### Case 101

**Workshop**  
1. Reactive cytopenia and dysplasia

**Title**  
CNL evolving from SAA

**Clinical History**

M, 41  
Clinical history of severe aplastic anemia (SAA) from 1997 till 2016  
Therapy with ATG and cyclosporin (CyA) in 1998 -> CR  
Relapses in 2006 and in 2009 -> ATG and CyA -> CR  
CyA given until 2015 -> ongoing CR  
August 2016: neutrophilia (14.8 G/L rising to 32G/L within 2 months) with 3-4% myeloid and metamyelocytes; hemoglobin and platelets - unremarkable (157g/L and 292G/L)

**Morphological findings**

Hypercellular packed marrow with increased megakaryopoiesis with smaller forms with increased nucleo-cytoplasmic ratios and hyperchromatic nuclei. Dysorganized erythropoiesis. Increased maturing myelopoiesis. Increased iron content. Myelofibrosis grade 0.

**Immunophenotype**

CD34: increased microvascular density, approx. 3% blasts with isolated ALIPs, single CD34+ megakaryocytes  
CD42b: occasional micromegakaryocytes (<10%)  
CD15: increased and dysmaturational myelopoiesis  
CD11c/CD14: increased and left-shifted monopoiesis  
CD123: discrete increase of plasmacytoid dendritic cells without collections/islets  
Tryptase: scattered mast cells  
TdT: scattered hematogones

**Cytogenetics**

46, XY  
(conventional karyotypization)

**Molecular studies**

CSF3R T618I allelic burden 29%  
SF3B1 G870S allelic burden 42%  
EZH2 E663C allelic burden 40%  
(NGS using a customized panel for MPN/MDS-specific mutations in 30 genes (IonTorrent, Thermo Fisher Scientific, Carlsbad, USA): ASXL1, CBL, CLSTN1, CLSTN2, DNMT3A, EPORE, EZH2, GSN, IDH1, IDH2, IKZF1, JAK2, KRAAS, MPL, NF1, NFE2, NRAS, PIAS1, PIAS2, PRPF40B, PTPRT, SF1, SF3B1, SH2B3, SRSF2, TET2, TP53, TPO, U2AF35 and ZRSR2)

**Proposed diagnosis**

Chronic neutrophilic leukemia (CNL)

**Interesting feature(s) of submitted case**

CNL developing in the background of SAA is a rare complication. Analogous mechanisms like those observed in patients with severe congenital neutropenia evolving into acute myeloid leukemia by acquiring CSF3R mutations can be assumed in long standing SAA. SAA puts the hematopoietic stem cell under high proliferative pressure, which may give advantage to any hyperproliferative subclones like those bearing the T618I mutation. Importantly, the latter is capable of inducing colony formation in the absence of G-CSF ligand, which suggests
constitutive ligand-independent activation of the receptor.

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<tr>
<th>Submitted by</th>
<th>Prof. Dr. Alexandar M Tzankov</th>
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<tr>
<td>Institute</td>
<td>University Hospital Basel</td>
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<td>Pathology</td>
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<td>Country</td>
<td>Switzerland</td>
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Panel Diagnosis
Case 102

**Workshop**
1 Reactive cytopenia and dysplasia

**Title**
12 year-old boy with severe anemia and dyserythropoiesis

**Clinical History**
12 year-old boy, who was in his general state of good health until 6 months ago, presented with "not feeling" well, and "feeling tired".

**Morphological findings**
Core biopsy:
- Cellularity: Variable, 40%-70%
- Blasts: Not increased
- Myeloid lineage: Decreased; exhibit full maturation
- Erythroid lineage: Markedly increased; exhibit full maturation
- Megakaryocytes: Mildly decreased. Morphologically unremarkable.

Aspirate smears:
- Erythroid precursors are markedly increased and show marked dysplasia in the form of binucleation, irregular nuclear contours, megaloblastoid change and occasional karyorrhexis. Myeloid precursor show maturation with no significant dysplastic features. Megakaryocytes are mildly reduced, and morphologically unremarkable.

**Immunophenotype**
Flow cytometry, peripheral blood:
- PNH clone detected on monocytes, granulocytes and red cells.

PNH clone on RBCs:
- TYPE III (complete deficiency) = 29.8%
- TYPE II (partial deficiency) = 5.2%
- PNH clone on Granulocytes = 66.2%
- PNH clone on Monocytes = 63.6%

**Cytogenetics**
Karyotype:
46,XY [20]

FISH:
- Negative for MLL gene (11q23) rearrangement.
- Negative for EVI1 gene (3q26) rearrangement.

**Molecular studies**
Next generation sequence for specific mutations in 30 genes, NEGATIVE

Gene tested: NPM1, FLT3, CEBPA, JAK2, MPL, KIT, DNMT3A, IDH1, IDH2, ASXL1, JAK1, RUNX1, CBL, SF3B1, JAK3, SH2B3, SUZ12, ETV6, KRAS, TET1, EZH2, TET2, TET3, HRAS, NRAS, TP53, PHF6, TYK2, PTEN, WT1.

**Proposed diagnosis**
Paroxysmal Nocturnal Hemoglobinuria

**Interesting feature(s) of submitted case**
Bone marrow shows marked erythroid predominant hematopoiesis and dyserythropoiesis, and dysplasia is not evident in megakaryocytic and granulocytic lineages. Laboratory findings were compatible with the presence of hemolysis. Molecular and cytogenetic analyses did not detect any aberrant findings. Peripheral blood flow detected PNH clones on RBCs, granulocytes, and monocytes. Prominent dyserythropoiesis could be seen in reactive conditions (hemolysis etc.) due to high turn-over of erythroid cells.

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<thead>
<tr>
<th>Submitted</th>
<th>Mariko Yabe, Attending hematopathologist</th>
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<tbody>
<tr>
<td>Institute</td>
<td>Memorial Sloan Kettering Cancer Center</td>
</tr>
<tr>
<td>Address</td>
<td>New York</td>
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<td>Country</td>
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**Panel Diagnosis**
# Case 103

**Workshop**
1. Reactive cytopenia and dysplasia

**Title**
PRCA post Ibrutinib

## Clinical History
Presented with lymphocytosis (80x10^9/L) and IgM paraproteinaemia; mantle cell lymphoma diagnosed on BM biopsy in Jan 2015 (massive involvement by then). No response to Chlorambucil; re-treated with Ibrutinib. Lymphocytosis reduced lymphocytosis (23 x 10^9/L), but developed marked anaemia (Hb 59 g/L). Jan 2016: re-biopsy to the cause of anaemia.

## Morphological findings
Persistent - though reduced - MCL infiltrates, near-complete disappearance of red blood cell precursors, as confirmed on CD71 and Glycophorin C immunostains.

## Immunophenotype
Lymphoid infiltrates - consistent with MCL CD71, GIC: hardly any nucleated erythroid cells.

## Cytogenetics
N/A

## Molecular studies
N/A

## Proposed diagnosis
Pure red cell aplasia following Ibrutinib therapy.

## Interesting feature(s) of submitted case
Rare and newly recognised complication of Ibrutinib treatment. Only once case reported so far.

## Panel Diagnosis

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<th>Title</th>
<th>Dr Zbigniew J Rudzki</th>
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<tr>
<td>Institute</td>
<td>Institute: Department of Histopathology Birmingham Heartlands Hospital Heart of England NHS Foundation Trust</td>
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<td>Birmingham</td>
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<tr>
<td>Co authors</td>
<td>Co-authors:</td>
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<td></td>
<td>Dr Humayun AHMAD</td>
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<td></td>
<td>Dr Jawaid CHANNA</td>
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<tr>
<td></td>
<td>Department of Haematology, Burton Hospital, Burton, UK</td>
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Workshop I Reactive cytopenia and dysplasia
## Case 104

**Workshop**  
I  Reactive cytopenia and dysplasia

### Title
52 year-old woman with aplastic anemia with trisomy 6

### Clinical History
52 year-old woman, who was recently diagnosed with breast ductal carcinoma, was incidentally found to have anemia and thrombocytopenia during her work up for a surgery. She has no prior history of cytotoxic chemotherapy or radiation therapy.

