrosis.
- Bone marrow aspirate frequently inadequate, due to fibrosis, with resultant challenges for immunophenotyping and assessment of dysplasia.
- The absence of splenomegaly and a myeloproliferative neoplasm driver mutation makes fibrotic progression of a myeloproliferative neoplasm a very unlikely differential diagnosis.

Panel Diagnosis: MDS-F / MDS-EB

EA4HP26-BMWS-387

Acute myeloid leukaemia with a novel *PRDM16::GATA2* fusion detected with optical genome mapping and associated bone marrow fibrosis

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Case Description

A routine laboratory examination of a 52 years old female revealed massive thrombocytosis and elevated LDH (WBC:12 G/L, Hgb: 101 g/L, Thr: 2445 G/L, LDH: 1200 U/L). Bone marrow (BM) biopsy lead to the diagnosis of acute myeloid leukaemia (AML) with *PRDM16::GATA2* fusion, as detailed below.

Follow-up: Besides cytoreduction, intensive treatment could not be initiated as the patient developed severe bilateral pneumonia. Flow cytometry (FCM) identified pulmonary manifestation of AML. In addition, a multidrug-resistant *Acinetobacter* was detected and the patient died within one month of the initial diagnosis.

Biopsy Fixation Details

Jamshidi BM biopsies fixed in Schaefer's fixative (4% neutral buffered formaldehyde containing methanol and glucose), decalcified overnight in 10% ethylenediaminetetraacetic acid disodium salt (EDTA-Na₂) and embedded into paraffin wax.

Frozen Tissue Available

No.

Details of Microscopic Findings

Biopsy: BM was markedly hypercellular (~95%) with at least 80% of the cells being dysplastic megakaryocytes, in addition to an intermixed small-to-medium-sized blast population. Both the granulocytic and erythropoietic lineages were suppressed. Silver staining revealed diffuse grade 2 fibrosis.

Aspirate smear: The hypocellular smear did not contain marrow particles. Most of the cellular elements represented the blast population, along with a few granulopoietic cells, without the evidence of the bizarre megakaryocytosis seen in the biopsy.

Immunophenotype

FCM: *Peripheral blood* - 44% blast with the following phenotype: **Pos:** CD34, CD117, CD99; **Het:** HLA-DR; **Dim:** CD45, CD38, CD13, CD33; **Neg/dim:** CD36, CD71; **Neg:** CD14, CD7, CD19, CD64, CD11b, CD15, CD4, CD56, CD5, sCD3, cyCD3, TdT, MPO. The phenotype of the *BM blasts* was nearly identical, however CD36 was completely negative.

IHC: CD61 highlighted dysplastic megakaryocytosis – this population was CD34 negative. In contrast, the intermixed blast population (~20%) was CD34 positive, lacking CD61. CD71 strongly expressed by the residual erythropoiesis.

Cytogenetics

Classic cytogenetics: structural rearrangement of chromosome 1 and a deletion of the long arm of chromosome 3. [46,XX,der(1p)t(1;3),del(3q21)[20]].

Molecular Studies

Optical genome mapping (OGM): identified the t(1;3)(p36.32;q21.3) translocation resulted in the *PRDM16::GATA2* gene fusion. 46,XX,t(1;3)(p36.32;q21.3)(3045979;128485475)(PRDM16::GATA2)

Myeloid NGS (59 gene QIaseq Targeted DNA panel) revealed pathogenic *PTPN11* (VAF39%), likely pathogenic *GATA2* (VAF 30%) and a variant of unknown significance *ASXL1* (VAF 48%) mutations.

Proposed Diagnosis

Acute myeloid leukaemia with *PRDM16::GATA2* fusion and associated bone marrow fibrosis

Interesting Feature(s)

- **Role of OGM in the detection of the novel fusion *PRDM16::GATA2*** - OGM is novel technology allowing the analysis of ultra-high molecular weight DNA molecules, provides high-resolution genome-wide information and able to detect copy number and structural abnormalities, including balanced translocations.
- **Similarities with *MECOM* rearranged and *PRDM16::RPN1* AMLs** - The case illustrates shared characteristics with *MECOM* rearranged and *PRDM16::RPN1* AMLs. The patient presented with marked thrombocytosis, the bone marrow expressed dysplastic megakaryocytosis and significant fibrosis and the clinical course was very aggressive.
- **Consideration regarding future classifications** – The case may provide further evidence to consider including *PRDM16*-rearranged AMLs into future classifications (as ICC already counts AML with *PRDM16::RPN1* among the group of 'AML with other rare recurring translocations').

Panel Diagnosis: AML defined by differentiation / AML, NOS (*PRDM16::GATA2*)

Myelodysplastic syndrome with progressive molecular and cytogenetic evolution to a proliferative/fibrotic neoplasm

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Case Description

75-year-old woman with low-grade myelodysplastic syndrome diagnosed in August 2017 (hypercellular marrow with erythroid/myeloid hyperplasia, micromegakaryocytes, ~2% blasts) presenting with chronic anemia. Founding mutations included *SRSF2* p.P95H and *TET2* p.H1380Y. From 2021–2024 she received supportive and disease-directed therapies with partial response. In May 2024 she developed worsening anemia, emerging leukocytosis/monocytosis and splenomegaly, prompting repeat marrow evaluation.

A repeat bone marrow biopsy in July 2024 showed persistent hypercellularity with multilineage dysplasia and significant fibrosis, supporting phenotypic progression along an MDS/MPN spectrum.

Biopsy Fixation Details

Formalin fixed paraffin embedded, decalcified.

Frozen Tissue Available

No

Details of Microscopic Findings

Markedly hypercellular marrow (>95%) with myeloid predominance, dysplastic megakaryocytic clusters and no overt increase in blasts. Aspirate was markedly hemodilute/aspiculate, limiting morphologic assessment.

Immunophenotype

Immunohistochemistry

No increase in CD34+/CD117+ blasts; CD61 highlighted atypical megakaryocyte clusters. Reticulin showed diffuse fibrosis (MF-2) with minimal/focal collagen by trichrome. Perls stain showed markedly increased iron stores.

Flow Cytometry

Differential: ~70% granulocytes, 24% lymphocytes, 2% monocytes, 0.6% blasts. Granulocytes: low side scatter with left shifted, abnormal maturation and aberrant CD56+. Monocytes: classical (CD14+, CD16–) with aberrant CD56+. Blasts: partial CD7+. Peripheral blood: small CD34+ population with dim CD7+ and partial CD56+; monocytes with aberrant dim CD123+, CD2+, CD7+ and CD56+.

Cytogenetics

Baseline interphase FISH and karyotype (46,XX) were normal. Follow-up showed emerging 20q12 loss and a balanced translocation t(X;12) in ~40% of metaphases.

Molecular Studies

Sequential molecular evolution included acquisition of *JAK2* p.V617F in a background of *SRSF2/TET2* with rising allele frequencies.

Proposed Diagnosis

Myelodysplastic syndrome with acquired *JAK2* mutation associated with fibrosis and progressive monocytosis.

Interesting Feature(s)

- This case demonstrates features of both fibrotic myeloproliferation and myelodysplasia without meeting the full criteria for either Myeloproliferative neoplasm (MPN) or a defined MDS/MPN subtype.
- It shows sequential acquisition of genetic abnormalities *SRSF2*, *TET2* → *JAK2* → 20q12 deletion/t(X;12) with corresponding morphologic and clinical progression.

- *SRSF2* and *TET2* mutations, particularly when co-occurring, are characteristic of MDS/MPN syndromes and AML [PMID: 26908133, PMID: 33349666]. The small populations of immunophenotypically aberrant blasts and progressive monocytosis in our case adds complexity by suggesting clonal hematopoiesis.
- The subsequent acquisition of a *JAK2* mutation represents a critical point: it being the dominant clone, drives the morphologic phenotype to exhibit MPN features; concurrent *SRSF2* mutations with classic MPN driver mutations were shown to be associated with dysplasia and monocytosis by Tashakori *et al.* [PMID: 35690645].
- The acquisition of further cytogenetic abnormalities (20q12 deletion and t(X;12)) raises concern for disease progression. 20q deletions in MDS are frequent and by themselves carry a low risk of progression [PMID: 24372512]. There is extremely limited literature on balanced whole-arm translocation t(X;12) in myeloid neoplasms, with one 2010 case report describing it in a patient with atypical chronic myeloid leukemia (aCML) [PMID: 19657750]. Chromosome 12 harbors ETV6/TEL (12p13), which is a known partner in leukemogenic translocations [PMID: 39017849].

Panel Diagnosis: MDS-LB / MDS-NOS with monocytic progression; WHO allows to reclassify as Myelodysplastic CMML-1

EA4HP26-BMWS-400

A dry tap with a myeloid neoplasm with fibrosis

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Case Description

68 year-old female with thrombocytosis.

Biopsy Fixation Details

Biopsy fixation involves 10% neutral buffered formalin (NBF).

Frozen Tissue Available

No

Details of Microscopic Findings

- Cellular marrow with focal grade 1 reticulin fibrosis
- The bone marrow core is suboptimal for evaluation due to extensive crush artifact with limited areas of hematopoiesis for evaluation
- The specimen was a dry tap and morphological evaluation of dysplasia could not be performed
- The peripheral blood showed no evidence of granulocytic dysplasia or circulating blasts

Immunophenotype

Concurrent flow cytometry showed no abnormal immunophenotypes or increase in blasts

Cytogenetics

No metaphases detected

Molecular Studies

- Positive for Calretinin mutation by PCR analysis
- PCR was negative for *JAK2* and *MPL*
- MPN FISH was negative including:
 - BCR-ABL1
 - Del 13q
 - Del 20
 - Gain of chr 8

- Gain of chr 9
- There is insufficient specimen left to run MDS FISH

Proposed Diagnosis

Myeloid neoplasm with grade 1 reticulin fibrosis

The differential diagnosis includes:

Myeloproliferative neoplasms (MPN) including essential thrombocythemia or prefibrotic primary myelofibrosis (PMF)

Myelodysplastic neoplasm (MDS) with deletion 5q

Interesting Feature(s)

- Thrombocytosis can occur in myelodysplastic neoplasms (MDS) versus myeloproliferative Neoplasms (MPN). MPNs generally involve overproducing cells like platelets in essential thrombocythemia (ET) and MDS often causing low counts due to ineffective maturation, but some rare MDS and MDS/MPN overlap syndromes, like MDS with deletion 5q and MDS/MPN with ringed sideroblasts and thrombocytosis (MDS/MPN-RS-T) can present with both features (anemia and thrombocytosis).
- MPL (myeloproliferative leukemia virus oncogene) mutations are associated with various hematological disorders including myelodysplastic neoplasms (MDS) and myeloproliferative neoplasms (MPNs).
- In the absence of a cellular aspirate smear, morphological evaluation of dysplasia could not be performed and an MDS could not be entirely ruled out.
- In addition, cytogenetics yielded no metaphases and an MDS FISH to include a del 5q probe could not be performed due to lack of sufficient material.

Panel Diagnosis: PMF, fibrotic / Overt PMF (MF3)

EA4HP26-BMWS-406

Myelodysplastic/myeloproliferative neoplasm with *SF3B1* mutation and thrombocytosis (MDS/MPN-*SF3B1*-T) with progression to myelofibrosis.

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Case Description

- 80-year-old male.
- Presented with anaemia, leukocytosis and thrombocytosis.
- Haemoglobin 116 g/L, White Cell Count $14 \times 10^9/L$, Platelets $591 \times 10^9/L$, Neutrophils $11.4 \times 10^9/L$, Lymphocytes $1.7 \times 10^9/L$, Monocytes $0.6 \times 10^9/L$.
- Leukoerythroblastic blood film.
- Spleen size: 13.7 cm.
- Previous history: Prostatic adenocarcinoma (treated with radical prostatectomy).

Biopsy Fixation Details

- 5% buffered formalin.
- EDTA decalcification.

Frozen Tissue Available

- No.

Details of Microscopic Findings

Bone marrow aspirate

- Aparticulate, partially haemodilute aspirate.
- At least single lineage (erythroid) dysplasia with ring sideroblasts, but sample otherwise suboptimal for assessment. Bone marrow trephine biopsy
- Markedly hypercellular for the patient's age (marrow cellularity > 95%).
- No new bone formation.
- Diffuse and dense increase in stromal reticulin fibres, along with focal deposition of collagen fibres; WHO fibrosis grade MF-2.
- M:E ratio: Increased.
- Granulopoiesis: Expanded and disorderly, with maturation to neutrophils.
- Erythropoiesis: Variably disrupted, left-shifted erythroid colonies, with dispersal of erythroid precursors.
- Megakaryocytes: Increased in number, with focal clustering. Predominance of large forms, including those with hyperlobated or bulbous nuclei. Very occasional small forms and rare possible micromegakaryocytes.
- CD14+ monocytes: < 5% of TNCs.
- CD34+ and/or CD117+ blasts: < 5% of TNCs.

Immunophenotype

Flow cytometry on bone marrow aspirate

- CD34+ myeloid progenitors: 0.7% of TNCs with phenotype CD13+, CD33 weak, CD117+ and HLA-DR weak, with no aberrant expression identified.
- Granulocytes: 70% of TNCs.
- Monocytes: 2.6% of TNCs.
- Granulocytes and monocytes show weak CD33 expression.
- Erythroid: 5.2% of TNCs.
- Integrated MDS Scoring System by flow cytometry: Multiple MDS-related changes detected.

Cytogenetics

SNP Array

- CN-LOH(1p36.33p21.2), del(12p13.2) detected.

Molecular Studies

Myeloid Gene Panel

- *JAK2* V617F mutation (VAF 40%).
- *SF3B1* mutation (VAF 44%).

Proposed Diagnosis

Myelodysplastic/myeloproliferative neoplasm with *SF3B1* mutation and thrombocytosis (MDS/MPN-*SF3B1*-T) with progression to myelofibrosis.

Interesting Feature(s)

- This case highlights the diagnostic challenges in classifying myeloid neoplasms when patients present at an advanced (fibrotic) stage of disease.
- In the setting of marked bone marrow fibrosis with dysplasia, the differential diagnosis includes MDS with fibrosis, fibrotic stage of MPN, or fibrotic stage of MDS/MPN. Distinction between these entities can be challenging due to overlapping clinical, morphological, and genetic features.
- In this case, a diagnosis of MDS with fibrosis is excluded given the presence of cytos and genetic findings (including the detection of an MPN-associated driver mutation). However, distinction between *BCR::ABL1*-negative MPN in fibrotic phase and MDS/MPN-*SF3B1*-T in fibrotic phase remains challenging, as *SF3B1* mutations have been reported to occur in 10% of *BCR::ABL1*-negative MPNs.¹ One such study which investigated the clinicopathological and molecular features of *SF3B1*-mutated MPNs showed that *SF3B1* mutations were enriched in cases of primary myelofibrosis in both prefibrotic and fibrotic stages and detected during late fibrotic stages of essential thrombocythaemia and polycythaemia vera, but were not associated with

myelodysplastic progression.¹ As a result, based on the presence of dysplasia in this submitted case, a diagnosis of MDS/MPN-SF3B1-T in fibrotic phase is favoured.

