**Case 201**

**Workshop**  
II CHIP, ICUS and IDUS

**Title**  
Isolated vertebral pain and 12% JAK2 V617F allelic burden: myeloproliferation of undetermined significance?

**Clinical History**

M, 47  
Clinical history of isolated lumbar vertebrum 1 pain  
PET-CT scan: FDG-avid lesion in the lumbar vertebrum 1 (SUVmax 3.7). No osteolysis, no osteosclerosis  
CT-guided biopsy: 34707  
Absolutely normal peripheral blood values  
Slightly decreased erythropoietin (EPO) to 3.6 IU/L (4.3-29.0)

**Morphological findings**

34707 lumbar vertebrum 1: Hyperplastic grouped/clustered megakaryopoiesis, erythroid predominance, left-shifted myelopoiesis and eosinophilia  
43104 "staging iliac crest biopsy": Hypocellular bone marrow (ca. 25 %). Megakaryopoiesis slightly increased, but neither clustered or grouped, occasional staghorn forms, occasional smaller forms. Erythropoiesis and myelopoiesis unremarkable. Myelofibrosis grade 0.  
Morphological findings: 34707 lumbar vertebrum 1: Hyperplastic grouped/clustered megakaryopoiesis, erythroid predominance, left-shifted myelopoiesis and eosinophilia  
43104 "staging iliac crest biopsy": Hypocellular bone marrow (ca. 25 %). Megakaryopoiesis slightly increased, but neither clustered or grouped, occasional staghorn forms, occasional smaller forms. Erythropoiesis and myelopoiesis unremarkable. Myelofibrosis grade 0.

**Immunophenotype**

not done

**Cytogenetics**

46, XY

**Molecular studies**

JAK2 V617F with an allelic burden of 12% in the peripheral blood and 2% in the bone marrow (staging iliac crest biopsy)

**Proposed diagnosis**

Focal bone pain-causing JAK2+ myeloproliferation of undetermined significance

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

The patient had a remarkable clinical history since suffering from isolated bone pain, having FDG-PET activity at that site and showing some myeloproliferative features in a CT-guided biopsy from the respective focus. In the histopathological report we advised the clinicians to correlate the findings at least with the peripheral blood values, which turned out being normal, to exclude myeloproliferative neoplasia (MPN). Because of our advice to exclude MPN, JAK2 has been studied as well and turned out being positive. Finally, EPO was slightly decreased.

Applying the WHO criteria (Blood 2016 127:2375-2390) the patient did not suffer from any MPN (completely normal peripheral blood values, particularly hemoglobin 147g/L, hematocrit 41%, platelets 371G/L, LDH 221U/L), yet he fulfilled the second two (of three) major criteria for the diagnosis of polycythemia vera, admittedly the morphologic criterion only at one site (the site of pain and PET-positivity, but not in the ilial crest), and the minor criterion (subnormal EPO level). All above and the symptomatic nature of his presentation prompted us, together with our clinical colleagues, to rank his diagnosis as a “focal bone pain-causing, JAK2+ myeloproliferation of undetermined significance”. The patient was given aspirin 100mg/d and a watchful-waiting
strategy with 6-monthly controls has been decided. The case illustrates that a scenario, in which a hematopoietic stem cell population located to a distinct bone might be first affected by an MPN-typical genetic lesion giving rise to a focal myeloproliferation (even causing symptoms) before an apparent disease evolves, may apply to MPN. In addition, it further underscores the higher sensitivity of JAK2 determination from the peripheral blood than from the bone marrow (textbook knowledge), which may be explained by the focal or at least discontinuous/partial involvement of the bone marrow by MPN progenies.