### Morphological findings
Core biopsy:  
- Cellularity: Variable, ranging from less than 10 to 60%  
- Blasts: Not increased  
- Myeloid lineage: Complete maturation  
- Erythroid lineage: Relatively increased. Complete maturation  
- Megakaryocytes: Markedly decreased

### Immunophenotype
Flow cytometry, peripheral blood PNH clone detected on Granulocytes, Monocytes and Red Cells PNH clone on RBCs:  
- Type III (complete deficiency)=0.10%  
- Type II (partial deficiency)=0.0076% PNH clone on Granulocytes=0.20% PNH clone on Monocytes=0.089%

Flow cytometry, bone marrow aspirate:  
- No abnormal myeloid blast, monocyte or maturing myeloid population identified.  
- No abnormal mature B-cell population detected.  
- No abnormal T cell population detected

### Cytogenetics
Karyotype:  
47,XX, +6 [20]  
FISH:  
Gain of MYB (6q23) and CEP 6 detected in 47.3% of cells.

### Molecular studies
Next generation sequence for specific mutations in 30 genes, NEGATIVE  
Gene tested: NPM1, FLT3, CEBPA, JAK2, MPL, KIT, DNMT3A, IDH1, IDH2, ASXL1, JAK1, RUNX1, CBL, SF3B1, JAK3, SH2B3, SUZ12, ETV6, KRAS, TET1, EZH2, TET2, TET3, HRAS, NRAS, TP53, PHF6, TYK2, PTEN, WT1.

### Proposed diagnosis
Evolving aplastic anemia in association with PNH clone

### Interesting feature(s) of submitted case
Bone marrow is variably cellular, ranging from less than 10% and up to 60%, and shows erythroid predominance, and marked megakaryocytic hypoplasia/aplasia. There are mild megaloblastoid changes in the erythroid lineage, however, no definite morphologic evidence of MDS is present. In the presence of
a small PNH clone, the overall findings may represent a marrow failure syndrome such as an evolving aplastic anemia in association with a PNH clone. Trisomy 6 is not a characteristic MDS related genetic abnormality and is reported in association with aplastic anemia.

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Panel Diagnosis
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<th>Case 105</th>
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<td><strong>Workshop</strong></td>
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<td>I Reactive cytopenia and dysplasia</td>
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<tr>
<td><strong>Title</strong></td>
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<td>SLE as MDS mimic</td>
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<td><strong>Clinical History</strong></td>
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<td>F16, with oculocutaneous albinism, hypothyroidism and coeliac disease presented with cytopenias (Plt 80x10^9/L, progressive anaemia - Hb down to 80 g/L, episodic neutropenia with a recent nadir of 0.1x10^9/L, recent lymphopenia 0.3x10^9/L). SLE diagnosed roughly at the time of the biopsy.</td>
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<tr>
<td><strong>Morphological findings</strong></td>
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<td>Marrow hypocellular for age, with impression of topographical chaos. Myelopoiesis relatively well preserved. Focal excess of megakaryocytes, megakaryocytic clustering, presence of manifestly dysplastic megakaryocytes. Mild dyserythropiesis. Stromal fibrosis (Bauermeister +2). No excess of blasts.</td>
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<td><strong>Immunophenotype</strong></td>
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<tr>
<td>CD34: no excess of blasts. CD20, CD3: no suspicious lymphoid infiltrates. CD61: highlights excess &amp; clustering of megakaryocytes, but does not reveal any micromegakaryocytes. P53 negative.</td>
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<td><strong>Cytogenetics</strong></td>
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<td><strong>Molecular studies</strong></td>
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<td><strong>Proposed diagnosis</strong></td>
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<td>Reactive changes in the course of SLE, mimicking MDS.</td>
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<td><strong>Interesting feature(s) of submitted case</strong></td>
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<td>SLE may be a very good morphological mimick of MDS or MDS/MPN. Challenge if the diagnosis of SLE not known or not yet established. Hints of deception: no ‘common denominator’ to megakaryocytic dysplasia, no excess of blasts.</td>
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<td><strong>Panel Diagnosis</strong></td>
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Case 106

Workshop
I  Reactive cytopenia and dysplasia

Title
Pancytopenia in the setting of marrow hypoplasia, a PNH clone, and a DNMT3A mutation

Clinical History
The patient is a 69 year-old female with a one-year history of peripheral neuropathy (upper extremities dysesthesias, tingling and neuropathic pain), refractory dermatitis and osteopenia. She developed bruising and petechiae on her arms about 6 weeks before presentation. Also, she had a few episodes of chest tightness a week before presentation that improved after a red blood cell transfusion. Recently, she was found to be pancytopenic.

Antinuclear Antibodies: positive, 1:80
Serum Ferritin: 240 ng/mL (Reference range: 12 to 150 ng/mL)
Serum Folate: 20.29 ng/mL (Reference range: 2.7 to 17.0 ng/mL)
Vitamin B12: 593 pg/mL (Reference range: 200 to 1100 pg/mL)
Haptoglobin: <30 mg/dL (low) (Reference range: 36-195 mg/dL)
LDH: 276 U/L (Reference range: 98-192 U/L)
Direct Antiglobulin (Coombs) Test: IgG and C3 negative
G6PD Screen: Normal

Morphological findings

Bone Marrow Biopsy:
H&E and PAS stained sections show cortical and trabecular bone with focal evidence of active remodeling and predominantly subcortical marrow that is hypocellular overall for age (10% cellularity; range 5-20%).
There is no overt increase in blasts.
The myeloid to erythroid ratio is 2:1.
Erythroid elements are decreased in number with orderly maturation.
Myeloid elements are decreased in number but show full maturation.
Megakaryocytes are markedly decreased and occasional dyspoiesis (hypolobation or irregular nuclear lobation, high n/c ratio, and/or small cell size).
Scattered small lymphocytes and plasma cells account for <5% of cellularity.
Lymphoid aggregates are not identified.
A reticulin stain shows grade 0 fibrosis (European Consensus System).

Bone Marrow Aspirate Clot:
H&E and PAS stained sections show that the aspirate clot is variably cellular (range 5-30%) with an overall cellularity of 20%.
Megakaryocytes are decreased in number and a few ones show dyspoietic features (small cell size with hypolobated and/or hyperchromatic nuclei).
Blasts do not appear increased in number.
Iron stain: storage iron is increased; ring sideroblasts are not identified.
Bone Marrow Aspirate Smears:

The aspirate smear contains cellular spicules with bilineage hematopoiesis without dysplasia. Megakaryocytes are not identified. Myeloid elements are present and show full maturation but no morphologic dyspoiesis. Erythroid elements are present with orderly maturation but no morphologic dyspoiesis. The myeloid to erythroid ratio is approximately 2:1.

Iron stain: storage iron is present; ring sideroblasts are not identified. A differential count of 200 nucleated cells shows the following percentages:

- 3% Blasts
- 4% Promyelocytes
- 19% Myelocytes/Metamyelocytes
- 20% Bands/Neutrophils
- 3% Promonocytes/Monocytes
- 1% Eosinophils
- 0% Basophils
- 21% Lymphocytes
- 2% Plasma cells
- 27% NRBCs

**Immunophenotype**

Flow cytometry for paroxysmal nocturnal hemoglobinuria (done on peripheral blood) identified a PNH clone on erythrocytes [0.8% with partial CD59 deficiency (Type II) and 1.7% with complete CD59 deficiency (Type III)] and granulocytes (11.1% FLAER/CD24 deficient clone).