Panel Diagnosis: MDS/MPN-SF3B1-T

EA4HP26-BMWS-407

Acute Myeloid Leukemia, NPM1-mutated, with Fibrosis

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Case Description

A 39-year-old woman with no significant past medical history presented to the emergency department with a 3-day history of dizziness and fatigue. She experienced heavier menstrual periods, clot passage, and intense cramping for two months. There was no reported history of abnormal blood counts.

Initial laboratory studies revealed pancytopenia and 11.9% circulating blasts.

Biopsy Fixation Details

Formalin-fixed.

Decalcified.

Frozen Tissue Available

None.

Details of Microscopic Findings

The bone marrow core biopsy sections show a markedly hypercellular marrow for age (~90-95% cellularity) with mildly increased blasts (10-15% of the cellularity; best seen on a CD34 immunostain). The background marrow shows an erythroid-predominance trilineage hematopoiesis with left-shifted myelopoiesis. The megakaryocytes are both singly-scattered and are present in small clusters, and range from small forms with hypolobated, hyperchromatic nuclei to large forms with widely separated nuclear lobes or complex patterns of nuclear lobation.

The aspirate is hemodilute, but reveals scattered blasts, erythroid precursors with nuclear membrane irregularities and megaloblastoid maturation, and occasional hypogranular neutrophils.

A reticulin stain shows moderately increased fibrosis (MF 2 out of 3).

Immunophenotype

Flow cytometric analysis, performed on a hemodilute specimen, detected approximately 15% blasts which are positive for CD45, CD34, CD117, CD13, CD33, CD38, HLA-DR, and partial cytoplasmic MPO.

Cytogenetics

Cytogenetic analysis of the peripheral blood revealed a normal female karyotype. An acute myeloid leukemia fluorescence in situ hybridization (FISH) panel and chromosomal microarray analysis detected no abnormalities.

Molecular Studies

Molecular analysis identified the following abnormalities: *NPM1* (W288Cfs12) frameshift mutation (variant allele frequency, VAF=21.9%), *STAG2* R216 (VAF=22.7%), a *DNMT3A* R882C (VAF=30.9%), and *FANCA* F1263del (VAF of 47.2%). No increase in chromosome breakage after DEB exposure (ARUP labs).

Proposed Diagnosis

Acute myeloid leukemia, *NPM1*-mutated, with fibrosis.

Interesting Feature(s)

The morphologic and immunophenotypic findings, including large megakaryocytes with clustering and fibrosis, raised the possibility of a myelodysplastic/myeloproliferative neoplasm or myeloproliferative neoplasm with increased blasts. Taken together, the blast count of 10-15% and presence of an *NPM1* mutation prompted re-classification as “acute myeloid leukemia (AML) with mutated *NPM1*.” The *STAG2* and *DNMT3A* mutations also seen in this case have been described in *NPM1*-mutated AML, and it is possible that the unusual megakaryocyte morphology is due, at least in part, to the *STAG2* mutation [PMID: 38780921]. Both the strong expression of CD34 on the blasts and significant fibrosis are somewhat unusual for *NPM1*-mutated AML [PMID: 36008542].

The possibility of evolution from MDS or MDS/MPN cannot be entirely excluded; however, there is no prior history of abnormal counts. Of note, mutated *NPM1* is strongly correlated with the emergence of AML shortly after the diagnosis of MDS and MDS/MPN [PMID:34575966].

The role played by the *FANCA* mutation in this case is unclear, but it may not be relevant given the normal chromosome breakage assay.

Panel Diagnosis: AML with *NPM1* mutation / AML with mutated *NPM1*

EA4HP26-BMWS-421

Fibrotic Transformation in MDS-to-AML Progression Mimicking Primary Myelofibrosis

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Case Description

01/2025: An 85-year-old woman on chronic psychotropic medication presented with persistent anemia and leukopenia. Blood counts showed Hb 10.2 g/dL, WBC $2.2 \times 10^9/L$ with ANC $0.56 \times 10^9/L$ and absolute monocytes $0.36 \times 10^9/L$, and platelets $334 \times 10^9/L$. BM biopsy was performed. 07/2025: She was followed as with ongoing cytopenias and concern for progression/AML transformation. She had received weekly erythropoietin and a single dose of G-CSF in the preceding week. Blood counts showed Hb 9.1 g/dL, WBC $3.97 \times 10^9/L$ with ANC $0.71 \times 10^9/L$, and platelets $134 \times 10^9/L$. A repeat BM biopsy was performed. 08/2025: She deteriorated with profound pancytopenia. Blood counts showed Hb 8.3 g/dL, WBC $0.66 \times 10^9/L$ with ANC $0.07 \times 10^9/L$, and platelets $73 \times 10^9/L$. A further BM biopsy and expanded molecular testing were performed.

Biopsy Fixation Details

BM decalcified in EDTA (6–12 h), FFPE processing.

Frozen Tissue Available

No

Details of Microscopic Findings

01/2025: Suboptimal and fragmented trephine. Marrow shows age-adjusted hypercellularity with dysplastic hematopoiesis, including markedly decreased granulopoiesis, disorganized left-shifted erythropoiesis, occasional small hypolobated megakaryocytes, and over 5% ring sideroblasts. MF0. 07/2025: Marrow is markedly hypercellular with extensive blast infiltration. There is increased megakaryopoiesis with large hyperlobulated MPN-like forms, dyserythropoiesis, and severely reduced granulopoiesis. Reticulin fibrosis is minimal (MF 0–1). 08/2025: Myelofibrotic

marrow with advanced fibrosis (MF-3) and architectural distortion. There is markedly increased atypical and clustering megakaryopoiesis, profound reduction of erythroid and granulocytic lineages, and persistent blasts.

Immunophenotype

In the sequential BM biopsies, the blast population was CD34-positive with weak CD117 expression, accounting for 10–15% of nucleated cells in 01/2025, 40–50% in 07/2025, and 15–20% in 08/2025.

Cytogenetics

Initial work-up in 01/2025 showed normal karyotype (46,XX). By 07/2025, cytogenetic analysis revealed an hyperdiploid karyotype with 47 chromosomes (23 out of 25 metaphases). Trisomy 13 and a 7q deletion were found in 7 of these metaphases.

Molecular Studies

NPM1 p.Trp288CysfsTer12 (Type A), VAF 4% (pathogenic)

RUNX1 p.Ala147GlnfsTer3, VAF 52% (clinically relevant)

SF3B1 p.Lys700Glu, VAF 43% (pathogenic/likely pathogenic)

FLT3-ITD, VAF ~0.2% (low-level; confirmation by fragment-length analysis)

FLT3 p.Gly583Arg, VAF 7% (VUS)

KIT p.Met541Leu, VAF 50% (benign/likely benign)

Proposed Diagnosis

Progression to AML with myelodysplastic-related changes and marked marrow fibrosis (MF-3).

Interesting Feature(s)

1. In MDS/AML, fibrosis may progress rapidly and be disproportionately severe relative to the blast count, resulting from alterations in the BM microenvironment, where MGK-derived profibrotic cytokines and inflammation promote fibroblast activation. Consequently, the marrow may exhibit an “end-stage MF” appearance even when blast count remains low.
2. Non-MPN myeloid neoplasms may resemble PMF, which can be misleading diagnostically. Additional confounding factors in this case, including EPO, G-CSF administration, or psychotropic medications, may influence blood counts and marrow morphology. Therefore, clinicopathologic correlation and repeated bone marrow biopsies are essential for accurate diagnosis.
3. Genetic alterations such as *SF3B1*, *RUNX1* mutations, del7q, or a complex karyotype support secondary AML with significant fibrosis.
4. Fibrotic BM may underestimate blast counts. BM trephine biopsy and assessing CD34-distribution can prevent misclassification as PMF or MDS/MPN.

Panel Diagnosis: MDS-EB2 / MDS/AML with progression to AML-MR / AML with MR gene mutations and subclonal *NPM1* progression

EA4HP26-BMWS-425

Myeloid neoplasm with fibrosis and concurrent chronic lymphocytic leukemia (CLL)

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

Case Description

77-year-old male with relative lymphocytosis, relative and absolute monocytosis, and anemia.

Biopsy Fixation Details

Biopsy fixation involves 10% neutral buffered formalin (NBF).

Frozen Tissue Available

Not available

Details of Microscopic Findings

Hypercellular marrow (>90%) with megakaryocytic hyperplasia and atypia

The megakaryocytes have tight clusters and atypical hyperchromatic nuclei

Grade 2-3 reticulin fibrosis

Increased myeloblasts comprising 5-7% by IHC and 4% by flow cytometry

Chronic lymphocytic leukemia involving 20% by IHC and 56% by flow cytometry

Immunophenotype

Concurrent flow cytometry shows abnormal CD5-positive lambda-restricted B-cells comprising 56% of total events and 4% myeloblasts.

Cytogenetics

45,XY,-7,del(12)(p13p11.2)[2]/46,idem+,mar[5]

Abnormal male karyotype with monosomy 7 and deletion 12p.

In the setting of unexplained cytopenias, monosomy 7 can be seen in myelodysplastic neoplasms or acute myeloid leukemias.

Molecular Studies

Insufficient material for send out for FISH and molecular studies

Proposed Diagnosis

Myeloid neoplasm with grade 2-3 reticulin fibrosis; pending ancillary studies

The differential diagnosis includes:

Myelodysplastic neoplasm (MDS) with fibrosis

Myeloproliferative neoplasms (MPN) including primary myelofibrosis (PMF)

Chronic myelomonocytic leukemia (CMML)

Acute myeloid leukemia with recurrent cytogenetic abnormalities

Interesting Feature(s)

Fibrotic marrows are hard due to the accumulation of fibrous connective tissue, which disrupts the normal process of hematopoiesis.

Marrow fibrosis can be primary as seen in conditions like idiopathic myelofibrosis, or secondary, stemming from other diseases or factors such as chronic inflammation and neoplastic changes.

The buildup of fibrous tissue can also be associated with various hematological disorders, including myelodysplastic neoplasms (MDS), myeloproliferative neoplasms (MPNs) including primary myelofibrosis (PMF), and MDS/MPN subtypes like chronic myelomonocytic leukemia (CMML).

This patient also had concurrent CLL which could have contributed to the fibrosis.

Panel Diagnosis: MN with fibrosis, morphologically suggestive of PMF; concurrent B-cell LPD with CLL-like phenotype

EA4HP26-BMWS-432

A Confusing Case of Systemic Mastocytosis with Associated Hematologic Neoplasm

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Case Description

The patient is a 67-year-old male who presented initially with palpable splenomegaly 6 cm below the left costal margin. Laboratory workup is as follows:

Complete blood count:

WBC: $1.48 \times 10^9/L$

RBC: $3.28 \times 10^{12}/L$

HGB: 9.6 g/dL

HCT: 30.7%

MCV: 93.6 fL

MCH: 29.3 pg

MCHC: - g/dL

RDW: 20.5%

PLT: $66 \times 10^9/L$

Elevated total bilirubin: 2.0 mg/dL and direct bilirubin: 0.67 mg/dL.

Elevated gamma-glutamyl transferase (GGT): 105 U/L.

Elevated serum tryptase: 637 ug/L.

Biopsy Fixation Details

Biopsy is fixed in buffered neutral 10% formalin and decalcified in EDTA.

Frozen Tissue Available

No frozen tissue is available.

Details of Microscopic Findings

Peripheral blood smear showed pancytopenia and a leukoerythroblastic picture. There were rare circulating mast cells (<1%). Touch preparation of the bone marrow core had erythroid dysplasia (nuclear irregularities, 30%) and ring sideroblasts (20%). There were increased mast cells (38%) including 85% immature (promastocytes) and 15% spindle-shaped. The core biopsy was hypercellular (95%) with trilineage hematopoiesis. There was myeloid hypoplasia, megakaryocytic hyperplasia with pleomorphic and hyperchromatic megakaryocytes. Additionally, there were multifocal confluent dense aggregates of mast cells composing 75% of the total marrow cellularity. Of these mast cells, 25% were atypical (spindle-shaped). Moderate reticulin fibrosis, moderate collagen fibrosis, and moderate osteosclerosis were present.

Immunophenotype

Mast cells:

CD117: 100% mast cells, strong expression

Tryptase: 100% mast cells, weak expression

CD25: 100% of mast cells, strong expression

CD30: 90% of mast cells, strong expression

Other:

CD34: CD34-positive blasts, 1% out of total marrow cellularity

Cytogenetics

None are available.

Molecular Studies

Myeloid panel by NGS:

Tier 1 (Likely oncogenic)

- BCOR G1380TX VAF 17.3%

- DNMT3A F755S VAF 30.5%

- JAK2 V617F VAF 3.8%

- MPL W515R VAF 6.8%

Tier 2

- TET 2 V218M VAF 50.9%

KIT D816V by droplet digital PCR (ddPCR)

- Initial VAF 0.10%

Proposed Diagnosis

The proposed diagnosis is systemic mastocytosis with associated hematological/myeloid neoplasm (SM- AHN/AMN). The SM component is mast cell leukemia (MCL) vs. aggressive systemic mastocytosis (ASM). The AHN/AMN component is most consistent with myeloproliferative neoplasm with myelofibrosis.

Interesting Feature(s)

The distinction of ASM and MCL is challenging as the diagnosis of MCL is based on the number of mast cells in well-prepared aspirate smears ($\geq 20\%$). Marrow fibrosis often results in suboptimal, hemodilute, aspirate or a “dry tap”, compromising this diagnostic criterion, as seen in the presented case. We favor MCL on account of the following:

- The presence of circulating mast cells, albeit rare ($<1\%$).
- A significant proportion of immature mast cells (promastocytes), 85%.
- Pattern of mast cell infiltration in the core biopsy specimen.

The subclassification of the AHN/AMN component is difficult and a myeloproliferative neoplasm is favored. Advanced marrow fibrosis precludes from a more precise subclassification (polycythemia vera vs. primary myelofibrosis, vs. other. Dysplastic features are likely secondary.

Panel Diagnosis: SM-AHN / SM-AMN (unclassifiable)

EA4HP26-BMWS-435

MDS/MPN with myelofibrosis MF-2 versus MDS with fibrosis

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Case Description

A 62-year-old woman with suspected MDS with significant history of alcoholism, mitral valve prolapse, hypertension, depression and Lyme's disease.

Investigated over 3 years. Multiple CBCs showing variable degrees of leukocytosis, normocytic anemia. Monocytes ranging between 4-7%, at times AMC reached 0.52.

Multiple BM specimens performed over the course of 3 years showed non-diagnostic findings. Outside mutation panel showed mutations in SRSF2 and TET2 at two time points. Patient continued on observation without a definitive diagnosis.

Last outside BM review (+3 years since initial suspicion):

CBC: WBC 8.12, Hgb 10.2, Hct 31%, and platelet count 121 with 5% monocytes. BM exam suggested MDS with multilineage dysplasia and MF-2 fibrosis and osteosclerosis.