<table>
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<tr>
<th>Submitted by</th>
<th>Prof. Dr. Alexandar Tzankov</th>
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<tbody>
<tr>
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<td>University Hospital Basel</td>
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<td>Country</td>
<td>Switzerland</td>
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Panel Diagnosis
**Case 202**

**Workshop**
II CHIP, ICU and IDUS

**Title**
Idiopathic pancytopenia of unknown etiology with a distinct small NK cell population

**Clinical History**
A 47-years old Chinese female presented chronic pancytopenia since March 2013. She did have complains of extreme fatigue and weakness but no B-symptoms. Rheumatology and infectious work-up were all negative. The concurrent CBC with bone marrow biopsy showed: Hgb 11.8 g/dL; WBC 2.3x 10^9/L (absolute neutrophils 0.94; absolute lymphocytes 1.09); Platelet 100 x 10^9/L.

**Morphological findings**
The bone marrow aspirate and biopsy showed variable cellularity; which ranged from empty (0%) to slightly hypocellularity (40%) with trilineage hematopoiesis. The erythropoiesis was qualitatively megaloblastoid; but no significant dysplasia or ringed sideroblasts were seen. The myelopoiesis was progress and complete without left-shifted maturation. Megakaryocytes were decreased. Lymphocytes are slightly increased and 18% mature lymphocytes are present.

**Immunophenotype**
A small distinct NK-cell population was identified in both peripheral blood (3%) and bone marrow biopsy (5% of total event), expressing CD16, CD2, CD7 (dim), CD56, CD57, NKG2a; negative for CD4, CD8, gamma/delta, CD3, CD5, and CD26. Flow cytometry analysis for PNH was negative.

No monotypic B cells or increased blasts were identified by flow cytometry analysis.

Immunohistochemical of CD2 and CD7 show only scattered interstitial lymphocytes; CD57 shows scattered interstitial NK cells.

**Cytogenetics**
46, XX [20]; FISH studies with MDS panel (see below probes) are negative
inv(3) RPN1/MECOM fusion; -5q(1)(D5S630x2,EGR1x1) ; -5(D5S630,EGR1)x1; -7q(1)(D7Z1x2,D7S486x1); -7(D7Z1,D7S486)x1; +8(D8Z2,MYC)x3 -13q14.3(D13S319x1,LAMP1x2); -13q14.3x2(D13S319x0,LAMP1x2) -20q12(D20S108x1,20qterx2); 11q23(MLL sep);
17p13.1(TP53x1,D17Z1x2)
-17(TP53,D17Z1)x1

**Molecular studies**
1. Molecular study by next gene sequencing (panel gene list below) did not identify pathogenic genetic alteration; however, a variant genetic alteration of unknown significance is present, SETBP1: c.1879C>T; p.Arg627Cys (50%).

OncoHeme Panel Gene list: ASXL1 (NM_015338.5) exons 11-14, BCOR (NM_001123385.1) exons 5-16, BRAF (NM_004333.4) exon 15, CALR (NM_004343.3) exon 9, CBL (NM_005188.3) exon 8, intron 8, and exon 9, CEBPA (NM_004364.4) exon 1, CSF3R (NM_000760.3) exons 14 and 17, DNMT3A (NM_022552.4) exons 8-23, ETV6 (NM_001987.4) exons 3-8, EZH2 (NM_004456.4) exons 3-21, FLT3 (NM_004119.2) exons 14-20, GATA1 (NM_002049.3) exons 2 and 4, GATA2 (NM_001145661.1) exons 4-8, IDH1 (NM_005896.3) exon 4, IDH2 (NM_002168.3) exon 4, JAK2 (NM_004972.3) exons 12-16, KIT (NM_000222.2) exons 8-11 and 17, KRAS (NM_003360.3) exons 2-3, MPL (NM_005373.2) exons 10-11, MYD88 (NM_002468.4) exon 5, NOTCH1 (NM_017617.3) exons 26, 27, and 34, NPM1 (NM_002520.6) exons 9, 11, and 12, NRAS (NM_002524.4) exons 2 and 3, PHF6 (NM_001015877.1) exons 2-10, PTPN11 (NM_002834.3) exons 3-4 and 12-13, RUNX1 (NM_001001890.2) exons 4-10,
SETBP1 (NM_015559.2) partial exon 6; amino acids 400-950, SF3B1 (NM_012433.2) exons 13-16, SRSF2 (NM_003016.4) exons 1 and 2, TERT (NM_198253.2) exons 2-16, TET2 (NM_001127208.2) exons 3-11, TP53 (NM_000546.4) exons 4-9, U2AF1 (NM_001025203.1) exons 2, 7, and 9, WT1 (NM_024426.449Aas.3) exons 1-11, and ZRSR2 (NM_005089.3) exons 1-11.