**Cytogenetics**

Karyotype: 46,XX[20] (normal female karyotype)

**Molecular studies**

DNMT3A c.1784T>C mutation (p.L595P). A missense variant in DNMT3A at amino acid 595 converting the wild type residue, Leucine, to Proline in 569 reads out of a total 2557 sequence reads for an allele frequency of 22%.

**Proposed diagnosis**

Evolving aplastic anemia (AA) with an associated paroxysmal nocturnal hemoglobinuria (PNH) clone and clonal hematopoiesis.

**Interesting feature(s) of submitted case**

Causes of pancytopenia in this patient:

The anemia has a hemolytic component that may be explained, at least in part, by the impaired red cell survival associated with the presence of a PNH clone. Furthermore, given that the low reticulocyte production index demonstrates inadequate marrow response to anemia and the marrow is mildly hypocellular, the anemia also has a component of defective red cell production.

Given the identification of mild marrow hypocellularity with marked megakaryocytic hypoplasia, the neutropenia and thrombocytopenia are due to defective granulocytic and megakaryocytic production.

The differential diagnosis of pancytopenia in this patient mainly includes an evolving aplastic anemia or hypocellular myelodysplastic syndrome (if a medication effect or an autoimmune process are excluded).

PNH clones may be found in ~20%-35% of patients with MDS but also in several patients with AA and may evolve into classic PNH. Aplastic anemia can be followed by classic PNH and later MDS with or without subsequent acute leukemia. The PNH clone may disappear in those who develop MDS after...
AA/classic PNH, but tend to persist in patients who show a PNH clone at diagnosis of MDS. The pathophysiology of PNH clonal expansion in AA or MDS is not well understood. Small PNH clones can be detected in AA and MDS and the prognostic value of its identification remains controversial. Approximately 1/3 to ½ of cases of AA have a small PNH clone, usually much lower than in classic PNH cases, in which the PNH cells tend to account for more than more than 50% of erythrocytes and granulocytes. The strong association of PNH with AA points to a possible immune mechanism of selection.

DNMT3A mutations can be observed in MDS, AML and AA.

DNMT3A is among the most frequently mutated genes in AA at 8.4% of cases. Other frequently mutated genes include BCOR and BCORL1 (9.4%), PIGA (7.5%) and ASXL (6.2%). DNMT3A mutations are associated with worse overall survival and progression-free survival in comparison to the unmutated cases. Clones carrying DNMT3A and other mutations in AA tend to increase in size over time.

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<tr>
<th>Submitted by</th>
<th>Dr. Gabriel C Caponetti</th>
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<tbody>
<tr>
<td>Institute</td>
<td>Hospital of the University of Pennsylvania</td>
</tr>
<tr>
<td></td>
<td>Pathology and Laboratory Medicine – Hematopathology Hospital of the University of Pennsylvania Philadelphia</td>
</tr>
<tr>
<td>Country</td>
<td>United States of America</td>
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Panel Diagnosis
### Case 107

#### Workshop

1. Reactive cytopenia and dysplasia

#### Title

Acquired idiopathic aplastic anemia with a missense variant in PRPF40B gene

#### Clinical History

76 year old man presented with new onset fatigue and pancytopenia.

Labs: WBC 1.1 (neutrophils 12%, lymphocytes 84%, monocytes 2%, eosinophils 0%, basophils 0%, blasts 0%), Hgb 9.3, Plt 47, MCV 95, Retic 0; ANC 0.15.

Findings were concerning for either an acquired idiopathic aplastic anemia or a myelodysplastic syndrome.

No PNH clone was detected by flow cytometry.

Bone marrow biopsy was performed.

#### Morphological findings

**Aspirate Smear:**
- The aspirate smear is markedly hemodilute and aspicular and the marrow differential count may not be representative.
- Myeloid and erythroid elements are markedly decreased.
- The myeloid to erythroid ratio cannot be assessed.
- Megakaryocytes cannot be assessed due to hemodilution.
- Lymphocytes are increased without atypia.
- An iron stain is non-contributory due to absence of marrow particles and erythroid precursors.
- A formal differential is not performed.

**Aspirate Clot:**
- H&E and PAS stained sections of the aspirate clot are predominantly composed of blood with few hematopoietic elements.
- Iron stores are moderately increased.

**Biopsy:**
- H&E and PAS stained sections show trabecular bone with a markedly hypocellular marrow for age (5-10%).
- The myeloid to erythroid ratio cannot be assessed due to marked hypocellularity.
- Erythroid elements are markedly decreased.
- Myeloid elements are markedly decreased.
- Megakaryocytes are not identified.
- Interstitially scattered lymphocytes and plasma cells are noted.
- No lymphoid aggregates are identified.
- A reticulin stain shows grade 1 fibrosis (European Consensus System).

#### Immunophenotype

**Immunohistochemistry:**
- Immunostains performed with adequate controls on the core biopsy show no increased CD34+ blasts (1%). CD20 and CD3 stain interstitially scattered B- and T-lymphocytes respectively. A pan-cytokeratin stain (pan-CK) is negative for metastatic carcinoma. Polyclonal CD138+ plasma cells with no kappa or lambda light chain predominance comprise approximately 20% of the total marrow cellularity.

#### Cytogenetics

**Karyotype: normal 46,XY[20]**
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<th><strong>Molecular studies</strong></th>
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<td>Sequencing studies revealed a variant of unknown (VUS) significance in PRPF40B gene:</td>
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<td>PRPF40B p.P751S c.2251C&gt;T</td>
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<th><strong>Proposed diagnosis</strong></th>
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<td>Acquired idiopathic aplastic anemia</td>
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<th><strong>Interesting feature(s) of submitted case</strong></th>
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<td>Presence of PRPF40 variant. PRPF40 mutations are described in patients with myeloid malignancies at a frequency of 1% or less (rare cases of MDS and AML) but no association with an aplastic anemia to date exists.</td>
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<th><strong>Submitted by</strong></th>
<th>Dr. Agata M Bogusz</th>
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<tr>
<td><strong>Institute</strong></td>
<td>Pathology and Laboratory Medicine</td>
</tr>
<tr>
<td><strong>Address</strong></td>
<td>University of Pennsylvania Philadelphia</td>
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| **Panel Diagnosis** |  |
Case 108

Workshop
I Reactive cytopenia and dysplasia

Title
hereditary bone marrow failure syndromes

Clinical History
18 months old child was referred to children hospital from NCI pediatric hospital after excluding a current acute leukemia diagnosis that was suspected by his presentation.
At our hospital the child presented with pancytopenia & some atypical lymphocytes in peripheral blood. His condition started 8 months earlier with pallor & easy fatigability& he received packed RBC transfusion 4 times during this period. The child had a history of post-circumcision bleeding.
On Examination, the child has dismorphic features (long ears, short philtrum); bilateral lower limb hypotonia; delayed motor& mental milestones. There were no organomegaly nor lymphadenopathy. Abdominal US was normal His family history was remarkable for positive consanguinity. 2 of his 3 siblings had history of blood transfusion at the age of 1 year (both male& female). He has a relative with abdominal tumor & is now under follow up. His grandfather had a history of skin cancer. Only supportive treatment is given. No specific therapy or growth factors were given The child died 3 weeks after admission LABORATORY INVESTIGATIONS:

1. CBC
Hb. 6.4 g/dl, MCV 72.5 fl, MCHC: 26 pg, WBCs: 4.3, Platelets: 17.000/ul, reticulocytic count: 0.2%
DIFFERENTIAL COUNT: baso 0%, Eos 0%, Neutr 28%, lymphocytes 70%, monocytes: 2%
ESR: 14/30
Hemolytic tests:
Coombs’ test: direct& indirect: negative
LDH: 1048
Coagulation profile:
PT: 14 seconds PC: 80% INR: 1.1
PTT: 31 sec.
Therapeutic trial of B12& folate: no improvement in his Hb or Rx count
Blood chemistry:
T bil: 0.4 mg/dl
D bil: 0.1 mg/dl
AST: 16 (N)
ALT: 4 (N)
ALP: 185 (N)
Alb: 3.2
GGT: 59 (increased)
BUN: 20
Creatinine: 0.3
Phosphorus : 3 (decreased)
K: 2.9
Ca: 9.2
Flow cytometry on BMA:
Didn’t reveal a definite abnormal pattern. It just revealed a rare primitive cell population (?Myeloblasts)
(2-3% CD33 positive, bright CD34, CD117+, DR+, CD13+, with large SS/FS
Cytogenetics: normal karyotype
Molecular: negative for t(8;21) & inv 16
Virology screening:
Negative antibody for: HBV, HCV, HAV, HIV 1& 2
Flow cytometry on BMA:
Didn’t reveal a definite abnormal pattern. It just revealed a rare primitive cell population (?Myeloblasts)
(2-3% CD33 positive, bright CD34, CD117+, DR+, CD13+, with large SS/FS
Cytogenetics: normal karyotype
Molecular: negative for t(8;21) & inv 16 Virology screening:
Morphological findings
Peripheral blood smear: marked thrombocytopenia, leukopenia with mild neutropenia & relative lymphocytosis, dysplastic & hypersegmented neutrophils, RBC changes (aniso & poikilocytosis, fragmented cells. Bone marrow Aspirate: reduced erythroid elements & megakaryocytes prominent megaloblastoid & dysplastic features of myeloid elements.
Bone marrow biopsy:
# Mildly hypocellular marrow for age.
# Megakaryocytes are markedly reduced with some early megakaryocytic forms.
# Erythropoietic elements are markedly reduced. Scattered early forms are seen, including giant forms & megaloblastoid changes & small cytoplasmic vacuolations.
# Myeloid elements predominate & reveal differentiation with moderate shift to left, increased eosinophils, many giant forms & prominent dysplastic changes & some show cytoplasmic vacuolations. Blast cells constitute <5% of total marrow cellularity.
# Scattered small lymphocytes are seen with occasional cytoplasmic vacuolation.
# Macrophage/histiocytic elements are slightly increased & show phagocytosed cellular debris, hemosiderin granules & occasional hemophagocytosis.

Immunophenotype
Flow cytometry on BMA:
Didn’t reveal a definite abnormal pattern. It just revealed a rare primitive cell population (?Myeloblasts) (2-3% CD33 positive, bright CD34, CD117+, DR+, CD13+, with large SS/FS IHC for CD34, CD68, CD61, glycophorin A: pending

Cytogenetics
normal karyotype

Molecular studies
negative for t(8;21) & inv 16

Proposed diagnosis
HEREDITARY BONE MARROW FAILURE SYNDROME WITH DYSPLASTIC FEATURES; PROBABLY AUTOSOMAL RECESSIVE

Interesting feature(s) of submitted case
# Early presentation of the patient & siblings in first year of life # Findings are not classic for the common hereditary bone marrow failure syndromes (FA, DKC, DB, SDS) nor of perdiatric MDS # Late onset solid malignancies in 2 family members (a grandfather & a relative) # Megaloblastoid changes are prominent together with the dysplastic features

Submitted by
Professor Samia Hassan Rizk
Institute
Cairo University School of Medicine, Medical Education Center
Country
Egypt

Panel Diagnosis
# Case 109

## Workshop

| Reactive cytopenia and dysplasia |

## Title

Bone marrow failure and skin lesions in a patient with USB1 gene mutation

## Clinical History

17 years old male refugee from Afghanistan, residing in Sweden for a year, was remitted because of neutropenia. He had recurrent infections in childhood, and was hospitalized several times then. He developed skin lesions first at 2 years of age. Patient’s parents are cousins, both healthy according to the patient, currently residing in Iran. Four siblings are also healthy.

Physical examination: normal stature and body weight. Dry skin with hyper- and hypopigmented macules and patches all over his body, which according to the patient increase in number over time and sometimes become itchy. Oral mucosa with white plaques. Clubbed fingers. Skin on palms and feet thickened, fingernails hard and malformed, one toenail missing. Eyes: epicanthus, mild blepharitis. No lymphadenopathy or organomegaly.

Peripheral blood status: Hgb 129 g/L, WBC 1.7 x10(9)/L (neutrophils 0.7, eosinophils <0.1, basophils <0.1, lymphocytes 0.8, monocytes 0.2), PLT 131 x10(9)/L, MCV 86 fL, CRP <3 mg/L Functional PB tests (FASCIA): decreased amounts of T- and B-cells.

## Morphological findings

**BM sample May 2016 (MC1047-16, L1261-16, not submitted):** cellularity in biopsy 40-50%, complete maturation with eosinophilia and single hypolobated megakaryocytes (MGK). Cytologically non-significant aberrations in all poeses (hypogranulated neutrophils, occasional megaloblastic erythroblasts, unlobated MGK). Blasts 1.5% by cytology. CD34+ cells 3%. PB with relative neutropenia.

**BM sample December 2016 (MC2376-16):** biopsy with cellularity of 40%, slightly thickened but regular trabecules. Iron deposits (+), ring sideroblasts (-). Slightly left-shifted myelopoiesis. Mild aberrations in all poeses, at the threshold for significant dysplasia (neutrophils with hyposegmented or clumpy nuclei, pseudo-Pelger forms, large metamyelocytes; intercytoplasmic bridging and occasional megaloblastic change in erythroid precursors; ordinarily sized but hypolobated MGKs). No Auer rods. Blasts by cytology 1.5% (flow cytometry 1.2%). PB: mild anisocytosis and leucopenia (62% lymphocytes, 26% neutrophils), 8% monocytes, 4% eosinophils). Normal amount thrombocytes.

## Immunophenotype

**Immunohistochemistry MC2376-16: 5% CD34+, no ALIPs. Up till 5% CD117+ precursors, less than 5% mast cells. Weak p53-positivity in approx. 10% cells. Lymphocytes and plasma cells dispersed (T-cells >B-cells, plasma cells). No increase in Tdt+ cells.**

**Flow cytometry L2945-16 (December 2016):** 59% granulocytes, 11% lymphocytes (5% T-cells, 1% NK-cells, 4% B-cells, 0.2% plasma cells), 1% GPA+ erythroid precursors. CD4/CD8 ratio: 1. Normal B-cells maturation, with polyclonal mature lymphocytes and plasma cells. Myeloblasts 1.8%, monocytes 3.6%. Discrete fraction of myeloid cells with aberrant CD56-expression.

## Cytogenetics

Normal karyotype.

Fanconi anemia excluded by chromosome breakage studies (Guy & St Thomas’, London).

## Molecular studies

Homozygous mutation in USB1 gene (C16orf57, NM_024598.3): c. 623A>G; p. His208Arg.