In-house BM aspiration and biopsy was performed (within 2 months).

Follow-up:

Following the diagnosis MDS/MPN with MF-2, the patient was treated with erythropoietin (non-transfusion dependent anemia) initially, followed by azacitadine (after 18 months) and 12 months later with decitabine and venetoclax. She progressed to AML with additional mutations involving ASXL1 and STAG2. She is lost for follow-up.

Biopsy Fixation Details

Formalin

Frozen Tissue Available

No

Details of Microscopic Findings

CBC: WBC: 20.6, Hgb 9.7, MCV 95 Platelets 145; Monocytes: 4%

PB smear:

RBCs showed anisopoikilocytosis, includes ovalocytes, basophilic stippling and circulating nRBCs. WBCs were decreased, hypogranular neutrophils with left-shift (~8%) and circulating blasts; no increased monocytes, eosinophils or basophils. Platelets were adequate in number, included many giant forms.

Bone marrow:

Bone marrow biopsy is adequate in quality with ~90% cellularity. Megakaryocytes are increased in number, with many small dysplastic forms. There is increased immature mononuclear cells and marked paratrabecular fibrosis.

Bone marrow aspirate smears show particles. There is trilineage hematopoiesis, dysmegakaryopoiesis and increased blasts (~7%).

Special stains:

Reticulin stain highlights multifocal areas of dense increase with coarse fibers

Trichrome stain showed patchy (multi-focal) areas with abnormal interstitial collagen deposits

MF grade 2

Immunophenotype

Flow cytometry: Aberrant CD34+ blasts (1.9%) with altered immunophenotype (CD34 inc, CD117 inc, CD13 partial/dec, CD33+, CD123+. CD38+). Absent hematogones. Granulocytes showed altered maturation. Monocytes showed increased CD56 expression.

Immunohistochemistry:

CD34: Rare scattered blasts

CD117: Immature cells and scattered mast cells (no tight aggregates)

CD71: Erythroid precursors

CD61: Highlights increased megakaryocytes

Cytogenetics

46,XX[20]

Molecular Studies

81-gene NGS panel: Mutations in SRSF2 (VAF: 32%), double TET2 mutations (biallelic) with high VAFs

Proposed Diagnosis

MYELOYDYLASTIC/ MYELOPROLIFERATIVE NEOPLASM WITH FIBROSIS (MF-2), 3% blasts

Interesting Feature(s)

At the time of diagnosis, the differential diagnosis between MDS and MDS/MPN was a challenge.

Leukocytosis was absent to mild; relative monocytosis ranged between 4% and 7%, and absolute monocytosis was <500 except in one instance which resolved shortly afterwards. However, the presence of SRSF2 and TET2 mutations was typical of MDS/MPN (CMML-like signature).

At the time of in-house biopsy, the leukocytosis was present, representing MDS/MPN, although not formally meeting the criteria for oligomonocytic CMML. The lack of monocytosis, ring sideroblasts and MF-2 fibrosis raised the differential diagnosis between MDS/MPN-unclassifiable and atypical CML.

Panel Diagnosis: MDS/MPN, NOS (potentially from CCUS)

EA4HP26-BMWS-453

Chronic myeloid leukemia with a concurrent CALR+ mutated myeloproliferative neoplasm.

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Case Description

A 30 year old male presents with abdominal pain in 2015. Imaging studies showed splenomegaly, and CBC studies showed leukocytosis with basophilia. A bone marrow demonstrated a hypercellularity, myelofibrosis (MF1-2), and atypical megakaryocytes, which raised the possibility of a BCR-ABL negative myeloproliferative neoplasm. However, FISH and cytogenetic studies confirmed a Philadelphia chromosome abnormality - t(9;22), and a diagnosis of chronic myeloid leukemia was made without mutational studies. The patient responded well to dasatinib and RT-PCR studies showed decreased in BCR-ABL1-ABL% (IS) levels. A subsequent bone marrow showed a negative FISH and cytogenetic studies for Philadelphia chromosome. However, patient's splenomegaly persisted, even after radiation treatment. An elective splenectomy was performed in 2018, which showed extramedullary hematopoiesis and possible splenic MPN. However, follow up studies showed a low RT-PCR study for BCR-ABL1. The patient develop thrombocytosis after the splenectomy.

In 2022, patient developed leukocytosis and his thrombocytosis continued. A repeat bone marrow showed hypercellularity, increased myelofibrosis (MF2), and atypical megakaryocytes (similar to initial diagnostic bone marrow in 2015). A FISH study for BCR-ABL and cytogenetic studies were negative for the Philadelphia chromosome [t(9;22)], and mutational studies demonstrated a CALR+ mutation. As a result, a concurrent CAPR+ MPN was diagnosed. CML with a concurrent CALR+ is a recently described phenomenon that needs to be recognized, since this case elucidates the pit fall in making this diagnosis and differences in treatment.

Biopsy Fixation Details

The bone marrow was process by buffered formalin, decalcified (10% formic acid), processed, dehydrated, and paraffin embeded.

Frozen Tissue Available

No

Details of Microscopic Findings

Bone marrow (2015) showed hypercellularity, myelofibrosis (MF1-2), and unusual atypical megakaryocytes (often seen with primary myelofibrosis).

Bone marrow (2015 - 4 months after CML diagnosis) was inadequate, but aspirate showed FISH and cytogenetic studies were negative for Philadelphia chromosome.

Splenectomy specimen in 2018 showed splenomegaly with extramedullary hematopoiesis. Splenic MPN involvement could not be excluded, due to patient's history and myeloid precursors in the spleen. However, RT-PCR studies for BCR-ABL1 were very low to zero. Patient soon developed thrombocytosis soon after splenectomy. Patient had a follow up bone marrow in 2022, since the patient developed leukocytosis and his thrombocytosis persisted. This bone marrow showed hypercellularity, increased myelofibrosis (MF2), and atypical megakaryocytes (clustered megakaryocytes with hypolobated, bulbous and hyperchromatic nuclei). FISH and cytogenetic studies were negative for the Philadelphia chromosome [t(9;22)]. CALR+ mutation study was positive.

Immunophenotype

Non-contributory

Cytogenetics

Philadelphia chromosome positive in initial bone marrow. Negative in subsequent bone marrows.

Molecular Studies

CALR+ mutation study was positive in most recent bone marrow.

Proposed Diagnosis

Chronic myeloid leukemia with a concurrent CALR+ myeloproliferative neoplasm, unclassifiable.

Interesting Feature(s)

- CALR-positive MPN and CML may appear concurrently with composite morphology or sequentially with either malignancy as revealed as a consequence of specific treatment for one of malignanies.
- Decreasing BCR-ABL transcript levels secondary to treatment, but progression of myelofibrosis with CALR+ mutation supports the coexistence of driver mutations from two different clones growing independently.

Panel Diagnosis: CML, chronic phase with subsequent overt PMF (MF3)

Bone Marrow Workshop Part III | Myeloid neoplasms (including AML) with concomitant clonal mature T- or NK-cell proliferations/T-cell lymphomas

EA4HP26-BMWS-43	Massive Infiltration Of Bone Marrow With T-Cell Lymphoma With TFH Immunophenotype, Secondary Hematophagocytic Lymphohistiocytosis And Subsequent CMML Development
EA4HP26-BMWS-51	Chronic myelomonocytic leukemia (CMML) with T-large granular lymphocytic leukemia (T-LGL) and Monoclonal B-cell lymphocytosis (MBL)
EA4HP26-BMWS-52	Chronic myelomonocytic leukemia with concurrent clonal natural killer cell expansion
EA4HP26-BMWS-93	<i>Chronic myelomonocytic leukemia with associated persistent NK-Cell Lymphoproliferative Disorder and extra-medullary manifestation of both processes.</i>
EA4HP26-BMWS-130	Multilineage Hematological Neoplasm with a Shared <i>STAG2</i> Mutation: A case of sytemic mastocytosis with associated hematological neoplasias (SM-AHN with myelodysplastic syndrome (MDS) and T-lymphoblastic lymphoma (T-LBL)).
EA4HP26-BMWS-142	CALR-mutated myeloproliferative neoplasm in association with T-cell large granular lymphocytic leukemia
EA4HP26-BMWS-213	Chronic Myelomonocytic Leukemia Preceding Angioimmunoblastic T-Cell Lymphoma: An Underrecognized Temporal Pattern of Divergent Clonal Evolution
EA4HP26-BMWS-218	T-LGL Expansion in Patient with MDS/MPN with <i>SF3B1</i> Mutation and Thrombocytosis
EA4HP26-BMWS-265	Chronic myelomonocytic leukemia (CMML) with concomitant mature T-cell lymphoma
EA4HP26-BMWS-291	Myelodysplastic syndrome with concomitant involvement by T-cell lymphoma
EA4HP26-BMWS-293	Hidden Thorns Behind the Petals: A Case of Concurrent Nodal T Follicular Helper Cell Lymphoma and Myelodysplastic Neoplasm Presenting with Flower-like Morphology
EA4HP26-BMWS-294	T-cell lymphoma occurring in the setting of concomitant myelodysplastic syndrome
EA4HP26-BMWS-304	Adult with concurrent diagnosis of myeloid neoplasm (MDS evolving to AML), CD4(+) T-follicular helper cell lymphoma, and abnormal NK cell proliferation
EA4HP26-BMWS-306	Peripheral T-cell lymphoma, NOS, arising in a patient with VEXAS syndrome
EA4HP26-BMWS-309	Acute myeloid leukemia, T-cell lymphoproliferative disorder with T prolymphocytic leukemia/lymphoma (T-PLL)-like immunophenotype, and chronic lymphocytic leukemia in a patient with germline <i>APC</i> mutation
EA4HP26-BMWS-328	CMPN with concomitant intestinal T cell lymphoma
EA4HP26-BMWS-342	MDS with concurrent clonal T-LGL population
EA4HP26-BMWS-355	Essential thrombocythemia with T-cell clone of uncertain significance
EA4HP26-BMWS-419	De Novo Mixed Phenotype Acute Leukemia, T/Myeloid Type, Presenting as Isolated Extramedullary-Only Myeloid Sarcoma: T/Myeloid Blasts Amid Maturing T-cell Proliferation Mimicking PTCL-NOS
EA4HP26-BMWS-424	Myeloid, T-Cell, and B-Cell Neoplasms Sharing a Common <i>TET2</i> -Mutated Origin: A Case of Divergent Clonal Evolution

Massive Infiltration Of Bone Marrow With T-Cell Lymphoma With TFH Immunophenotype, Secondary Hematophagocytic Lymphohistiocytosis And Subsequent CMML Development

Dr. Gorana Gasljevic^{1,2}, PhD/MD Panna Dora¹, Prof. Veronika Kloboves Prevodnik^{3,2}, Dr. Biljana Grcar Kuzmanov¹

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Case Description

53-yrs-old male, otherwise healthy, admitted to the hospital in the end of July 2024 for 3-weeks lasting fever up to 39° C, unresponsive to antibiotics. Lab at the admission: Leu $3.6 \times 10^9/L$, Hbg 92 g/L, Trc $31 \times 10^9/L$, ferritin 18 073 ug/L (normal 41-400), triglycerides 580 mg/dL (normal <150), D-dimer 3329 ng/mL (normal <500). CT imaging showed increased lymph nodes in the thorax and abdomen and hepatosplenomegaly. Extensive infectological work up was negative. PET CT showed increased metabolic uptake with SUV up to 7 in LNs and BM. BM biopsy was performed together with aspiration, flow cytometry, PCR Biomed2 and NGS. The diagnosis of massive BM infiltration by T-cell NHL with TFH phenotype and associated secondary HLH was established. Patient received therapy with dexamethason and etoposide and then 8 cycles of CHOEP chemotherapy. At the end of the treatment, complete remission was achieved. After the few months CNS relapse was proved and 3 cycles of methotrexate were administered. Absolute and relative monocytosis were noticed in the end of January. In April BM trephine was repeated. That time Leu 3.54×10^9 , Hbg 114 g/L, Trc 134×10^9 , Mono 1.42×10^9 (40%), LDH 5.59 ukat/L (nor<4.2). BM aspiration and biopsy were repeated together with NGS. The diagnosis of CMML type 1 was established. Patient underwent stem cell transplantation but unfortunately passed away because of sepsis.

Biopsy Fixation Details

Schaffer fixative, decalcification in EDTA and HCL

Frozen Tissue Available

No

Details of Microscopic Findings

1st BM packed with atypical lymphocytes positive for (by FCM and/or IHC) CD3, CD4w, CD7w, bcl6, PD1, ICOS; negative for CD5,CD2,CD8,CD10,CD30,EBER. Dysplasia in cells of all three hematopoietic lineages. Focal hematophagocytosis. Reticuline fibrosis grade I.

2nd BM. Hypercellular BM, dysplasia in E-poesis, left shifted G-poesis with increased monocytoid forms being CD4, CD14 and PGM1 positive (18% in FCM). Megakaryopoiesis with proliferative and dysplastic features. 0.01% of aberrant T cells positive for CD3 and PD1. Reticuline fibrosis grade II.

Immunophenotype

In microscopic findings

Cytogenetics

46,XY

Molecular Studies

PCR Biomed 2 1st BM: Monoclonal T cells with rearrangement in TCRB and TCRG.

2nd BM: polyclonal T cells.

NGS from the BM aspirate twice, in 2024 and 2025. Both times

DNMT3A p(Gly543Cys): 1st VAF 31%; 2nd VAF 49.03% both class I

TET2 p(Ser1586Ter) 1st VAF 27%; 2nd VAF 47.67% both class I

TET2 p(His1912Tyr) 1st VAF 32%; 2nd VAF 49.82% both class I

Proposed Diagnosis

Massive Infiltration Of Bone Marrow With T-Cell Lymphoma With TFH Immunophenotype, Secondary Hematophagocytic Lymphohistiocytosis And Subsequent CMML Development.

Interesting Feature(s)

A clonal relationship between nodal TFH-lymphomas and myeloid neoplasms (MNs) is well-known. *TET2* and *DNMT3A* mutations are frequently shared by both neoplasms indicating a divergent clonal evolution. Most frequently MN occurs months or years after cytotoxic treatment for nTFHL, suggesting a potential role of selective treatment-related pressure and clonal expansion. However, in this case this period was extremely short. Poor patient condition due to the secondary HLH didn't allow LN biopsy to be performed so the diagnosis of T-NHL with TFH phenotype was established from the BM. Two diseases in our patient are very probably clonally related with same set of mutations being proved in 2024 with high VAFs when BM was packed with lymphoma cells and in 2025 when there was no limfoma in the BM any more. However, since no cell sorting was performed, this remains only presumption.

Panel Diagnosis: 1. Mature T-cell neoplasm, consistent with TFH lymphoma phenotype 2. Myelodysplastic CMML-1 / CMML-1, myelodysplastic type

EA4HP26-BMWS-51

Chronic myelomonocytic leukemia (CMML) with T-large granular lymphocytic leukemia (T-LGL) and Monoclonal B-cell lymphocytosis (MBL)

Dr. Sheren Younes, Dr. Oscar Silva

Stanford University, Pathology, Palo Alto, USA

Case Description

39 year-old male with anemia and persistent monocytosis.