2. Molecular study for T cell receptor gene rearrangement on previous outside bone marrow biopsy a month ago was reported to be positive for clonal at outside institute. We repeated the TCR gene rearrangement on peripheral blood and the result is negative; while on bone marrow specimen it is equivocal.

**Proposed diagnosis**
Idiopathic cytopenia of unknown etiology; cannot exclude aplastic anemia. A distinct small NK cell population is identified, favor reactive change.

**Interesting feature(s) of submitted case**
- The case is very puzzling in the clinical setting since no obvious etiology can be identified via various clinical investigation and particularly bone marrow pathology examination.
- The distinct small NK cell population and a transient clonal T cell gene rearrangement in peripheral blood do raise the suspicion of LGL related cytopenia; however, the small volume of the NK cell population (3% in peripheral blood and 5% in bone marrow) cannot rationalize the clinical presentation of chronic pancytopenia.

<table>
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<tr>
<th>Submitted by</th>
<th>Dr. Liuyan (Jennifer) Jiang</th>
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<tbody>
<tr>
<td>Institute</td>
<td>Mayo Clinic Florida</td>
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<td></td>
<td>Department of Pathology and Laboratory Medicine</td>
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<td>Jacksonville</td>
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<td>Country</td>
<td>The United States of America</td>
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**Panel Diagnosis**
**Case 203**

**Workshop**  
II CHIP, ICUS and IDUS

**Title**  
78 yo male with CCUS

**Clinical History**  
78 y.o male who was recently found to have pancytopenia at the time of pacemaker placement. He is asymptomatic. His past medical history is notable for hypertension, hyperlipidemia, and atrial fibrillation. His physical examination was unremarkable.

**Morphological findings**  
A CBC at the time of the bone marrow examination showed Hgb 10.5 g/dL; RBC 3.03 x 10(12)/L; MCV 109.6 fL; RDW 15.0%; WBC 2.6 x 10(9)/L; PLT 142 X 10(9)/L.

The peripheral blood smear showed RBC macrocytosis with slight poikilocytosis with occasional elliptocytes and dacrocyes. The white blood cells and platelets showed no cytologic abnormalities.

The bone marrow was slightly hypercellular due predominantly to a mild granulocytic hyperplasia. Granulocytic maturation was normal and blasts were not increased. There was slight megaloblastoid erythroid maturation and slight dysmegakaryopoiesis consisting of a few hypolobated megakaryocytes. The iron stain showed decreased storage iron without ring sideroblasts.

**Immunophenotype**  
Flow cytometry studies performed on the bone marrow aspirate specimen showed no increase in CD 34 positive blasts or a monoclonal B-cell population. There was equivocal loss of blast heterogeneity (HLA-DR/CD13 plot). There was no expression of CD2/CD7/CD56 on the blast population. Granulocytic maturation was normal (CD13/CD16 plot).

Immunohistochemical stains performed on the bone marrow biopsy showed no increase in CD34 positive blasts. CD61 positive megakaryocytes were quantitatively normal with an occasional hypolobate form.