## Proposed diagnosis

Workshop I Reactive cytopenia and dysplasia
Neutropenia due to bone marrow failure in a patient with USB1 mutation

**Interesting feature(s) of submitted case**

Poikiloderma with neutropenia (PN, Clericuzio-type) is a rare autosomal recessive genodermatosis with associated bone marrow failure (BMF), predisposing to malignancy. The syndrome is due to mutation in USB1 (C16orf57, OMIM*613276) gene, coding for a protein active in RNA processing (spliceosome). Based on clinical presentation and symptom constellation, differential diagnoses include dyskeratosis congenita (DC, various genetic defects and inheritance patterns implicated) and Rothmund-Thomson syndrome (mutation in RECQL4 gene), although these entities can have overlapping features. Biallelic loss-of-function variants of the gene have been associated with both poikiloderma with neutropenia and DC.

The patient presents signs of developing BMF, currently on the verge of MDS-diagnosis. Hematopoietic tissue is hypocellular, and morphological abnormalities in myelopoiesis and megakaryocytes, were observed in both BM samples. Neutropenia is prominent in PB, although the patient does not suffer currently from serious infections. Automated PB assessment points also to thrombocytopenia, although no such feature was observed in smears.

Variants in USB1 gene have also been identified in 3/141 BM samples from patients with MDS, MDS/MPN or AML, and germline character of the mutation was confirmed in one case. No gene variants/mutations were identified in 145 control samples (Negri et al. BJH 2015). Mutation in the gene is a predisposing factor for BM malignancy but its frequency and impact on prognosis remains to be determined in larger cohorts of patients with acquired BMF.

Negri G. et al. BJH (2015); 557-565.

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<tr>
<th>Submitted by</th>
<th>Monika A Klimkowska MD PhD</th>
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<td>Institute</td>
<td>Department of Clinical Pathology &amp; Cytology</td>
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<td>Karolinska University Hospital, Stockholm</td>
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**Message**

Clinical pictures (skin lesions) may be submitted later.
## Case 110

### Workshop

1. Reactive cytopenia and dysplasia

### Title

Dyserythropoiesis associated with hemophagocytic lymphohistiocytosis (HLH)

### Clinical History

A 39 year old male with a history of severe depression, asthma and obesity presented with 72 hour history of flu-like symptoms. He was drowsy on admission and became increasingly agitated requiring sedation and intubation for CT scan which did not show any acute intracranial abnormalities. He was started on empirical antibiotic treatment and required hemofiltration for acute kidney injury. A CSF sample showed no abnormality. A CT scan of the chest, abdomen and pelvis showed evidence of cirrhosis and portal hypertension and no other abnormalities.

He became increasingly coagulopathic and developed a marked neutrophilia. The blood film appeared leukoerythroblastic. A bone marrow aspirate showed haemophagocytosis. He was given intravenous immunoglobulin (IVIG) followed by high dose methylprednisolone. Despite maximal support, he continued to deteriorate and died of multiple organ failure 14 days after admission. Extensive infective screens including Hepatitis A, B, C, CMV, EBV and respiratory virus PCR were negative. Soluble CD25 was reported as 6552 U/ml [<2500] supporting a diagnosis of haemophagocytic syndrome.

### Morphological findings

A normocellular marrow which shows dyserythropoiesis in the form of nuclear budding and multinuclearity, as well as haemophagocytosis.

### Immunophenotype

Staining for CD68P highlights haemophagocytic macrophages. Staining for CD61 confirms normal megakaryocyte frequency and morphology.

### Cytogenetics

Not requested.

### Molecular studies

Not requested.

### Proposed diagnosis

Dyserythropoiesis associated with hemophagocytic lymphohistiocytosis (HLH).

### Interesting feature(s) of submitted case

Although rare, this association has been reported.

### Refs:


### Submitted by

Dr Penny A Wright

Institute

Cambridge University Hospitals NHS Trust

Histopathology Department
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<th>Addenbrookes Hospital, Cambridge</th>
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**Panel Diagnosis**
### Case 111

**Workshop**

1. Reactive cytopenia and dysplasia

**Title**

T-cell mediated cytopenia

**Clinical History**

The patient is a 41-year-old man who initially detected to have thrombocytopenia (80,000) in 2011. In January of 2013, he underwent bone marrow work-up for anemia and thrombocytopenia and was diagnosed with myelodysplastic syndrome (MDS). The patient was treated with 8 cycles of azacitidine, following which the transfusion requirements decreased. He subsequently presented to our hospital for a second opinion and treatment options.

BM assessment at MDACC (11/2013) showed variably cellular marrow with decreased megakaryocytes, dysplastic trilineage hematopoiesis, 3% blasts and diploid cytogenetics. Flow cytometry identified a small atypical T-cell population of uncertain significance; there was no support for MDS. Review of peripheral blood smear showed rare large granular lymphocytes. Next generation sequencing showed no mutations. A monoclonal T-Cell receptor gamma chain gene (TCRG) rearrangement was detected by PCR analysis on subsequent sample. Based on these findings and the relatively young age of the patient, a diagnosis of bone marrow failure syndrome was considered. DEB-induced chromosomal breakage and measure of telomeres length showed no clinically significant increased incidence of spontaneous or DEB-induced chromosomal breakage. No GPI-deficient (PNH-phenotype) clones were identified. RUNX1 and GATA2 mutations were negative.

The patient received three cycles of eltrombopag and azacitidine from November 2013 to May 2014, without any response, and continued to require platelet transfusions.

The patient received danazol from 04/2014 to 10/2014 followed by prednisolone without any improvement in platelets counts. Despite negative findings for BM failure syndrome, he was empirically started on cyclosporine, and achieved favorable response and has been transfusion independent for two years. The patient continues to have thrombocytopenia (38,000). Subsequent BM assessments showed hypocellular marrow with trilineage hypoplasia, and mild dyserythropoiesis, negative for MDS. There is no aberrant T-cell clone by flow cytometry. No large granular lymphocytes on peripheral blood smears or lymphocytosis on serial WBC’s were developed during last 2 years of follow up.

**Morphological findings**

**BM sample (11/2013):**

Variably cellular (~60%) marrow; left-shifted granulopoiesis with nuclear: cytoplasmic asynchrony, hypogranular cytoplasm; mature neutrophils showed dysmorphic nuclear lobes. Erythrocytes showed nuclear: cytoplasmic asynchrony, binucleation and karyorrhexis; megakaryocytes were markedly decreased with some hypolobated forms. No increased lymphocytes. Blast count was 3%. No ring sideroblast were identified.

**BM after azacitidine, eltrombopag and danazal therapy (08/2014):**

Cellular marrow (~40%) with mild dyserythropoiesis (nuclear: cytoplasmic asynchrony, cytoplasmic vacuolization and binucleation). Granulocytes and megakaryocytes showed complete orderly maturation. Blast count was 0%. No ring sideroblasts were identified. No increased or atypical lymphocytic infiltrates were noted by immunohistochemical stains. Anti-CD3 antibody demonstrated scattered small T-cells. Anti-CD4 and anti-CD8 antibodies demonstrated predominance of CD4-positive T-cells. Anti-CD20 antibody decorated scattered small B-cells. No clusters of B-cells are detected. Anti-CD34 antibody demonstrated scattered single immature cells. No clusters of CD34 positive cells were detected. Anti-CD61 antibody demonstrated scattered megakaryocytes. Mast cell tryptase stain did not demonstrate
any positive cells. Staining with antibodies specific for CMV and Parvo Virus B19 did not demonstrate any positive cells. Staining with antibody specific for TCL-1 demonstrated scattered single cells accounting for <1% of all cellular elements.

BM (02/2016):
Hypocellular (10-20%) bone marrow with trilineage hypoplasia; increased, mildly dyspoietic erythrocytes with megaloblastoid maturation and rare nuclear irregularities. Granulocytes and megakaryocytes showed complete orderly maturation. Blast count was 1%. No ring sideroblasts were identified. Anti-CD3 was positive in 10% of marrow cells in interstitial pattern; Anti-CD20 was positive in 1% of marrow cells.