Biopsy Fixation Details

The core biopsy was placed in Bouin's solution, covered with formalin, followed by Immunocal decalcification.

Frozen Tissue Available

na

Details of Microscopic Findings

Peripheral blood: Red cells are decreased, macrocytic with polychromasia. Leukocytes are adequate with increase in monocytes (1.44 K/uL). Hypogranulated and hyposegmented neutrophils and a prominent population of large granular lymphocytes are present (1.5 K/uL). Platelets are decreased and normal in appearance.

Bone marrow aspirate: Erythroid cells are normal with full spectrum maturation and with rare nuclear to cytoplasmic dyssynchrony. Myeloid cells are increased with full spectrum maturation and with expected nuclear segmentation and cytoplasmic granularity. Megakaryocytes are normal with occasional small hypolobated forms.

Bone marrow biopsy: Hypercellular for age (70%). Erythroid cells are slightly decreased with full spectrum maturation. Myeloid cells are increased with full spectrum maturation. The M:E ratio is 3:1. Megakaryocytes are normal in number and normal in appearance.

Immunophenotype

Peripheral blood, flow cytometry: Abnormal T-cell population detected, 40% of CD45+ events, expressing CD3 (bright), CD2 (dim), CD5 (dim), CD7 (dim), CD8, CD16 (dim), CD38 (dim), CD43, CD57 (partial) and TRBC1; negative for CD4, CD10, CD23, CD56, CD79b, and CD200. Abnormal lambda-restricted B-cell population, 3.8% of CD45+ events, expressing CD5, CD19, CD20, CD22, CD23 (dim), CD38 (moderate), CD79b, CD81, and CD200; negative for CD5, CD10, and CD34. Immunohistochemistry on core biopsy: CD3, CD8, TIA1 and granzyme highlights CD8+ T-

cells in sinusoidal linear arrays. CD4 staining highlights scattered T-cells without a sinusoidal distribution. PAX-5 highlights scattered B-cells. CD14 highlights monocytes. CD34 does not show increase in blasts. CD117 highlights mast cells and erythroid precursors.

Cytogenetics

Normal

Molecular Studies

NGS: SRSF2 P95L (VAF-24%), TET2 V1846fs (VAF-42%), TET2 W1291* (VAF-12%); and likely pathogenic mutation of STAT3 H410R (VAF-2%). FISH: Negative for del(5q)/monosomy #5, del(7q)/monosomy #7, trisomy #8, and del(20q)

Proposed Diagnosis

Chronic myelomonocytic leukemia with T-large granular lymphocytic leukemia and monoclonal B-cell lymphocytosis with CLL-like immunophenotype.

Interesting Feature(s)

Although concurrent MDS and T-LGL has been reported, the coexistence of CMML and T-LGL is poorly characterized. One rare case described CMML with an underlying T-LGL–type clonal proliferation in a patient with long-standing anemia and new pancytopenia. Whether these entities are unrelated or share a pathogenic pathway involving immune dysregulation remains unclear. A study of 7 MDS cases with associated T-LGL showed that LGL cells often harbor myeloid mutations consistent with CHIP; higher allele burdens or more penetrant hits may indicate association with more advanced disease. Common age-related mechanisms may promote outgrowth of both LGL and CHIP. Conversely, LGL may arise as an immune-surveillance response to CHIP or myeloid neoplasms, possibly leading to partial or complete clone eradication. In our case, NGS identified an SRSF2 mutation associated with CMML; STAT3 mutations are linked to T-LGL, and TET2 mutations occur in both disorders. Rare associations between CMML and B-cell lymphoproliferative disorders have also been reported, mechanisms remain unclear. Our patient is managed with observation. Two years after diagnosis, CMML, T-LGL, and MBL persist, and follow-up NGS data are unavailable to assess genetic evolution.

Panel Diagnosis: 1. Myelodysplastic CMML-1 / CMML-1, myelodysplastic type 2. T-LGL 3. MBL, atypical CLL-type

EA4HP26-BMWS-52

Chronic myelomonocytic leukemia with concurrent clonal natural killer cell expansion

Dr. Joshua Menke, Dr. Aaron J. Wilk

Stanford University School of Medicine, Department of Pathology, Stanford, Germany

Case Description

An 84 year old female presents for evaluation of a 6 year history of progressive anemia (nadir 9.4 g/dL), neutropenia (nadir 0.38 K/ μ L), thrombocytopenia (nadir 83 K/ μ L), absolute and relative monocytosis (maximum 1.7 K/ μ L [34.9%]), and lymphocytosis (maximum 3.9 K/ μ L).

Biopsy Fixation Details

Formalin fixed, paraffin embedded.

Frozen Tissue Available

None available.

Details of Microscopic Findings

Bone marrow aspirate:

Erythroid precursors are unremarkable. Myeloid precursors include a subset with hypogranularity and nuclear hyposegmentation (<10%). Blasts are not increased (<5%). Megakaryocytes include a subset with dysplastic features, including small hypolobated forms and forms with separate nuclear lobes (>10%).

Bone marrow core biopsy:

The core biopsy is variably cellular (0-40%). Erythroid and myeloid precursors are unremarkable, and there is no increase in blasts. Megakaryocytes include hypolobated forms. No atypical lymphoid aggregates or infiltrates are observed.

Immunophenotype

Flow cytometry:

Abnormal NK cell population identified (55% of lymphocytes). Immunophenotypic aberrancies include: loss of CD7, uniform expression of CD2, uniform bright expression of CD94/NKG2A, absence of all killer cell immunoglobulin-like receptors (KIR; CD158a/b/e), dimmer CD16 expression than background NK cells. Additionally, the population is positive for CD56 (dim), CD8 (dim), and CD57. It is negative for T and B cell lineage markers.

Subsequent peripheral blood flow cytometry re-demonstrated the abnormal NK cell population, constituting 64% of lymphocytes (2.5 K/ μ L). In this subsequent peripheral blood specimen, monocytes were skewed towards CD14⁺/CD16⁻ classical subsets (94%).

Immunohistochemistry: TIA1 and granzyme B stain small scattered cells, comprising ~10% of marrow cellularity. EBER *in situ* hybridization is negative.

Cytogenetics

Negative for rearrangements involving *BCR* and *ABL1*, *FIP1L1* and *PDGFRA*, *PDGFRB*, and *FGFR1*.

Molecular Studies

Mutational profiling:

IDH2 R140Q mutation identified, VAF 46%

ASXL1 G646fs mutation identified, VAF 37%

SRSF2 R94dup mutation identified, VAF 29%

T cell receptor (TCR) and B cell receptor (BCR) gene rearrangement studies:

Polyclonal

Proposed Diagnosis

Chronic myelomonocytic leukemia-1

NK-large granular lymphocytic leukemia

Interesting Feature(s)

CMML may result in significant immune dysregulation and has accordingly been occasionally associated with concurrent lymphoid expansions. This case is diagnostic of CMML and also reveals a marked concurrent expansion of clonal NK cells meeting diagnostic criteria for NK-LGLL. This population is immunophenotypically atypical for a reactive NK cell expansion (Wilk, et al. *medRxiv* 2025) and appears to be persistent and progressive. Some groups have proposed that identification of MDS-associated mutations should exclude diagnosis of NK-LGLL (Pastoret, et al. *Blood Adv* 2025). However, both CMML and NK-LGLL may be associated with underlying clonal hematopoiesis (CH), and both conditions can be driven by *TET2* mutations (Hirsch, et al. *Leukemia* 2018; Olson, et al. *Blood* 2021). This raises the possibility that NK-LGLL with a concurrent myeloid neoplasm could represent divergent evolution of a CH clone, rather than an outsized reactive clonal expansion to the inflammatory milieu of a myeloid neoplasm. We argue there is insufficient evidence to distinguish between these two possibilities, and that identification of MDS-associated mutations should not automatically exclude the diagnosis of NK-LGLL. Careful single-cell analyses combining immunophenotyping and molecular studies will be required to clarify this association.

Panel Diagnosis: 1. Myelodysplastic CMML-1 / CMML-1, myelodysplastic type 2. NK-LGLL / chronic LPD of NK cells

Chronic myelomonocytic leukemia with associated persistent NK-Cell Lymphoproliferative Disorder and extra-medullary manifestation of both processes.

Dr. Lily Benedict, Dr. Siba El Hussein

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

Case Description

70-year-old male with history of prostate adenocarcinoma presented with erythrocytosis, leukocytosis, absolute monocytosis, & thrombocytopenia.

Biopsy Fixation Details

10% neutral buffered formalin for a minimum of six hours

Frozen Tissue Available

N/A

Details of Microscopic Findings

Bone marrow (2021)

Hypercellular with panmyelosis, erythroid predominance, & mild fibrosis (MF-1).

Bone marrow (2023)

Hypercellular with maturing trilineage hematopoiesis. The morphology & fibrosis were similar to the previous biopsy.

Bone marrow (3/2025)

Hypercellular marrow, dysplastic megakaryocytes, increased blasts (11%), & increased fibrosis (MF-2).

Bone marrow (7/2025)

Hypercellular marrow with trilineage dysplasia & increased fibrosis (MF-2).

Right Perinephric Mass

Extra-medullary manifestation of patient's known neoplasm with dysplastic megakaryocytes.

Immunophenotype

Bone marrow (2021)

No evidence of an aberrant population by flow cytometry.

By IHC, CD34 highlights scattered immature cells (<5% of total cellularity).

Reticulin highlights mild fibrosis (MF-1).

Bone marrow (2023)

Aberrant expression of CD56 and CD16 in a subset of blasts and monocyte populations & an aberrant NK cell population (<1% of total marrow cellularity), expressing CD56 with loss of CD16 by flow cytometry.

Bone marrow (3/2025)

By IHC, CD34 highlights increased immature cells (~11% of total cellularity).

Bone marrow (7/2025)

Aberrant NK cell population identified by flow cytometry (~9% of total cellularity), expressing CD7, CD8 (subset) and CD56 with loss of CD57, CD16, and CD2.

Right Perinephric Mass

No evidence of an aberrant cell population by flow cytometry.

By IHC,

E-cadherin highlights erythroid precursors

Myeloperoxidase highlights myeloid cells

CD68 highlights monocytic cells

CD34 highlights scattered immature cells (<5% of cellularity)

CD3 highlights T-cells
CD20 highlights B-cells

Cytogenetics

Diploid male karyotype.

Molecular Studies

Peripheral blood (concurrent with first marrow)

Positive for JAK2 p.Asn542_Glu543del

Negative for JAK2 V617F

Bone marrow (2023)

Tier IA

JAK2 p.N542_E543del c.1624_1629del6 NM_004972.3 (VAF 38.8%)

TET2 p.N1387K c.4161C>G NM_001127208.2 (VAF 50.8%)

Tier IIC

STAT5B p.N642H c.19NM_012448.3 24A>C (VAF 6.9%)

KDM6A p.N845Lfs*23 NM_021140.2 c.2530_2531dupCT (VAF 7.7%)

Bone marrow (3/2025)

Tier IA

JAK2 p.N542_E543del c.1624_1629del6 NM_004972.3 (VAF 42.4%)

TET2 p.N1387K c.4161C>G NM_001127208.2 (50.4%)

Tier IIC

STAT5B p.N642H c.19NM_012448.3 24A>C (VAF 4.3%)

PTPN11 p.E76G c.227A>G NM_002834.3 (VAF 1%)

KDM6A p.N845Lfs*23 NM_021140.2 c.2530_2531dupCT (VAF 8.1%)

Bone marrow (7/2025)

Tier IA

JAK2 p.N542_E543del c.1624_1629del6 NM_004972.3 (42.4%)

TET2 p.N1387K c.4161C>G NM_001127208.2 (50.4%)

Tier IIC

STAT5B p.N642H c.19NM_012448.3 24A>C (VAF 4.3%)

KDM6A p.N845Lfs*23 NM_021140.2 c.2530_2531dupCT (VAF 12.2%)

Proposed Diagnosis

Chronic myelomonocytic leukemia with MF-2 & associated NK-Cell Lymphoproliferative Disorder with extra-medullary manifestation.

Interesting Feature(s)

Extramedullary manifestation of the patient's myeloproliferative neoplasm in soft tissue.

Persistent aberrant NK cell population associated with the myeloproliferative neoplasm, with marrow and extra-medullary presence, detectable by flow cytometry only, with persistent associated STAT5B mutation by NGS.

Potential role of the aberrant NK-cell population in the dynamic stability of the underlying myeloproliferative neoplasm in relationship to the background normal hematopoiesis?

Panel Diagnosis: 1. PV with monocytosis with post-PV-MF progression and EMH; 2. CUS, most likely NK-CUS

Multilineage Hematological Neoplasm with a Shared *STAG2* Mutation: A case of systemic mastocytosis with associated hematological neoplasias (SM-AHN with myelodysplastic syndrome (MDS) and T-lymphoblastic lymphoma (T-LBL)).

PhD/MD Andreas Mock¹, Dr. Gesa Schuebbe², Prof. Johannes Ruebenthaler³, Prof. Hans-Peter Horny¹, Prof. Karsten Spiekermann², **Prof. Martina Rudelius**¹

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Case Description

We report a complex case of concurrent mast cell, myeloid, and lymphoid neoplasm with overlapping molecular features in a 54-year-old female presenting with generalized lymphadenopathy and splenomegaly. Peripheral blood counts showed bicytopenia with neutropenia ($1.8 \times 10^9/L$) and normocytic anemia (hemoglobin 8 g/dL).

Lymph node biopsy established the diagnosis of T-lymphoblastic lymphoma (T-LBL), with no involvement of the bone marrow or leukemic manifestation. Bone marrow examination revealed systemic mastocytosis with associated myeloid neoplasia (SM-AHN). The mast cell component formed compact aggregates accounting for approximately 5% of the bone marrow area. The associated myeloid neoplasm fulfilled criteria for myelodysplastic syndrome with multilineage dysplasia (MDS-MLD).

Molecular analysis by whole-exome sequencing demonstrated variants of uncertain significance (VUS) in *STAG2* in the bone marrow specimen, while the T-LBL component harbored VUS in *STAG2* and *BAX*, suggesting partial molecular overlap between the disease components. No *KIT* mutation or *PDGFR*-rearrangement was detected.

The patient was treated with chemotherapy according to the GMALL protocol followed by whole-brain radiotherapy. Restaging by computed tomography after three months showed regressive lymphadenopathy.

This case illustrates the diagnostic and biological challenges posed by concurrent multilineage hematologic neoplasms and highlights shared molecular alterations suggesting a clonal hierarchy.

Biopsy Fixation Details

FFPE

Frozen Tissue Available

none

Details of Microscopic Findings

Enlarged lymph node with disrupted architecture. Expanded T-zone with a diffuse infiltrate of lymphoblasts. The lymphoblasts with irregular nuclear contours and finely dispersed chromatin.