**Cytogenetics**  
46,XY [20]/[20]

**Molecular studies**  
Next Generation Sequencing studies performed on the bone marrow aspirate specimen revealed the following mutations:

1. TET2: c.421_424dup; p.Ser142Cysfs*6 (VAF 17%)
2. TET2: c.3594+2dup; p.? (VAF 24%)
3. ZRSR2: c.376C>T; p.Arg126* (VAF 57%)

**Proposed diagnosis**  
Clonal cytopenias of undetermined significance (CCUS)

**Interesting feature(s) of submitted case**  
The morphologic features in this case were insufficient for a morphologic diagnosis of a myelodysplastic syndrome, and cytogenetic studies showed a normal karyotype. However, Next Generation Sequencing demonstrated two separate loss of function TET2 mutations and a loss of function ZRSR2 mutation with a very high variant allele frequency. Therefore, this case was classified as clonal cytopenias of undetermined significance (CCUS).
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<tr>
<th>Submitted by</th>
<th>Dr. James D Hoyer</th>
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<tr>
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<td>Mayo Clinic</td>
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<td>Country</td>
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Panel Diagnosis

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Case 204

Workshop
II CHIP, ICUS and IDUS

Title
68 Year Old Man with Anemia and Thrombocytopenia

Clinical History
68 year old man, in May 2016, presented with:
2 months of night sweats, fatigue, loss of appetite.

Past Medical History (including Medications):
Type II Diabetes Mellitus (Glyburide-Metformin / Sitagliptin)
Cholesterolemia (Atorvastatin)
Gastroesophageal reflux disease (Pantoprazole)
Cigarette smoking (until 2012)
No alcohol.

Physical Exam:
Unremarkable. No hepatosplenomegaly. No lymphadenopathy.

Imaging:
PET/CT Scan: No evidence of pathology.

Labs, Peripheral Blood, May 2016:

<table>
<thead>
<tr>
<th>WBC</th>
<th>4.8</th>
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<tbody>
<tr>
<td>RBC</td>
<td>2.19 (L)</td>
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<td>HGB</td>
<td>8.6 (L)</td>
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<tr>
<td>HCT</td>
<td>24.5 (L)</td>
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<tr>
<td>MCV</td>
<td>112 (H)</td>
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<tr>
<td>RDW</td>
<td>17 (H)</td>
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<tr>
<td>PLAT.</td>
<td>111 (L)</td>
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Normal Differential Cell Count.

Decreased Reticulocyte Production Index 0.48 (REF >3)
Increased EPO levels 845 (REF: 4 - 27)
Normal Folate, B12, Methylmalonic acid levels

Monoclonal IGG Lambda Detected: 0.4 g/dL

Morphological findings
Mildly hypercellular marrow.
Mild relative myeloid hyperplasia (increased M:E ratio).
Occasional clusters of atypical plasma cells.
Occasional hypolobated megakaryocytes
Occasional subtle dysplasia in maturing granulocytes.
No increase in blasts or monocytes.
Megaloblastoid erythroid features; no ring sideroblasts.
Overall, the morphologic features were not sufficient for a diagnosis of MDS.

Immunophenotype
CD138+, CD56+, Lambda restricted Plasma Cells (5% of all cells, <5% of intertrabecular space) detected by IHC and Flow Cytometry.
<table>
<thead>
<tr>
<th>Cytogenetics</th>
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<tbody>
<tr>
<td>Normal 46, XY [20]</td>
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<tr>
<td>Negative MDS FISH Panel</td>
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<td>Negative Myeloma FISH Panel</td>
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<table>
<thead>
<tr>
<th>Molecular studies</th>
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<tbody>
<tr>
<td>ASXL1  pS577* (35% variant allele frequency; pathogenic)</td>
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<tr>
<td>EZH2   p.R25* (37% variant allele frequency; pathogenic)</td>
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<tr>
<td>SETBP1 p.I871T (34% variant allele frequency; pathogenic)</td>
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<tr>
<td>TET2   c.4044+1G&gt;C (72% variant allele frequency; pathogenic)</td>
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<th>Proposed diagnosis</th>
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<tr>
<td>Clonal Cytopenia of Undetermined Significance (CCUS)</td>
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<tr>
<td>Concomitant Low Level Plasma Cell Neoplasm</td>
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<tr>
<th>Interesting feature(s) of submitted case</th>
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<tbody>
<tr>
<td>Mutation Status by Myeloid NGS Panel permits diagnosis of Clonal Cytopenia of Undetermined Significance in setting of a concomitant plasma cell neoplasm and borderline morphologic myelodysplastic findings and negative cytogenic work up. A subsequent biopsy revealed persistent myelodysplastic features and also persistent presence of the myeloid gene mutations as well as persistent plasma cell neoplasm.</td>
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<tr>
<td>Dr. Michael Kluk</td>
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<td>Weill Cornell Medicine</td>
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<td>Weill Cornell, New York-Presbyterian</td>
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<td>Dep’t of Pathology &amp; Laboratory Medicine</td>
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<td>New York</td>
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<td>USA</td>
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<th>Panel Diagnosis</th>
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Case 205