Immunophenotype
BM sample (11/2013):
No immunophenotypic support for myelodysplastic syndrome (rare CD34+ myeloblasts (<0.1%) with normal immunophenotype; normal myelomonocytic maturation pattern; a distinct subset of hematogones noted) Reversed CD4:CD8 ratio (1:2) with a small population of atypical T cells (~1% of total cells): positive for CD3, CD8dim, CD57, and negative for CD5.
No monoclonal B cells were noted.
No GPI-deficient (PNH-phenotype) clones identified.

BM samples (3/2013, 8/2014, 11/2014 and 02/2016):
Subsequent BM flow immunophenotypic studies were negative for aberrant T cell population and myelodysplastic syndrome.

Cytogenetics
Cytogenetic studies showed diploid karyotype in all bone marrow specimens.
DEB-induced Breakage Study for Fanconi’s anemia showed no clinically significant increased incidence of spontaneous or DEB-induced chromosomal breakage. Measure of telomeres length was 10% for his age which is no diagnostic of dyskeratosis congenital (diagnostic <1%).

Molecular studies
BM sample (11/2013):
Next-generation sequencing based somatic mutation analysis using a 28-gene panel was negative for mutations (including RUNX1 and GATA2).
Sanger sequencing on CEBPA gene showed a 6 base pair insertion was in the C-terminal of CEBPA that results in addition of Histidine (His)-Proline (Pro) to three His-Pro repeats normally present between codons 191 and 196 (reported as a germ line polymorphism in non-tumor samples in the population).

BM sample (08/2014):
T-Cell Receptor-Gamma Chain Gene (TCRG) Rearrangement Analysis by PCR showed a monoclonal T-Cell receptor gamma chain gene (TCRG) rearrangement utilizing the V-gamma 2 family. T-Cell Receptor-Beta Chain Gene (TCRB) Rearrangement Analysis by PCR showed several prominent TCRB rearrangements (oligoclonal pattern) without a monoclonal peak.

Proposed diagnosis
T-cell mediated thrombocytopenia

Interesting feature(s) of submitted case
This case identifies an infrequent clonal cause of peripheral blood cytopenia and bone marrow dysplasia. The case also emphasizes the need for work-up of bone marrow failure syndromes.

Submitted by Juliana E Hidalgo Lopez MD
Institute MDAnderson Cancer Center
Hematopathology department Houston
Country USA
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### Case 112

**Workshop**
- Reactive cytopenia and dysplasia

**Title**
- A young boy with fever and pancytopenia.

**Clinical History**
- Four year old boy with pancytopenia, fever up to 41°C for 12 days and dry cough. Transabdominal ultrasound shows hepatosplenomegaly. Rash on both legs and arms. Antibiotic therapy with erythromycin did not improve symptoms. High levels of ferritin, s-IL-2-R (CD25), LDH and triglycerids in additional blood tests.

**Morphological findings**
- Normocellular bone marrow trephine biopsy with reduced erythropoiesis, reduced granulopoiesis with a decrease in mature granulocytes and dysmegakaryopoiesis. A few macrophages show signs of hemophagocytosis. There is a dense infiltration of T–lymphocytes and a normal distribution of CD34-positive blasts.

**Immunophenotype**
- T-lymphocytes express CD3, CD8 and Perforin. Double staining with EBER –in situ hybridization and CD8 immunohistochemistry shows that almost all CD8 positive T-cells are also EBER positive. The EBV infected cells did show neither expression of the EBV encoded proteins LMP-1, EBNA-2 and BZLF1 nor of CD30.

**Cytogenetics**
- None.

**Molecular studies**
- EBV-PCR EDTA blood 1.410.000 copies/ml.

**Proposed diagnosis**
- Secondary EBV- associated hemophagocytic lymphohistiocytosis (HLH).

**Interesting feature(s) of submitted case**
- Secondary HLH is a rare disease. However EBV-associated HLH is the most common form of secondary HLH and comprises about 25 cases per year in Japan, where the disease is most common. The morphologic changes in trephine biopsies may be subtle and since T-lymphocytes do not show morphologic changes, diagnosis may be missed without additional test like EBER–in situ hybridization.

**Submitted by**
- Dr. Philipp Lohneis

**Institute**
- Institut für Pathologie, Charité-Universitätsmedizin Berlin

**Country**
- Germany

**Panel Diagnosis**
## Case 113

### Workshop

1. Reactive cytopenia and dysplasia

### Title

Post-traumatic leukocytosis and marrow myeloid predominance complicating the diagnosis of MDS

### Clinical History

88 year old man with a history of prostate cancer (on therapy), hypertension and probable congestive heart failure was sent to the emergency room by his cardiologist for shortness of breath, fatigue and bilateral lower extremity edema. Patient had been recently started on Lasix and 1 week prior to admission had been prescribed antibiotics (Augmentin) for a foot infection at an outside institution. On admission the patient was in atrial fibrillation, but no fever, chills or other systemic symptoms. Pertinent laboratory values on admission: WBC: 19K/uL with absolute neutrophilia, hemoglobin 4.8g/dL, MCV 112.4 fL, platelets 16K/uL; differential showed 16 bands, 4 metamyelocytes, 24 myelocytes and 1 promyelocyte, but no blasts and absolute lymphopenia. The patient was transfused red cells and platelets resulting in a hemoglobin of 8.6g/dL and platelets of 35K/uL. Ultrasound, done to evaluate for hepatosplenomegaly, showed a cystic/solid mass (12x10x10.3cm) in Morton's pouch. Upon questioning, patient had recent trauma to that area.

Subsequent clinical information:

1. WBC over the next few days decreased to normal levels with a decrease in immature forms; hemoglobin stabilized in the 8g/dL range. Platelet count continued to be low.
2. Additional clinical information from the primary care physician indicated that the patient had had anemia and thrombocytopenia for ~6 months.
3. The prostate cancer was diagnosed 15 years ago and he has been only on Lupron since then.
4. Molecular panel results were obtained (see below)

### Morphological findings

Hypercellular marrow (80-90%); increased granulopoiesis with immature forms, markedly decreased erythropoiesis, decreased megakaryopoiesis. Myeloid to erythroid ratio on aspirate count: 29.7; blasts 2%. Peripheral blood smear: thrombocytopenia, macrocytic anemia, leukocytosis with left shifted granulocytes and a few hypo-segmented forms.

### Immunophenotype

Predominately granulocytes/myeloid cells based on forward and side scatter characteristics (80-90% of viable cells). Predominantly polyclonal B cells with a small CD5+ B cell population (0.1% all cells) with over-expression of kappa light chain; T cells without loss of antigen; polyclonal plasma cells; blast population (3-5% of viable cells); Distinct CD34+/CD117+ blast population (2-3%) with skewing of antigen expression to the CD34+ cells in the blast gate; some evidence of abnormal myeloid maturation with CD117 positive cells, some CD56 expression on granulocytes, some CD56 expression on monocytes. Immunostaining for CD61 and CD42b shows decreased numbers of small to few normal sized megakaryocytes; CD71 confirms the low number of erythroid precursors; parvovirus negative

### Cytogenetics

Cytogenetics bone marrow: 46,XY[20] FISH bone marrow: Normal MDS FISH panel (no monosomy 5 or 7, no trisomy 8, no deletions of 5q31, 7q31, 20q12 regions; no BCR-ABL1 rearrangement)

### Molecular studies

Molecular (RT-PCR; AMRS-PCR) bone marrow: JAK2 normal alleles; BCR-ABL1, e13a2 and e14a2 not detected; BCR-ABL1 e1a2 (p190) negative Molecular (myeloid molecular panel; allele frequency): RUNX1 (47%), TET2 (46%), ZRSR2 (94%)
**Proposed diagnosis**

Myelodysplastic syndrome, unclassifiable, obscured by infection and trauma.