Hypercellular bone marrow with disturbed architecture. Dysplastic granulopoiesis with increase in immature precursors. Dysplastic erythropoiesis with expanded, confluent erythronemes and increase in immature precursors.

Dysmegakaryopoiesis with hypo- / monolobulated megakaryocytes and few micromegakaryocytes.

Compact mast cell infiltrates of neoplastic mast cells with numerous spindle-shaped mast cells with aberrant expression of CD25 (5% of bone marrow area).

Immunophenotype

Lymph node (lymphoblasts): CD3+, CD2+, CD7+, CD1a+, TdT+, CD4+, CD8-, CD56-, T-bet-

Bone marrow: mast cells positive for CD117, mast cell tryptase and CD25.

no excess of blasts (CD34)

Cytogenetics

none

Molecular Studies

whole exome sequencing:

	Bone marrow (VAF %)	Lymphoblasts (VAF %)
<i>KIT</i> -Mutation	0	0
<i>PDGFR</i> -rearrangement	0	0
<i>STAG2</i> c.658A>C; p.T220P	10,8	25,8
<i>BAX</i> c.121del; p.G41R fs Ter190		6

Proposed Diagnosis

Systemic mastocytosis with associated hematological myeloid (MDS-MLD) and lymphoid (T-LBL) neoplasm

Interesting Feature(s)

This case represents a rare coexistence of systemic mastocytosis with associated myeloid and lymphoid neoplasia (MDS with multilineage dysplasia and T-LBL). The SM was *KIT*-negative and characterized by compact mast cell infiltrates involving approximately 5% of the bone marrow area.

Molecular analysis revealed a shared *STAG2* variant across disease components, supporting the presence of a common precursor with subsequent multilineage clonal evolution, while additional lineage-restricted variants (*BAX* in the T-lymphoblastic component) were consistent with clonal diversification.

The case posed significant diagnostic complexity, necessitating integrated evaluation of clinical presentation, morphology, immunophenotype, and molecular findings.

Panel Diagnosis: MLN-eo with *FGFR1* rearrangement, presenting as T-LBL in LN and chronic myeloid neoplasm in BM

EA4HP26-BMWS-142

CALR-mutated myeloproliferative neoplasm in association with T-cell large granular lymphocytic leukemia

Dr. Clarissa E. Jordan, Dr. Rebecca L. King

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Case Description

- A 67-year-old man with no relevant past medical history presented to our institution in early 2025 for second opinion
- CBC studies performed at his local institution revealed thrombocytosis since in 2020
 - Platelet counts were persistently elevated in the 700s x10⁹/L
- In December 2024, the patient endorsed night sweats and oral ulcers, and laboratory testing revealed a decreased absolute neutrophil count (0.522 x 10⁹/L). A bone marrow biopsy was subsequently performed at an outside institution.
- In January 2025, he was hospitalized for neutropenic fever and treated for human metapneumovirus. Imaging at that time revealed splenomegaly (17.4 cm).

Biopsy Fixation Details

Formalin-fixed, paraffin-embedded

- Bone marrow biopsy decalcified

Frozen Tissue Available

N/A

Details of Microscopic Findings

- Peripheral blood: Large granular lymphocytes and thrombocytosis with occasional hypogranular forms

- Bone marrow:
 - Hypercellular with increased erythropoiesis, decreased granulopoiesis, and increased, abnormal megakaryopoiesis, including large hyperlobated megakaryocytes forming clusters. Increased reticulin fibrosis (MF-2).
 - Abnormal lymphoid infiltrate with intrasinusoidal growth (10% of bone marrow cellularity) was best appreciated by immunostains

Immunophenotype

- By IHC, the abnormal CD3-positive infiltrate marks as CD8-positive T-cells, which show partial co-expression of TIA1 and granzyme B.
- Flow cytometry performed at an outside institution showed no increase in marrow blasts, and an immunophenotypically distinct T-cell population
- Peripheral blood flow cytometry performed at our institution confirmed this population as abnormal CD8-positive T-cells with co-expression of NK-cell antigens (CD2 dim, CD3+, CD5 dim, CD7 dim, CD4-, CD8+, TRBC1-, CD16 dim, CD56-, CD57+, CD94-, NKG2a-, no KIR restriction)

Cytogenetics

46,XY[20]

Molecular Studies

- STAT3, p.Y640F c.1919A>T, VAF 9.8%
- CALR, p.E386Cfs c.1154_1155ins5, VAF 27.8%
- TET2, p.H1380P, VAF 15.6%

Proposed Diagnosis

1. CALR-positive myeloproliferative neoplasm with no increase in bone marrow blasts (<5%), no circulating blasts, and MF-2 myelofibrosis
2. T-cell large granular lymphocytic leukemia.

Interesting Feature(s)

- We describe an unusual case of a 67-year-old male with long-standing thrombocytosis and splenomegaly, who developed neutropenia, fever, and oral ulcers. Bone marrow biopsy showed findings of both a myeloproliferative neoplasm (MPN) and T-cell large granular lymphocytic leukemia (T-LGL).
 - The hypercellular marrow with abnormal, large hyperlobated megakaryocytes in clusters and grade 2 reticulin fibrosis is supportive of post-essential thrombocythemia myelofibrosis vs primary myelofibrosis
 - Other bone marrow findings, including the aberrant CD8-positive T-cell population with co-expression of NK-cell antigens by flow cytometry, intrasinusoidal infiltrates in the bone marrow, and STAT3 mutation (which is not typically found in MPNs), are diagnostic for a concurrent T-LGL
- While LGL clones are increasingly reported in patients with bone marrow failure syndromes, they have also been occasionally seen co-occurring with myelodysplastic syndrome, and rarely with acute myeloid leukemias and MPNs.
 - Myeloid neoplasms with LGL clones are often associated with more severe cytopenias and autoimmune manifestations.
 - More studies are needed to elucidate if LGL clones develop in reaction to myeloid neoplasms, or if both processes share a common pathogenic origin
- Our case is interesting because of the rarity of these two diagnoses in combination, as well being an excellent example of the histopathologic, immunophenotypic, and molecular features of each diagnosis

Panel Diagnosis: 1. PMF, prefibrotic (MF1) / Prefibrotic PMF 2. T-LGL

Chronic Myelomonocytic Leukemia Preceding Angioimmunoblastic T-Cell Lymphoma: An Underrecognized Temporal Pattern of Divergent Clonal Evolution

Dr. Ozgur Can Eren, Dr. Ying Liu, Dr. Mikhail Roshal, Dr. Yanming Zhang, Dr. Ahmet Dogan, Dr. Wenbin Xiao

Memorial Sloan Kettering Cancer Center, Pathology, New York, Germany

Case Description

- A 76-year-old man initially presented with thrombocytopenia and monocytosis.
 - Bone marrow biopsy: chronic myelomonocytic leukemia with <5% blasts and RUNX1, TET2 and SRSF2 mutations.
- 2 years in follow up, he developed lymphadenopathy.
 - Excisional biopsy: angioimmunoblastic T-cell lymphoma with EBV+ polymorphic kappa-restricted lymphoplasmacytic proliferation
 - The marrow showed persistent CMML-1 without lymphoma involvement.
- Marrow and lymph node shared ancestral mutations (TET2 and SRSF2), while the lymph node acquired additional alterations (additional TET2, EP300, NF1, PCBP1, PPP2R1A), indicating clonal evolution.
 - After BV-CHP and allogeneic transplantation, he remains disease-free through 2025.

Biopsy Fixation Details

Bone marrow cores were formalin-fixed, decalcified, and paraffin-embedded. Lymph node excisional biopsy was formalin-fixed and paraffin-embedded.

Frozen Tissue Available

Not available.

Details of Microscopic Findings

- Initial marrow: Hypercellular bone marrow, erythroid hypoplasia with mild left shift, mild monocytosis, mild dysmegakaryopoiesis, and no increase in blasts
- Lymph node: effaced architecture, diffuse atypical T-cell infiltrate with irregular nuclei, vesicular chromatin, numerous small vessels, admixed histiocytes, eosinophils, and prominent plasmacytic component.
- Staging marrow: Hypercellular marrow, mild erythroid dysplasia, monocytosis, megakaryocytic dysplasia, and 5% blasts (CMML-1). No evidence of AITL.

Immunophenotype

LN: abnormal T cells expressed CD3, CD4, CD2, CD5, CD7, PD-1, ICOS, CD10 (subset), BCL6 (subset), CXCL13 (subset), TCRα. Abnormal B cells and plasma cells expressed CD20 and CD38 respectively; both kappa light chain restricted. EBER-ISH is positive.

Cytogenetics

- Bone marrow: 46,XY[20], no clonal abnormalities.
- Lymph node cytogenetics not performed.

Molecular Studies

- Pre-transplant marrow and lymph node shared somatic mutations: *TET2* p.Q742* and *SRSF2* p.P95L, consistent with a common progenitor. LN acquired additional mutations (*EP300*, *NF1*, *PCBP1*, *PPP2R1A*, *TET2* (x2)) demonstrating clonal evolution.
- Clonal rearrangements were demonstrated involving both Ig heavy chain and TCR genes in the LN.

Proposed Diagnosis

- BM (2021 and 2023): Chronic myelomonocytic leukemia-1 (CMML-1)

- LN (2023): Follicular helper T-cell lymphoma, angioimmunoblastic type (TFHL-AI) with EBV+ polymorphic kappa-restricted lymphoplasmacytic proliferation.

Interesting Feature(s)

- CMML and AITL with shared clonal hematopoiesis mutations (*TET2* and *SRSF2*), supporting a divergent clonal evolution from a common ancestral hematopoietic progenitor clone as shown by us and others.
- Myeloid neoplasm develops prior to AITL, an underrecognized temporal pattern, emphasizing the intrinsic drive by clonal hematopoiesis for the development of myeloid neoplasm (rather than therapy-related), as shown by our recent studies.
- Although *RHOA/IDH2/VAV* mutations are not detected in AITL, additional *TET2* mutations are common; *EP300*, *SRSF2*, and even *NF1* mutations have been infrequently reported as well. Some of these mutations are likely present in B-cell components based on the high VAF.

Panel Diagnosis: Myelodysplastic CMML-1 / CMML-1, myelodysplastic type with subsequent nodal follicular helper T-cell lymphoma, angioimmunoblastic type, and EBV+ monoclonal B-cell proliferation with plasmacytic differentiation

EA4HP26-BMWS-218

T-LGL Expansion in Patient with MDS/MPN with *SF3B1* Mutation and Thrombocytosis

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Case Description

The patient was an 81-year old man with a history of a myeloid neoplasm with mutated *SF3B1*. His diagnostic bone marrow was interpreted as refractory anemia with ring sideroblasts and thrombocytosis in 2015 (not reviewed at our institution) with CBC as follows: "WBC 7; Hgb 11.3...PLT 825". He was treated with hydroxyurea but developed worsening cytopenias and was transitioned to lenalidomide in 2018. He was dependent on RBC transfusions, with hemoglobin always below 10 g/dL. A bone marrow biopsy was performed 04/2023 with WBC $3.72 \times 10^6/L$, HGB 8.4 g/dL, PLT $382 \times 10^6/L$.

Lenalidomide was discontinued 12/23 and the patient was started on luspatercept without improvement in his anemia. In 10/2024 he developed aspiration pneumonia and was experiencing declining performance status. He was then re-hospitalized due to acute renal failure. Blasts appeared in peripheral blood up to 6%, confirmed myeloid by flow cytometry, which also showed atypical T cells similar to those in the bone marrow (355 cells/uL). He was discharged to palliative care and expired 3 days later.

Biopsy Fixation Details

Trephine biopsy and clot section fixed in formalin. Trephine acid decalcified.

Frozen Tissue Available

No

Details of Microscopic Findings

Peripheral blood showed anisopoikilocytosis and thrombocytosis including giant and hypogranular platelets. Aspirate smears were paucispicular with a low-normal M:E ratio (1.4) and dyserythropoiesis including approximately 50% ring sideroblasts. Granulopoiesis was orderly and blasts were not increased (2%). The trephine was hypercellular (90%) due to panhyperplasia including increased and clustered abnormal megakaryocytes. Both hypersegmented and hyposegmented, hyperchromatic megakaryocytes were present. Reticulin stain showed MF2 reticulin fibrosis.

Immunophenotype

Flow cytometric analysis of bone marrow aspirate showed 53% of lymphocytes (11% of total events) were abnormal T cells. Absolute lymphocyte count (blood) at this time was $1.08 \times 10^9/L$.

Positive: CD2, CD3, CD5 (dim), CD7, CD8 (dim), CD45, CD56, CD57

Negative: CD4, CD13, CD16, CD19, CD34 and TRBC1

IHC performed on the trephine showed T cells were positive for CD3 and subset TIA1 and granzyme B. They were negative for perforin.

Cytogenetics

46,XY[20]

Molecular Studies

NGS (bone marrow) showed no relevant fusions (RNA seq) and the following variants of strong clinical significance which had reportedly been previously reported with similar VAFs (peripheral blood 01/2021):

MPL p.S505N, NM_005373.2, c.1514G>A VAF: 36.2%

SF3B1 p.K666N, NM_012433.3, c.1998G>T VAF: 41.3%

SRSF2 p.P95R, NM_003016.4, c.284C>G VAF: 39.2%

There was also a variant of potential clinical significance not previously reported:

MPL p.V501M, NM_005373.2, c.1501G>A VAF: 37.4%

T-cell clonality (peripheral blood 04/2023)

TCRB A (V-J1+J2): Nonclonal

TCRB B (V-J2): Nonclonal

TCRB C (D-J1+J2): Nonclonal

TCRG D: Clonal (189bp)

Proposed Diagnosis

T-cell lymphoproliferative disorder with immunophenotype corresponding to large granular lymphocytes in setting of MDS/MPN with *SF3B1* mutation and thrombocytosis.

Interesting Feature(s)

This a clonal T cell process with an immunophenotype consistent with T large granular lymphocytes, however the atypical lymphocytes were likely $<0.5 \times 10^9/L$. This process may have contributed to his refractory anemia. The underlying MDS/MPN is interesting as it has a *MPL* mutation rather than the more common *JAK2*.

Some studies have reported T-large granular lymphocyte expansions in up to 30% of MDS patients, but they are much less frequently reported in other myeloid neoplasms such as acute myeloid leukemia and myeloproliferative neoplasms.

Panel Diagnosis: MDS/MPN-SF3B1-T and TCUS with LGL phenotype ($<0.5\%$)

EA4HP26-BMWS-265

Chronic myelomonocytic leukemia (CMML) with concomitant mature T-cell lymphoma

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Case Description

The patient was an 89-year-old male who was admitted for evaluation and management of generalized weakness. A CBC with differential counts done on admission revealed leukocytosis (WBC: 91 K/uL) with absolute lymphocytosis (73 K/uL), neutrophilia (11 K/uL) with left shifted neutrophilic maturation, monocytosis (3K/uL), anemia (Hgb: 10.6 g/dL), and thrombocytopenia (platelets: 88K/uL). The combined findings on an initial peripheral blood smear and flow

cytometry were compatible with leukemic involvement by a mature T-cell neoplasm. Bone marrow biopsy was performed.