**Interesting feature(s) of submitted case**

Interesting features:

- Morphologic findings, particularly the marked myeloid proliferation suggested MDS/MPN 1. Low hemoglobin and low platelets initially thought to be secondary to trauma.
- Unknown prostate therapy possibly thought to result in some of the morphologic findings.

However, there were some clues to the underlying MDS:

1. Abnormal megakaryocytes seen by IHC
2. Abnormal antigen expression by flow
3. Normalizing WBC on serial CBCs
4. Stabilized low hemoglobin at 7-8g/dL

Once complete clinical information and molecular genetic data became available, the underlying MDS “masked” by the leukocytosis and marrow myeloid predominance became manifest. The morphologic findings in the bone marrow, particularly the marked myeloid proliferation (M:E ratio of 29.7) suggest a MDS/MPN process. However, the molecular genetic findings of RUNX1, TET2 and ZRSR2, which are MDS-related mutations, and the lack of MPN-type mutations, such as JAK2, CALR, MPL, BCR-ABL1, in conjunction with the serial CBCs showing a downward trend in the WBC to within normal range indicated that this was a myelodysplastic process. As highlighted in the upcoming WHO revision (and reported in Blood 2016; 127; 2391), it is becoming clear that specific molecular alterations are associated with different myeloid neoplasms. In MDS the most commonly mutated genes are SF3B1, TET2, SRSF2, ASXL1, DNMT3A, RUNX1, U2AF1, TP53 and EZH2, two of which were identified in our patient. This case shows that cases of MDS, even in the presence of MDS driving-mutations, in specific clinical settings, such as those that are rich in cytokines and chemokines, as infection, can result in a clinical picture that mimics a MDS/MPN process.

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<th>Submitted by</th>
<th>Dr Amy. Chadburn</th>
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<td>Weill Cornell Medical College</td>
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<td>Immunopathology</td>
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**Message**

Dr. Attilio Orazi is a co-author

**Panel Diagnosis**
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<td>Reactive cytopenia and dysplasia</td>
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<tr>
<td><strong>Title</strong></td>
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<tr>
<td>Drug related cytopenia and myelofibrosis</td>
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<tr>
<td><strong>Clinical History</strong></td>
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<td>45 years-old, male, with bilateral inguinal pain, for which he overused non-steroidal anti-inflammatory drugs Complained of increasing fatigue in the last month; Peripheral blood count (at onset)/Laboratory: Haemoglobin 10.3 mg/dl, leukocyte count 2710/dL, neutrophils 800/dL, lymphocytes 1590/dL, 290/dL, platelet count 86,000/dL; LDH 971 U/L Pb smear: 3-4% myeloid blasts; no organomegaly</td>
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<td><strong>Morphological findings</strong></td>
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<td>1rst biopsy: diffuse edema/scleredema and fibrosis; decreased and patchy cellularity; erythropoiesis decreased; granulopoiesis in different stages of differentiation but maturation preserved; megakaryopoiesis expanded with dysplastic features and clustering; abundant plasmacells and lymphocytes</td>
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<td><strong>Immunophenotype</strong></td>
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<tr>
<td>CD34+/LAT+/-; CD3+/CD8+</td>
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<td><strong>Cytogenetics</strong></td>
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<td>Normal karyotype</td>
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<td><strong>Molecular studies</strong></td>
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<tr>
<td>Routine molecular/cytogenetic analyses for MDS/AML: negative JAK2/abl-bcr unmutated</td>
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<td><strong>Proposed diagnosis</strong></td>
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<tr>
<td>Reactive drug-induced self-regressing cytopenia and myelofibrosis</td>
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<td><strong>Interesting feature(s) of submitted case</strong></td>
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<td>Abnormal and threatening excess of immature cells and megakaryoblasts and subsequent restored morphology</td>
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Case 115

Workshop I  Reactive cytopenia and dysplasia

Title
Congenital Dyserythropoietic Anemia Type 1

Clinical History
32-years-old pregnant women presented with severe anemia (Hemoglobin: 4.5 gr/dl). She diagnosed with hemolytic anemia and treated with erythrocyte transfusions, methylprednisolone and vitamin B12 at another hospital. In a month –postpartum- hemoglobin level was 2.5 gr/dl. After 4 months she was referred to the hospital with severe anemia, jaundice and splenomegaly. On examination she had fever (38.6 C) and looked distressed. The conjunctivae were pale and icteric. A massively enlarged spleen was palpated. The remainder of the examination was unremarkable. She didn’t have any skeletal dysmorphic features. The patient had a history of bipolar disorder, first diagnosed in 2006. Medications included olanzapine and sertraline. She did not use alcohol and tobacco. She had two healthy children and previous pregnancies were unremarkable. Her father had lung cancer and her mother had endometrial cancer. Her uncle was diagnosed with Wilson’s disease. The family history revealed no consanguinity. The complete blood count revealed a hemoglobin: 2.93 g/dl, hematocrit: 8.2 %, MCV: 91 fl, RDW: 16 %. Leukocyte and platelet levels were normal, as was the differential count. The corrected reticulocyte was 1.47 %. Biochemical tests revealed total bilirubin: 5.4 mg/dl, conjugated bilirubin: 0.94 mg/dl, lactate dehydrogenase: 1049 U/liter (240–480 U/liter), iron: 232 lg/dl, total iron binding capacity: 240 lg/dl, transferin saturation: 96 % and ferritin: 4598 ng/ml. Direct and indirect antiglobulin tests were negative. Haptoglobin was measured 128 (normal range 41–165 mg/ml). Vitamin B12 level was 476 pg/ml and folate 8.8 ng/ml. Copper level was normal. During follow-up the fever resolved. Infectious causes such as brucellosis, malaria, leishmaniasis, human immunodeficiency virus, cytomegalovirus, parvovirus and Epstearr virus infections were excluded with specific laboratory tests. Wilson’s disease was also excluded (no Kaiser-Fleischer ring, normal serum copper and seruloplasmin levels and normal liver biopsy results). Red cell enzyme, membrane disorders and thalassemias were excluded. G6PD and pyruvate kinase screening were normal. Hemoglobin electrophoresis showed HbA at 97.5 %, HbA2 at 2.4 % and HbF at 0.1 %. Osmotic fragility, sucrose lysis, acidified serum lysis (Ham’s test) tests and FLAER-based assays were unremarkable. Peripheral blood film revealed macrocytic red blood cells with aniso-poikilocytosis and basophilic stippling. Ultrastructural erythroid features included spongy (Swiss-cheese) heterochromatin

Morphological findings
Bone marrow was hypercellular with erythroid hyperplasia (myeloid/erythroid ratio of 0.13). There was notable megaloblastic development of erythroid precursors and dyserythropoiesis. Inter-nuclear chromatin bridges involving polychromatic erythroblasts were prominent (Fig. 2). At least 500 nucleated erythroblasts were examined and binucleated erythroblasts were less than 5 %. An iron stain of the bone marrow aspirate identified the presence of abundant storage iron and ringed sideroblasts (15 %). Myeloid and megakaryocytic lineages were unremarkable with a lack of atypical bone marrow cells. Grade 1 reticulin fibrosis was seen.

Immunophenotype
Flow cytometric analysis of the bone marrow aspirate was nondiagnostic. Glycophorin A showed evident increase in Erytroid precursors, MPO and CD61 showed relative decrease in myeloid cells and megakaryocytes.