Biopsy Fixation Details

Bone marrow core biopsy fixed in zinc formalin following decalcification.

Frozen Tissue Available

N/A

Details of Microscopic Findings

A peripheral smear review revealed absolute lymphocytosis (with 80% lymphocytes) with frequent medium-sized atypical lymphoid cells with central to eccentric oval to irregular nuclei, coarse chromatin, inconspicuous nucleoli, and moderate amounts of agranular cytoplasm. Bone marrow aspirate shows frequent small to medium-sized atypical lymphocytes at 29% of nucleated cells. Neutrophils show full maturation and dyspoiesis in >10% of lineage. Erythroid precursors show full maturation and significant dyspoiesis (>10% of lineage). Monocytes display condensed chromatin and atypical nuclear lobation. Megakaryocytes show occasional dyspoiesis in approximately 10% of lineage. Bone marrow flow cytometry shows an aberrant CD4- CD8- CD25+ CD30- TCRa/b+ T cell population (32% of total events). Core biopsy shows a markedly hypercellular marrow (80-90%) for age. Scattered small to medium-sized and rarely large atypical lymphoid cells are observed. Erythroid and myeloid precursors are increased in number and show full orderly maturation. Megakaryocytes are mildly increased in number and show mild cytologic atypia in approximately 10%.

Immunophenotype

CD3+ CD25(weak)+ CD30- small to medium-sized and rarely large scattered and loosely clustered T-cells are present at approximately 30% of the marrow cellularity. A significant sinusoidal distribution of these T-cells is not identified. CD34+ blasts are scattered and account for <5% of the total cellularity. Scattered CD61+ megakaryocytes are seen.

Cytogenetics

Karyotype (peripheral blood): 40-44,X,-Y,-3,add(5)(p15),-8,add(8)(q24),add(?9)(p22),-10,-13,add(?13)(p11.2),add(19)(p13.3),-21, +2-4mar,inc[cp16]/46,XY[11] .

FISH for TCL1 probe (14q32.13): within normal limits, not detected.

Molecular Studies

NGS detects pathogenic mutations in *ASXL1*, *SRSF2*, *STAT3B*, *TP53*, *TET2* (x2).

TRG gene arrangement study shows evidence of T cell clonality detected.

HTLV I II abs testing is negative.

Proposed Diagnosis

1. Leukemic peripheral T-cell lymphoma, NOS
2. Chronic myelomonocytic leukemia (CMML-1)
3. Flow cytometric evidence of a minute CD5- CD10- mature B-cell clone, compatible with MBL non-CLL

Interesting Feature(s)

We report an unusual case of a leukemic mature T-cell lymphoma and concurrent CMML-1, initially showing profound lymphocytosis, followed by absolute neutrophilia and monocytosis. The initial differential diagnosis for the leukemic mature T-cell neoplasm was broad as the clinical, morphologic, immunophenotypic, and genomic features were not completely specific for any particular T-cell neoplasm. After a diagnosis of exclusion, a diagnosis of leukemic peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS) is favored. The co-occurrence of CMML and T-cell neoplasm is rare with only a few cases reported in the literature. Similar cases of leukemic aggressive PTCL-NOS and concurrent primary chronic myeloid neoplasm have not been described in the literature.

Panel Diagnosis: 1. Myeloproliferative CMML-1 /CMML-1 myeloproliferative type. 2. PTCL, NOS versus T-PLL (TCL1-negative by ICC) 3. MBL, non-CLL type

Myelodysplastic syndrome with concomitant involvement by T-cell lymphoma

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Case Description

The patient is a 65-year-old man with a reported history of angioimmunoblastic T-cell lymphoma (AITL) and a myeloid neoplasm who presented for further evaluation and consideration for bone marrow transplantation. Complete blood counts revealed a white blood cell count of $5.1 \times 10^9/L$, hemoglobin of 10.4 g/dL, and a platelet count of $14 \times 10^9/L$. Computed tomography demonstrated progressive lymphadenopathy above and below the diaphragm, along with worsening splenomegaly. The patient underwent a bone marrow biopsy for diagnostic confirmation. Ruxolitinib therapy was considered; however, it was felt that this approach would be unlikely to provide significant clinical benefit. Unfortunately, the patient's white blood cell count continued to decline, and he subsequently died.

Biopsy Fixation Details

The bone marrow core biopsy was fixed in acetic zinc formalin, followed by HCl-based decalcification.

Frozen Tissue Available

Not available

Details of Microscopic Findings

The marrow is hypercellular (90%) with 2% blasts. Myeloid elements demonstrate dysplastic features, including hypogranular cytoplasm. Megakaryocytes show dysplastic features, including hypolobated forms with abnormal chromatin patterns. Atypical lymphocytes are present.

Immunophenotype

CD3 highlights variably sized atypical lymphocytes, which are positive for CD4. PD-1 stains scattered cells. CD34 stain shows no increase in blasts.

Cytogenetics

Bone marrow: Abnormal complex karyotype with gain of 1q.

46,XY,der(22)t(1;22)(q21;p11.1)[9]/46,XY,der(15)t(1;15)(q21;p11.1)[5]/46,XY,dup(1)(q21q42)[2]/46,XY[2].

FISH: Shows tetrasomy 5, 7, 8, and 4-6 copies of 20q12.

Molecular Studies

Bone marrow:

Myeloid NGS panel: Demonstrates variants in TET2 (VAFs 15%, 82%), STAG2 (VAFs 2%, 14%, 9%), and SRSF2 (VAF 32%). For genes assayed, see associated PowerPoint.

Proposed Diagnosis

Myelodysplastic syndrome with concomitant involvement by T-cell lymphoma

Interesting Feature(s)

- Marked dysplastic features are evident in both the peripheral blood and bone marrow, including abnormalities involving the myeloid and megakaryocytic lineages, consistent with an underlying myelodysplastic syndrome. In addition, the bone marrow demonstrates an atypical lymphoid infiltrate with irregular nuclear contours.
- Immunophenotypic evaluation supports persistence of the previously reported T-cell lymphoma, in keeping with residual/persistent disease. The coexistence of marrow dysplasia and persistent T-cell lymphoma highlights a complex disease process and likely contributes to the patient's progressive cytopenias and adverse clinical course.

Panel Diagnosis: Myeloid neoplasm, most consistent with CMML, myelodysplastic type (CMML-1) and engagement by patient's known nodal follicular helper T-cell lymphoma, angioimmunoblastic type

Hidden Thorns Behind the Petals: A Case of Concurrent Nodal T Follicular Helper Cell Lymphoma and Myelodysplastic Neoplasm Presenting with Flower-like Morphology

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Case Description

A 77-year-old male was found to have incidental, asymptomatic macrocytic anemia (Hgb 11.8 g/dL, MCV 108.1 fL) and thrombocytopenia ($130 \times 10^9/L$). Within four months, he presented to the hospital with a 15 lb weight loss, poor appetite, new rash, severe fatigue, and worsening anemia (Hgb 7.4 g/dL, MCV 112.9 fL). Physical exam and imaging found diffuse lymphadenopathy and splenomegaly. After request, HTLV-1/2 antibody testing was negative.

Biopsy Fixation Details

Bone marrow specimen: B-plus fixative and the specimen was submitted following decalcification with Immunocal. Lymph node specimen: Submitted in 10% buffered formalin.

Frozen Tissue Available

Not performed.

Details of Microscopic Findings

The peripheral blood smear showed macrocytic anemia with polychromasia and circulating atypical lymphocytes with flower-like morphology. The core biopsy was hypercellular for age (60-70%) with multiple interstitial lymphoid aggregates, increased megakaryocytes with occasional hypolobated forms and clustering, and 5-10% blasts. An enlarged right cervical lymph node showed disrupted architecture with proliferation of high endothelial venules and an infiltrate of atypical T-cells in parafollicular and perivascular areas. The same process was also found in a left thigh skin biopsy where he developed a rash.

Immunophenotype

The atypical lymphoid infiltrate in the bone marrow was positive for CD3, CD5, CD4, and CD43 (subset). It was negative for CD7, CD8, CD16, CD20, CD25, CD30, CD56, and EBER by in-situ hybridization, immunohistochemistry, and flow cytometry.

The enlarged lymph node involved by the same process also showed T-cell positivity for PD1, CXCL13, and ICOS. CD21 highlighted the follicular dendritic expansion in the high endothelial venules and in the few residual germinal centers.

Cytogenetics

The karyotype displays two unrelated abnormal clones in 5/20 cells: 46,XY,del(5)(q22q35)[3] and 45,XY,add(1)(p36.1),del(3)(q12q25),del(6)(q21),del(7)(q11.2),-8,add(10)(q26),add(13)(q34),i(18)(q10),add(21)(p11.2)[2].

FISH analysis (MDS panel) with DNA probes for chromosome 5 and 7 showed 26.5% of cells with a deletion of 5q (EGR1) and 7% of cells showed deletion of 7q31 (D7S486). Targeted FISH for *KMT2A* (MLL) rearrangement at 11q23 was negative.

Molecular Studies

Next generation sequencing revealed pathogenic variants in *TET2* (p.S354* and p.Q916*, VAF 9.5% and 7.5%, respectively) and *ASXL1* (p.E537*, VAF 18.8%). Targeted PCR testing for *IDH1/IDH2*, *NPM1*, and *FLT3* mutations were negative.

Proposed Diagnosis

Myelodysplastic neoplasm with increased blasts-1 and concurrent nodal T-follicular helper cell lymphoma, angioimmunoblastic type (nTFHL-AI), involving lymph node, skin, and bone marrow.

Interesting Feature(s)

The flower-like lymphocytes found on the peripheral blood smear are highly atypical for Tfh lymphoma and are classically associated with aggressive HTLV-1-induced Adult T-cell Leukemia/Lymphoma. Furthermore, while the median survival for MDS and nTFHL-AI ranges from 1.6 to 2 years, this patient experienced a precipitous decline, passing within two months of symptom onset and only six months after incidental laboratory value changes. Such rapid deterioration suggests a synergistic effect. The concurrent presentation is exceedingly rare, highlighting the need for comprehensive broad immunophenotypic panels together with molecular and cytogenetic testing in challenging cases.

Panel Diagnosis: 1. MDS-IB1 /MDS-EB 2. Nodal T-follicular helper cell lymphoma (WHO-5)/follicular helper T-cell lymphoma (ICC), angioimmunoblastic type

EA4HP26-BMWS-294

T-cell lymphoma occurring in the setting of concomitant myelodysplastic syndrome

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Case Description

The patient is a 78-year-old man with cardiovascular problems presented to with complaints of progressive generalized weakness and shortness of breath. Further work-up revealed an elevated uric acid and LDH in the setting of macrocytic anemia. Complete blood counts showed WBC $5.3 \times 10^9/L$, Hgb 7.9 g/dL, PLT $140 \times 10^9/L$. CT chest/abdomen/pelvis showed multi-station adenopathy and splenomegaly, raising concern for a lymphoproliferative disorder. The patient underwent a lymph node biopsy, which showed nodal T follicular helper cell lymphoma, angioimmunoblastic type. He subsequently underwent a bone marrow biopsy for staging and further evaluation. Following the final diagnosis, he received one cycle of CEOP chemotherapy (cyclophosphamide, etoposide, prednisone). At the most recent follow-up, he was admitted for failure to thrive and died shortly thereafter.

Biopsy Fixation Details

The bone marrow core biopsy was fixed in acetic zinc formalin, followed by HCl-based decalcification.

Frozen Tissue Available

Not available

Details of Microscopic Findings

The marrow is hypercellular (50%) with 1% blasts. Megakaryocytes show dysplastic features, including hypolobated forms with abnormal chromatin patterns. Core biopsy demonstrates atypical lymphoid aggregates comprising 10% of core biopsy cellularity.

Immunophenotype

Core biopsy demonstrates atypical lymphoid aggregates, which are CD2(+), CD3(-), CD5(+), CD10(-), CD20(-), CD25(-), BCL-6(-), PD-1(+). CD34 stain shows no increase in blasts. Megakaryocytes also show aberrant cytoplasmic expression with CD34.

Corresponding flow cytometric studies also identify an abnormal CD4 positive T-cell population, which is CD1a(-), CD2(+), cCD3(+), CD3(-), CD4(+), CD5(+), CD7(-), CD8(-), CD16(-), CD25(-), CD30 predominantly(-), CD56(-), TRCB1(-; restricted), TCRab(-), and TCRgd(-).

Cytogenetics

46,XY,del(5)(q22q35)[3]/45,XY,add(1)(p36.1),del(3)(q12q25),del(6)(q21),del(7)(q11.2),-8,add(10)(q26),add(13)(q34),i(18)(q10),add(21)(p11.2)[2]/46,XY[15]

FISH: Deletion of 5q (EGR1) and deletion of 7q31.

Molecular Studies

Myeloid NGS panel: Demonstrates variants in TET2 (VAFs 8%, 10%) and ASXL1 (VAF 19%). For genes assayed, see the associated PowerPoint.

Proposed Diagnosis

T-cell lymphoma occurring in the setting of concomitant myelodysplastic syndrome

Interesting Feature(s)

- The bone marrow demonstrates prominent dysplastic changes, most notably involving the megakaryocytic lineage, including nuclear hypolobation. Cytogenetic evaluation revealed deletions of 5q and 7q, abnormalities that are well-established in myelodysplastic syndromes. In addition, myeloid NGS showed variants in TET2 and ASXL1. Notably, TET2 mutations are frequently identified in angioimmunoblastic T-cell lymphoma and other nodal T-cell lymphomas of follicular helper T-cell type, reflecting a shared clonal hematopoietic origin between the myeloid and lymphoid compartments in a subset of cases.
- Collectively, these morphologic, cytogenetic, and molecular findings support the presence of a coexisting myeloid neoplasm in this patient with T-cell lymphoma. The concurrence of AITL and MDS is increasingly recognized and is thought to arise from clonal hematopoiesis involving early hematopoietic progenitors, with subsequent divergent evolution into myeloid and T-cell neoplasms. Clinically, the coexistence of these entities is associated with an aggressive disease course and an overall poor prognosis.

Panel Diagnosis: 1. MDS-LB / MDS-NOS 2. Involvement by patient's known nodal follicular helper T-cell lymphoma, angioimmunoblastic type

EA4HP26-BMWS-304

Adult with concurrent diagnosis of myeloid neoplasm (MDS evolving to AML), CD4(+) T-follicular helper cell lymphoma, and abnormal NK cell proliferation

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Case Description

The patient is a 70 year old male with a history of pulmonary non-tuberculous mycobacterial infection. He presented with pancytopenia and underwent a bone marrow biopsy.