Cytogenetics
Conventional cytogenetic studies on the marrow aspirate showed a normal female karyotype

Molecular studies
Codanin-1 sequencing results did not show any mutation.

**Proposed diagnosis**

| Findings of ineffective erythropoiesis, morphologically abnormal typical bone marrow erythroid precursors and exclusion of other causes of dyserythropoiesis although genetically not confirmed suggested the diagnosis of congenital dyserythropoietic anemia type 1. |

**Interesting feature(s) of submitted case**

| Congenital dyserythropoietic anemias are a highly heterogeneous group of rare, hereditary anemias characterized by abnormalities during late erythropoiesis leading to refractory anemia, reticulocytopenia, hypercellular bone marrow with ineffective erythropoiesis, distinctive dysplastic changes in the erythroblasts and development of secondary hemochromatosis. Congenital dyserythropoietic anemia is a diagnosis of exclusion. The correct diagnosis is often delayed leading to ineffective treatments and tissue damage related to iron overload. |

| **Submitted by** | Profesor Nükhet N Tüzüner |
| **Institute** | Cerrahpasa Medical School Pathology Department |
| **Country** | Istanbul |

**Panel Diagnosis**
### Case 116

**Workshop**  
I  Reactive cytopenia and dysplasia

**Title**  
Autoimmun myelofibrosis

**Clinical History**  
A 60 years old female with 13 years history of primary biliary cirrhosis and 8 years history of Hashimoto thyroiditis, presented to hospital with fatigue. Hemoglobine levels was 3.2 g/dl, RBC: 1.020.000, hematocrit: 8.8%, WBC: 4810/ml, Platelets: 311300/ml, LDH: 783U/l, haptoglobin: 94mg/ml, ESR: 100mm/h, direct/indirect coombs: positive, kidney and liver function tests, endoscopy, colonoskopy were normal, serum iron, folic acid and vitamin B12 levels are normal. PET-CT showed that there are cervical and mediastinal few subcentimetric lymph nodes (SUVmax2.8-5.1), and slightly increased metabolic activity in bone marrow and spleen. Viral serology is normal. Autoantibody screening was positive for ANA and cryoglobulin, but RF, antidsDNA, antiSM, antiSS-A, antiSS-B were negative. C3 and C4 were lower than normal range. She had xerostomi and xeroftalmia but Schirmer test and minor salivary gland biopsy were negative for Sjögren disease. Patient had no adequate clinical or laboratory signs for diagnosis for sistemic lupus erithomatosis, Sjögren Disease or another connective tissue diseases.

**Morphological findings**  
In contrast to bone marrow aspiration hypocellularity, biopsy was hypercelluler (95%). There was notable erytroid hyperplasia. Myeloid cells and megakaryocytes were relatively fewer but both lineages are active. There were scattered 6 small lymphoid noduls, consisted of small/medium sized lymphocytes. Reticulin fibrosis was Grade III-IV(Bauermeister 1971).

**Immunophenotype**  
Lymphoid nodules have both CD3(+) Tcells and CD20(+) B cells. MPO showed paratrabecular and perivascular myeloid precursors. CD61 signed megakaryocytes. CD117 and CD34 proved there were not blastic cell increase.

**Cytogenetics**  
Not done

**Molecular studies**  
Not done

**Proposed diagnosis**  
Autoimmun myelofibrosis

**Note:** After the diagnosis, patient had received steroid treatment, and in 3 months hemoglobin levels became 12.3 g/dl

**Interesting feature(s) of submitted case**  
Cytopenia, myelofibrosis and small lymphoid nodules are the only evidents for autoimmun myelofibrosis. Differential diagnosis consists MDS, CMPN, metastatic malignancy, local or sistemic infections. The patient was already followed with long term Autoimmun diseases. Exclusion of other diagnosis, having long term autoimmun disease and the morphology leaded us to think of autoimmune myelofibrosis.
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<tr>
<th>Submitted by</th>
<th>Ahu S Demiröz MD</th>
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<tr>
<td>Institute</td>
<td>Istanbul University, Cerrahpasa Medical School Pathology Department</td>
</tr>
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<td>Country</td>
<td>Istanbul</td>
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<td>Turkey</td>
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Panel Diagnosis
Case 117

Workshop
I Reactive cytopenia and dysplasia

Title Pancytopenia due T-LGL, TCRgamma/delta positive variant.

Clinical History
2012: CLL (RAI stage II, Binet stage B) with lymphadenopathy en Coombs positivity without hemolysis. Treated with FC-R after which complete remission.
2015: Rectal ulcer
2016-Feb: Neutropenia e.c.i. splenomegaly
2016-April: Rectal ulcer, EBV and CMV IgM positive
2016-July: Progressive pancytopenia with severe thrombocytopenia and massive splenomegaly.

Morphological findings
Hypercellular marrow (90-95% cellularity) with left shifted granulopoiesis, prominent erythropoiesis without significant dysplasia, and megakaryocytes without significant dysplasia. Fibrosis grade I-II. No CLL.

Aspirate: repeatedly try tap.

Immunophenotype
Immunohistochemistry: increased amounts of T-cells with some loss of CD2 and CD5 expression. The T-cells lie interstitial and appear to lie intrasinusoidal as well. TIA is focally positive. GranzymB, CD56 and CD57 are negative.

Flow cytometry:
Peripheral blood: 10% T-cells with normal CD4/8 ratio, CD5 partially negative, CD2 positive though partially weak. On Vbeta analysis on 8, 3, 38 and 2 no signs of clonality

Cytogenetics
Normal

Molecular studies
IdentiClone T-cell rearrangement study on the bone marrow show T-cell clonality in the gamma/delta T-cells:

TCRB-A (blue): irregular
TCRB-A (green): polyclonal
TCRB-B: irregular
TCRB-C (blue): irregular
TCRB-C (green): irregular
TCRG-A (blue): irregular
TCRG-A (green): clonal (163bp)
TCRG-B (blue): clonal (172bp)
TCRG-B (green): clonal (172bp)

The same clone was found in the later removed spleen and confirmed by rearrangement studies on the T cell receptor Delta (TRD).

Proposed diagnosis
Pancytopenia due to T-cell large granular lymphocytic leukemia (T-LGL) of the TCRgamma-delta type.

Interesting feature(s) of submitted case
Although T-LGL is a known cause of (pan)cytopenia it can be difficult to recognize. In this case the infiltration of T-LGL in the bone marrow was quite subtle compared to the severe cytopenias. It took 6 months and 3 bone marrow biopsies before the final diagnosis was made. The clinicians thought primarily of an infection and hadn’t considered T-LGL. It was also not picked up by flow cytometry as only clonality of the alfa/beta T-cells was investigated. It is important to consider T-LGL in all cases with cytopenia, especially when there is splenomegaly.

Interesting features:
- Unusual case of T-LGL of gamma/delta TCR type
- Severe cytopenias and splenomegaly despite relatively small clone in bone marrow
- Typical features of T-LGL in the bone marrow: left shifted granulopoiesis, mild-moderate fibrosis, interstitial and intrasinusoidal infiltration difficult to identify by morphology. The cellularity of 90-95% is remarkable though, as most cases are described to be normocellular, hypocellular or only slightly hypercellular.
- Previous CLL (known association with T-LGL).

The later removed spleen (1637 g) showed infiltration with T-cells with loss of CD2 and CD5 and the same clone. Furthermore, it showed hyperplasia of the white pulp which is often seen in cases with LGL (in contrast to the hepatosplenic T-cell lymphoma which typically shows atrophy of the white pulp).

Patient showed a remarkable recovery after splenectomy with normalization of blood counts and recovery of body weight.