Bone marrow biopsy revealed

- Myelodysplastic neoplasm with increased blasts-2 (MDS-IB2)
- CD4(+) T-follicular helper cell lymphoma
- NK cell proliferation
- Rare mycobacteria

PET/CT performed soon after the bone marrow biopsy revealed

- 1 Sigmoid colon diverticulitis with small, contained perforations.
2. Hypermetabolic cervical, thoracic and abdominopelvic adenopathy (max SUV 6.7)
3. Diffuse marrow uptake
4. Splenomegaly with diffusely increased uptake

One month later he had a colonic perforation. A mesenteric lymph node showed paracortical expansion with EBV reactivation, polytypic plasmacytosis, and proliferation of immunophenotypically aberrant NK-cells, without evidence of involvement by T-cell lymphoma

He also developed dyspnea with large pleural effusions. Multiple pleural fluid reviews showed involvement by abnormal lymphoid cells and flow cytometry was positive for abnormal T-cells and NK cells.

He was treated with ventoclox and azacitidine. Repeat bone marrow three months after the diagnostic marrow showed hypocellular marrow with evolution to AML as well as persistent T-cell lymphoma and atypical NK cells. The patient was transitioned to hospice and died two weeks later.

Biopsy Fixation Details

Bone marrow:

- Core biopsy is fixed in Bouin's (picric acid, formaldehyde, and acetic acid) and 4 hour decalcification with formic acid solution.

Lymph node: 10% buffered formalin

Frozen Tissue Available

No

Details of Microscopic Findings

Bone marrow: Hypercellular with multilineage dysplasia, 15% blasts, and large nodules of medium sized CD10+CD4+ abnormal T-cells. Additionally, nodular proliferation of small NK cells.

Lymph node: Overall preserved architecture, with paracortical expansion. There are scattered small secondary follicles with partially regressed germinal centers. There is no overt morphologic lymphoid atypia. Interfollicular/sinusoidal proliferation of polytypic plasma cells. Interfollicular proliferation of NK-cells, which are mature-appearing.

Immunophenotype

T-cells: CD2(dim+), CD3(-), CD4(partial+), CD5(very dim+), CD7(dim+), CD8(-), CD10(+), CD38(variably+), CD45(+).

Myeloid blasts: CD34+, CD117+, MPO+, CD38(variably dim+), CD10(subset dim+)

NK cells: CD2(bright+), CD3(-), CD4(-), CD5(-), CD7(bright+), CD8(-), CD16(partial+), CD56(bright+), CD57(-)

Cytogenetics

Diagnostic bone marrow

- Culture failure
- FISH: Monosomy 7 observed in 33% of 200 cells analyzed

Molecular Studies

Bone marrow sample

SRSF2 p.P95L, VAF 23.3%

NRAS p.G12D, VAF 17.9%

TET2 p.C1633* VAF 4.0%

JAK2 p.V617F, VAF 3.5%

TET2 p.S825*, VAF 1.8%

Proposed Diagnosis

- Myelodysplastic neoplasm with increased blasts-2 (MDS-IB2), evolving to AML
- CD4+ T-follicular helper cell lymphoma
- Abnormal NK cell proliferation
- Nontuberculous mycobacterial infection, likely reflecting immune dysfunction

Interesting Feature(s)

Patient with a myeloid neoplasm (MDS evolving to AML) concomitantly identified with a mature CD4+ T lymphoma and abnormal NK cell proliferation.

A clonal relationship between T-follicular helper cell lymphomas (TFHCL) and myeloid neoplasms (MN) has previously been described. However, these cases typically involve sequential identification of two neoplasms, not concurrent.

Concurrent NK cell proliferations are less commonly associated with TFHCL and MN.

Panel Diagnosis: 1. Myelodysplastic neoplasm with increased blasts (MDS-IB2)(WHO5)/ Acute myeloid leukemia (AML) (ICC) 2. Atypical lymphoid infiltrate, consistent with Bm involvement by T-follicular helper cell lymphoma 3. NK-CUS

EA4HP26-BMWS-306

Peripheral T-cell lymphoma, NOS, arising in a patient with VEXAS syndrome

Dr. Sarah Gibson

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Case Description

An 89-year-old male presented with a 4-year history of skin rash and arthralgias, which had been treated with prednisone and methotrexate. A previous skin biopsy was compatible with Sweet syndrome, at which point treatment was transitioned to prednisone and dapsone. Imaging studies subsequently identified FDG-avid mediastinal and iliac lymphadenopathy. Skin, lymph node, and bone marrow biopsies were performed.

Biopsy Fixation Details

Formalin

Frozen Tissue Available

No

Details of Microscopic Findings

Right upper arm skin biopsy: Neutrophilic dermatosis compatible with Sweet syndrome.

Right level IV lymph node biopsy: Diffuse proliferation of medium-sized lymphoid cells with moderately abundant pale cytoplasm.

Bone marrow: Peripheral blood shows macrocytic anemia (Hb 8.8 g/dL, MCV 115.2). Bone marrow demonstrates myeloid and erythroid precursors with cytoplasmic vacuolization, mild dyserythropoiesis, and atypical megakaryocytes with condensed eosinophilic cytoplasm. Iron stain shows 8% ring sideroblasts.

Immunophenotype

Right level IV lymph node biopsy: T cells positive for: CD45, CD3, CD2, CD5, CD4, Beta F1, CD30 (subset); negative for: CD7, CD8, CD10, BCL6, PD1, CXCL13, CD56, TIA1, Granzyme B, ALK1, TCR delta, CD34, TdT, EBER

Bone marrow: No monoclonal B-cell or aberrant T-cell population detected by flow cytometry

Cytogenetics

Bone marrow: 45,X,-Y[19]/46,XY[1]

Molecular Studies

Bone marrow:

Myeloid NGS panel: No pathogenic mutations or variants of unknown significance detected

UBA1 sequencing: p.Met41Leu (c.121A>C) detected

Proposed Diagnosis

Peripheral T-cell lymphoma, NOS, arising in a patient with VEXAS syndrome

Interesting Feature(s)

VEXAS syndrome (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) is a disease of adulthood caused by somatic mutations in *UBA1* in hematopoietic progenitor cells leading to progressive rheumatologic and hematologic manifestations. This patient has typical clinical and pathologic features of VEXAS syndrome, including a several year history of Sweet syndrome and arthralgias, macrocytic anemia, classic vacuolated myeloid and erythroid precursors and atypical megakaryopoiesis with condensed eosinophilic cytoplasm in the bone marrow, and a *UBA1* p.Met41Leu variant. Patients with VEXAS syndrome are predisposed to other hematologic malignancies, most frequently myelodysplastic neoplasms (MDS) and plasma cell neoplasms. Rare mature B-cell neoplasms have also been

reported in patients with VEXAS syndrome, but associated T-cell neoplasms have not been described. Although *UBA1* mutation analysis was not performed on the T-cell lymphoma from this patient, previous studies have shown that *UBA1* mutations are only infrequently present in mature T cells from patients with VEXAS syndrome. Nevertheless, T cell clonal expansion has been described in patients with VEXAS syndrome, mainly among CD8+ T cells compared to CD4+ T cells. The altered and dysfunctional immune responses in patients with VEXAS syndrome appear to provide a milieu for clonal T-cell expansion, which in rare patients may ultimately lead to the development of T-cell neoplasia.

Panel Diagnosis: 1. VEXAS 2. PTCL, NOS

EA4HP26-BMWS-309

Acute myeloid leukemia, T-cell lymphoproliferative disorder with T prolymphocytic leukemia/lymphoma (T-PLL)-like immunophenotype, and chronic lymphocytic leukemia in a patient with germline *APC* mutation

Dr. Pallavi Galera, Dr. Mariko Yabe, Dr. Shenon Sethi, Dr. Menglei Zhu, Dr. Yanming Zhang, Dr. Mikhail Roshal

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Case Description

69/ Female presented with fatigue, headache, and overall functional decline for a few weeks

PMH: Breast DCIS, Papillary thyroid carcinoma and Chronic lymphocytic leukemia (CLL) currently on zanabrutinib

Biopsy Fixation Details

The specimen was fixed in 10% formalin and was decalcified in EDTA solution

Frozen Tissue Available

None

Details of Microscopic Findings

BONE MARROW

Variably cellular, overall hypercellular for age (50-60%) with increased blasts and atypical lymphoid infiltrate

Immunophenotype

Flow cytometric evaluation:

1. Abnormal blast population represents 30.9% of WBC.

Abnormal: CD5 (partial), CD11b (partial), CD13 (variable, negative to bright), CD19 (partial, dim), CD22 (variable, negative to dim), CD33 (variable, negative to intermediate), CD34 (dim), CD38 (variable, dim to intermediate), CD45RA (uniform, bright), CD64 (partial), CD371 (clec12a) (variable, negative to intermediate), cytoplasmic CD79a (partial, dim), cytoplasmic MPO (partial)

Normal: CD36, CD45, CD71, CD105, HLA-DR

Negative: CD2, CD3, CD4, CD7, CD8, CD10, CD14, CD16, CD20, CD25, CD26, CD56, CD117, cytoplasmic CD3, CD42b, CD61, CD90

2. Abnormal B cell population with CLL/SLL-like immunophenotype representing 41.9% of WBC.

Abnormal: CD5 (positive), CD20 (dim), CD22 (dim), CD23 (positive), CD43 (positive), CD79b (dim), CD81 (dim), CD200 (bright), ROR1 (positive), no definite light chain expression

Normal: CD19, CD40, CD45

Negative: CD38, CD10, CD3, CD14, CD25, CD279 (PD1)

3. Abnormal T cell population with immunophenotype is suggestive of T prolymphocytic leukemia/lymphoma (T-

PLL)-like proliferation representing 19.7% of WBC.

Abnormal: CD2 (dim), CD5 (bright), CD7 (bright), TCRCB1 (restriction, monotypic negative)

Normal: CD3, CD4, CD26, CD38, CD45

Negative: CD8, CD10, CD14, CD25, CD56, CD279 (PD1), TCR gamma/delta

Cytogenetics

Chromosome analysis of PHA stimulated peripheral blood sample detected a complex karyotype including inv (14) characteristic of T-PLL

FISH ANALYSIS:

Rearrangement of TRA/D (14q11.2) detected

Rearrangement of TCL1 (14q32.13) detected

Molecular Studies

Next generation sequencing revealed somatic mutations in *TET2*, *WT1*, *CHD2*, *BCORL1*, *ATM*, *BTK*, *MYD88*, and *SAMHD1*. Due to the presence of multiple malignancies, a germline testing was performed which detected a pathogenic variant in *APC* c.3920T>A (p.Ile1307Lys)

Proposed Diagnosis

Acute myeloid leukemia post-cytotoxic therapy, T-cell lymphoproliferative disorder with T prolymphocytic leukemia/lymphoma (T-PLL)-like immunophenotype, and chronic lymphocytic leukemia in a patient with germline *APC* mutation

Interesting Feature(s)

Adenomatous polyposis coli is a protein encoded by a gene of the same name (*APC*)

Originally described in the context of sporadic colorectal cancer and familial adenomatous polyposis, *APC* was later described as a key tumor suppressor gene in the WNT pathway, playing a central role in the pathogenesis of multiple neoplasms

Both somatic and germline alteration of *APC* has been reported in various neoplastic processes

Although somatic mutations in *APC* have been described, germline variants have not been reported to predispose to hematological malignancies

LEF1, one of the key players in the pathogenesis of CLL/SLL is intertwined with WNT pathway. However, *APC* mutation is not known as a predisposing factor for development of CLL/SLL

Hence, how the germline mutation of *APC* is related to three distinct hematological malignancies in this patient is unclear.

Panel Diagnosis: 1. AML without maturation / AML, NOS, post-cytotoxic therapy/therapy related 2. CLL 3. T-PLL

EA4HP26-BMWS-328

CMPN with concomitant intestinal T cell lymphoma

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Case Description

51 y/o female patient was diagnosed in 2013 with Polycythemia Vera. In 2022, when spleen started to enlarge and leukoerythroblastosis appeared BM biopsy showed progression to post-PV MF with MF grade 3. Treatment with ruxolitinib started. The spleen size decreased, no trombopenia or leukopenia. In 2024 abdominal pain, diarrhoea and nausea appeared. In February 2025 when she underwent surgery due to small intestine tumor. CT showed no lymphadenopathy and the diagnosis of intestinal lymphoma consistent with composite intestinal T cell lymphoma, NOS with DLBCL was rendered out.

Biopsy Fixation Details

10% buffered formalin overnight

Frozen Tissue Available

Yes

Details of Microscopic Findings

1. BM – PV, 2. BM – MF

Intestinal tumor: Transmural diffuse, cohesive proliferation of monotoneous medium-sized lymphoid cells with clear to basophilic cytoplasm, round nuclei with finely clumped chromatin. Focally, there are morphologically different cells: larger, with vesicular nuclei and prominent nucleoli. Ulceration with necrosis is present. No angiotropism.

Immunophenotype

Intestinal tumor Medium sized cells: CD4 +, CD8 +, CD2 +, CD3 +, CD5+, CD7 +, CD2 +, PD-1 +, ICOS focally +, TCRB+, GATA 3 +, TIA + in cca 60-70% cells, CD30 + 1-2% cells, CD10 -, BCL-6 -, BCL-2 -, CXCL13 -, CD56 -, EBV -, ALK -, TBX21 -, CCR4 -. MIB-1 labels more than 90% cells. The part of the tumor with larger cells: CD20+, BCL2+, BCL-6 +, MUM1 90%+, c-myc 10%+, CD10-, EBV ISH -, CD3-, MIB-1 90%

Cytogenetics

NA

Molecular StudiesPCR: monoclonal T and B lymphocytes in the intestinal tumor and pleural effusion

NGS: Class II mutations of CREBBP, JAK2, TET2, TNFAIP3 and Class III of ANKRD11, BCR, EML4, EPHA5, PAK7, PIK3CA, PRDM1.

Proposed Diagnosis

Proposed Diagnosis: PV with progression to post-PV MF and concomitant intestinal T cell lymphoma consistent with composite intestinal T cell lymphoma, NOS and DLBCL, NOS, non GC type and with probable MBL, non-CLL type

Interesting Feature(s)

- Although the same *JAK2* mutation was identified in PB and intestinal lymphoma, two diseases are most probably clonally unrelated: NGS of the peripheral blood in 2024 confirmed *JAK2* V617F mutation with VAF of 12,27% while in intestinal tumor *JAK2* V617F VAF of 2,54% was found, later one most probably representing contamination from peripheral blood. Subclonal proliferation of initially *JAK2* positive clone with additional *TET2* hit in lymphoma is much less probable: only single cell sequencing could answer this dilemma. However, presence of identical mutation in both specimens could lead to the misinterpretation about clonal relationship
- Our patient had primary intestinal lymphoma consistent with composite intestinal T cell lymphoma NOS and diffuse large B cell lymphoma, non GC type. This type of composite lymphoma in the intestine is extremely rare. An aberrant expression of CD20 on TCL could be seen in certain number of MEATLs (20%) and more rarely in other subtypes of peripheral TCLs; on the basis of different morphology, immunophenotype and monoclonal T as well as B cells by PCR, we think that this case represents composite lymphoma.
- Small monoclonal B cells found in low amounts (2-3% in each) in ascites, pleural fluid and bone marrow could fit into a diagnosis of MBL, non-CLL type. PCR Biomed2 showed the same monoclonal B and T cell products in all compartments (ascites, pleural fluid, BM, intestinal lymphoma) pointing toward probable minimal infiltration by intestinal lymphoma that was missed by FCM.

Panel Diagnosis: 1. Post-PV-MF 2. Intestinal T-cell lymphoma, NOS, with focal large B-cell proliferation in the setting of MBL; processes are unrelated

MDS with concurrent clonal T-LGL population

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Case Description

We report a case of newly diagnosed myelodysplastic neoplasm (MDS) with concurrent clonal T-large granular lymphocyte (T-LGL) population. The patient is an 86-year-old male with no known haematological history presenting with increasing fatigue, anaemia and neutropenia. At presentation, his full blood count was HB 74g/dL, WBC 6.1 X10⁹/L, neutrophils – 0.8 X10⁹/L and PLT 329 X10⁹/L. In view of suspicion of underlying bone marrow pathology, the patient underwent a diagnostic bone marrow biopsy.

Biopsy Fixation Details

The bone marrow trephine biopsy was fixed in acetic acid–zinc–formalin fixative, decalcified in 10% formic acid–5% formaldehyde and embedded in paraffin wax.

Frozen Tissue Available

Not applicable

Details of Microscopic Findings

The bone marrow aspirate showed significant trilineage dyshaematopoiesis with no excess of blasts. Perl's iron stain revealed grade 3-4 storage iron, but no ring sideroblasts present.

The bone marrow core biopsy showed hypercellular marrow for age (60-70% cellularity) with trilineage haematopoiesis and dysplasia, particularly noted in the megakaryocytic lineage, with no excess of blasts. Additionally, there was an interstitial increase in small to medium lymphocytes, some in linear arrays suggestive of intrasinusoidal localisation, as well as in a single aggregate.

Immunohistochemistry revealed these lymphocytes were predominantly CD3-positive T-cells, accounting for 25-30% of total marrow cellularity, with an inverted CD4:CD8 ratio, mildly attenuated expression of CD5 with retention of other pan T-cell markers (CD2 and CD7) and expression of CD57 in a large proportion. Three haematopoietic cell lines are represented.

Immunophenotype

Concurrent flow cytometry on the aspirate revealed no increased or abnormal myeloid progenitors, but detected multiple MDS related changes (integrated MDS scoring system) as well as an expanded T-cell population (30.8% of total nucleated cells).

Cytogenetics

SNP array studies detected a normal male karyotype.

Molecular Studies

Clonality studies were performed on the bone marrow biopsy, revealing clonal TCRG and TCRB gene rearrangements.

The lymphoid gene panel revealed a pathogenic STAT3 variant (NM_139276.3:c.1842C>G) at 6% allele frequency. No mutations found on myeloid gene panel.

Proposed Diagnosis

Despite the lack of myeloid associated molecular aberrations, the degree of dysplasia warranted a final diagnosis of myelodysplastic neoplasm with low blasts and concurrent T-large granular lymphocytic leukaemia.

Interesting Feature(s)

Myelodysplastic syndrome is a heterogeneous group of disorders characterised by clonal haematopoietic disorder with ineffective haematopoiesis leading to cytopenia, particularly affecting elderly population.

T-large granular lymphocytic leukaemia is a type of a chronic T-cell lymphoproliferative disorder characterised by clonal expansion of cytotoxic T cells associated with cytopenia (particularly neutropenia), recurrent infections, splenomegaly and autoimmune phenomena.

The co-occurrence of these two entities is rare and can be challenging both diagnostically and with treatment planning.

Panel Diagnosis: T-LGL with background morphologic dysplasia, favor reactive

EA4HP26-BMWS-355

Essential thrombocythemia with T-cell clone of uncertain significance

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Case Description

72 year old female presenting with thrombocythemia (incidental finding).

Asymptomatic and in good health apart from osteoporosis. HGB 129 g/L. WBC 5 K/uL. PLT 719 K/uL.

Biopsy Fixation Details

10% formaldehyde and decalcification with EDTA.

Frozen Tissue Available

No.

Details of Microscopic Findings

Peripheral blood smear:

RBC normal. 57% neutrophils. 33% lymphocytes. 9% monocytes. No blasts.

Increase in thrombocytes with anisocytosis.

Bone marrow biopsy:

Normal cell density for age. No fibrosis. Megakaryocytes increased in size and numbers, with a tendency to form loose groups and show partly hyperlobated nuclei. A few megakaryocytes with compact nuclei. Granulopoiesis, erythropoiesis, lymphocytes and plasma cells are seen without any significant anomalies. No increase in blasts.

Immunophenotype

IHC showed some diffusely spread out CD3+ CD5- T-cells, but no lymphoma-like infiltrations.

FC analysis of BM shows 1% CD34+ blasts, 22% lymphocytes, 3.6% monocytes and 70% granulocytes.

2.4% of viable cells are B-lymphocytes with normal kappa/lambda-distribution and normal maturation.

T-lymphocytes make up 13% of the viable cells with a CD4/CD8 ratio of 1.0.

A small portion of these (about 1% of the sample) consists of an aberrant population with the immune phenotype CD3++ CD5- CD7+ CD2+ CD4- CD8+ CD16-/dim CD56+ (CD57 not performed).

The population shows a suspected clonal pattern when staining for TCR-Cb1.

Cytogenetics

JAK2 mutation demonstrated.

Molecular Studies

-

Proposed Diagnosis

The morphological picture may be consistent with essential thrombocythemia.

The small aberrant T-cell population is of unknown significance; it may be reactive to viral infection or ET or may represent a pre-malignant population.

Interesting Feature(s)

The aberrant T-cell population does not have a T-LGL phenotype, which is otherwise most commonly seen in T-CUS.

EA4HP26-BMWS-419

De Novo Mixed Phenotype Acute Leukemia, T/Myeloid Type, Presenting as Isolated Extramedullary-Only Myeloid Sarcoma: T/Myeloid Blasts Amid Maturing T-cell Proliferation Mimicking PTCL-NOS

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Case Description

78y.o Caucasian male presented in sept 2025 with diffuse multistation lymphadenopathy (3.6 cm right axillary lymph node; SUV max 5.2) and pancytopenia (WBC 1.4 K/cmm, Hb 11.6 g/dL, platelets 32 x K/cmm). An excisional biopsy of the right axillary lymph node initially revealed peripheral T-cell lymphoma (PTCL). The patient showed a partial response to two cycles of CHOP chemotherapy, followed by re-emergence of multistation lymphadenopathy with interval enlargement. Bridge therapy with duvelisib, a dual PI3K inhibitor approved for PTCL, was initiated and resulted in the significant reduction of lymphadenopathy prior to further work up that revealed biphenotypic blasts of T/myeloid type in the diagnostic specimen.

Biopsy Fixation Details

FFPE

Frozen Tissue Available

No

Details of Microscopic Findings

Right axillary LN: Partial effacement of the architecture by paracortical and interfollicular expansion of T-cells. Bone marrow and PB : Hypercellular (70%-70%) without leukemic involvement.

Immunophenotype

Right axillary LN

Blasts: Positive for CD3, CD4, CD5, CD7, TdT+, CD33, CD34+ (partial), MPO+ (subset), CD99, Lysozyme and negative for CD1a-.

Abnormal maturing CD3+ CD4+ T-cells with partial CD2 and CD7 loss.

CD123/CD303+ mature plasmacytoid dendritic cells (negative for TCL1-/CD56-).

CD20/PAX5/CD21/CD23 show lack of follicular dendritic meshwork expansion

Cytogenetics

CMA

Right axillary LN: cnLOH 10q (66 Mb; encompassing PTEN), 2q loss and 6q gain.

Bone marrow: Diploid

Molecular Studies

Biallelic TET2 [c.4109G>A/p.Gly1370Glu, c.822delC/p.Asn275fs; axillary LN VAF 32-42%, marrow 6%]

Biallelic DNMT3A variants [c.1238delG/p.Gly413fs, c.1667+1_1667+2insA; node 32-42%, marrow 30-39%]

Site-specific RAS alterations (axillary LN NRAS c.37G>C/p.Gly13Arg 9.3%; cervical LN KRAS c.38_40dupGCG 36.7%), high-VAF germline-like CEBPA (c.724G>A/p.Gly242Ser ~55%) and SOCS1 (c.59G>T/p.Arg20Leu ~50%), and low-level KMT2C (c.2294A>G/p.Glu765Gly 10%)

Proposed Diagnosis

De Novo Mixed Phenotype Acute Leukemia, T/Myeloid Type, Presenting as Isolated Extramedullary Myeloid Sarcoma

Interesting Feature(s)

This unprecedented extramedullary-only T/myeloid MPAL presented as multifocal nodal myeloid sarcoma without marrow or peripheral blood leukemia, creating profound diagnostic challenges from lineage ambiguity. Nodal paracortical infiltrates—dominated by aberrant maturing T-cells (CD4+, partial CD2/CD5/CD7 loss/CD33+) with subtle, diffuse blast aggregates poorly resolved on flow cytometry—initially suggested GATA3-like PTCL-NOS, bolstered by biallelic TET2/DNMT3A (node VAF 32-42%), NRAS G13R, and high-complexity cytogenomics (10q cnLOH/PTEN, 2q/6q alterations). Definitive MPAL hinged on biphenotypic blasts (TdT+/cyCD3 weak subset + MPO/lysozyme/CD33/CD117/CD34 subsets), distinguishing from TdT-/myeloid-negative PTCL-NOS via limited TCRβ usage (Dβ-Jβ only, immature) and myeloid commitment excluding T-ALL/LBL (absent NOTCH1/FBXW7/IL7R-JAK-STAT5B, EZH2/SUZ12, CDKN2A/B deletions, TAL1/TLX/HOXA TCR translocations). BPDCN was excluded by CD56-/TC11- in CD123/CD303+ mature plasmacytoid dendritic cell aggregates (TFH-reactive) plus rare TET2/DNMT3A co-occurrence and arm-level CNAs atypical for BPDCN's usual focal pattern. Notably, duvelisib responsiveness harnessed PI3K/mTOR hyperactivation (PTEN loss synergizing TET2/DNMT3A hypermethylation, shared across PTCL/BPDCN), while CH substrate and AML-like/ETP-overlap genomics (lacking classic MPAL focal deletions IKZF1/ETV6/RB1) highlight genotype-tailored therapeutic convergence bridging diagnostic boundaries.

Panel Diagnosis: Extramedullary mixed phenotype acute leukemia, T/myeloid

EA4HP26-BMWS-424

Myeloid, T-Cell, and B-Cell Neoplasms Sharing a Common TET2-Mutated Origin: A Case of Divergent Clonal Evolution

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Case Description

- A 73-year-old man initially presented in 2016 with widespread lymphadenopathy and was diagnosed with angioimmunoblastic T-cell lymphoma.
- Although his staging marrow was inadequate for full evaluation, mild cytopenias and borderline monocytosis were already present.
- Over the next several years, he developed progressive monocytosis, cytopenias, and ultimately chronic myelomonocytic leukemia, along with EBV-positive clonal B-cell proliferations.
- Sequencing of the T-cell lymphoma, CMML, and B-cell lymphoproliferative lesions demonstrated shared *TET2* mutations with additional unique lesion-specific mutations, illustrating divergent evolution to the 3 lineages from a common clonal precursor.

Biopsy Fixation Details

Bone marrow cores were formalin-fixed, decalcified, and paraffin-embedded. Lymph node excisional biopsy was formalin-fixed and paraffin-embedded.

Frozen Tissue Available

Not available.

Details of Microscopic Findings

LN: Sections showed nodal architecture effaced by a diffuse polymorphous lymphoid infiltrate predominately comprising of many small to intermediate sized atypical cells with round to angulated nuclei and clumped chromatin. Vascular proliferation and background sclerosis was noted.

BM: Hypercellular marrow with an M:E of 3.6. Dysplasia was noted in erythroid and megakaryocytic cells, with increased monocytosis.

Duodenum: Small intestinal mucosa with an atypical polymorphous lymphoid infiltrate composed of small to medium sized lymphoid cells with irregular nuclei, condensed chromatin and inconspicuous nucleoli with foci of increased plasmacytoid cells and plasma cells with rare Dutcher bodies. Numerous admixed granulocytes and scattered eosinophils are noted.

Immunophenotype

LN: Neoplastic cells express CD2, CD3, CD4, CD5, CD7 (weak), PD1, CXCL13 (subset) and CD30, while they were negative with CD8, CD56, CD10 and CD21. EBER-ISH is noted in scattered large cells. CD21 highlights residual FDC meshwork.

BM: CD15 and MPO stain myeloid lineage, while E-Cadherin and CD71 demonstrate erythroid lineage. CD61 marks megakaryocytes highlighting the small, hypolobated forms.

Duodenum: CD79a highlights the atypical infiltrate including the B cells, plasmacytoid cells and the plasma cells. CD20 and PAX5 highlight fewer B-cells whereas MUM1 stains numerous plasma cells. The plasma cells are kappa light chain restricted. A subset of the atypical B cell proliferation is positive for EBER-ISH. CD30 stains rare scattered cells.

Cytogenetics

Not applicable.

Molecular Studies

Targeted NGS (MSK-IMPACT)

- AITL (LN, 2016): *TET2* (x3), *VAV1*, *SETBP1*, *CUX1*, *DNMT3A*
- CMML (BM, 2019): *TET2* (x2), *SRSF2*, *CHEK2*, *FBXO11*, *KRAS*
- B-LPD (Duodenum, 2020): *TET2* (x3), *SRSF2*, *CHEK2*, *FBXO11*, *KRAS*, *CBFB*, *SETD1A*, *SETD5*, *SOCS1*, *ARID1B*, *DNMT3A*

Proposed Diagnosis

In alignment with the theme of "Myeloid neoplasm with concomitant clonal mature T-cell lymphomas" we propose:

- LN: Angioimmunoblastic T-cell lymphoma (AITL)
- BM: Chronic myelomonocytic leukemia (CMML)

Interesting Feature(s)

1. In retrospect, the monocyte count in 2016 (0.7 K/ μ L, 10.8% of WBC) likely reflected borderline monocytosis and served as an early harbinger of an emerging clonal myeloid neoplasm. However, the initial marrow biopsy was inadequate for definitive assessment precluding an adequate diagnostic evaluation.
2. Sequencing of bulk samples involved by the 3 disease processes demonstrated the same founding *TET2* mutation in myeloid, T and B-cell proliferations, proving a common clonal relationship of these neoplastic processes with different lineage phenotypes. Additional unique mutations further demonstrated branching clonal evolution with.

Panel Diagnosis: Nodal follicular helper T-cell lymphoma, angioimmunoblastic type with subsequent myelodysplastic CMML-1 /CMML-1, myelodysplastic type and EBV+ clonal B-cell proliferation with plasmacytic differentiation