**Title**
Unexplained peripheral cytopenia with clonal hematopoiesis

**Clinical History**
50-year-old male in good health, liver and spleen normal size.
First diagnosis of peripheral cytopenia in 2000 (!), 15 years prior, currently bicytopenia. Leukocytes 2840 G/L, Hb 147 g/L, Thrombocytes 70.000 G/L Neutrophils 47.5%, Lymphocytes 36.6%, Monocytes 10%, Eosinophils 5.3%, Basophils 0.4%, Band forms 0.4% MCV 99.3

**Morphological findings**
BM cytology: no dysplasia, increase in mast cells, 1.5% blasts BM histology: normocellular marrow, reactive changes, no dysplasia

**Immunophenotype**
no increase in blasts, no increase in B- or T-cells flow cytometry unremarkable

**Cytogenetics**
47, XY, +8(4), 46, XY (15), 45, X-Y(6)

**Molecular studies**
NGS panel sequencing with a 15 gene panel performed on the FFPE BM biopsy showed a ZRSR2 mutation

**Proposed diagnosis**
Idiopathic cytopenia of uncertain significance (ICUS)

**Interesting feature(s) of submitted case**
The long standing, clinically stable cytopenia in a patient without features of dysplasia and non-diagnostic cytogenetic changes is compatible with a diagnosis of ICUS. Although the ZRSR2 mutation is not very common in clonal hematopoiesis of indetermined significance (CHIP), we believe that the presence of this mutation is not sufficient for a diagnosis of MDS in the light of the other findings. We will present the results of NGS panel sequencing on cases of suspected MDS, ICUS and reactive BM for comparison.

**Submitted by**
Prof. Falko Fend

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Institute of Pathology Tübingen University Hospital

**Country**
Germany

**Panel Diagnosis**
**Case 206**

**Workshop**
II CHIP, ICUS and IDUS

**Title**
JAK2 V617F of low allelic burden associated with intermittent erythrocytosis.

**Clinical History**
A 64-year-old woman with a history of breast cancer in 2002, status post lumpectomy, radiation therapy and chemotherapy, was found to have erythrocytosis of unclear etiology in 7/2013. Subsequent hematocrit and hemoglobin (Hb/Hct) levels were unremarkable until 2/2015 when CBC showed Hb/Hct of 15 g/dL and 46%, respectively. The most recent CBC in 7/2016 demonstrated Hb/Hct of 16.3 g/dL and 49.6%. Serum EPO level, which was measured multiple times, was not decreased.

**Morphological findings**
Bone marrow examination performed in 11/2016 showed a normocellular bone marrow with maturing trilineage hematopoiesis. The M:E ratio was normal. There was no clear morphologic evidence of a myeloproliferative neoplasm.

**Immunophenotype**
Not relevant.

**Cytogenetics**
Karyotype was normal.

**Molecular studies**
Genetic analysis detected a JAK2 V617F mutation with low allelic burden of 3.26%. The patient also has a CALR mutation of unknown pathogenic significance.

**Proposed diagnosis**
In this case, the intermittent nature of the erythrocytosis, low variant allele frequency and normal bone marrow morphology support a “reactive” cause. It most likely represents clonal hematopoiesis of indeterminate potential (CHIP), and suggests that JAK2 V617F-positive CHIP may present with hematologic abnormalities that should be differentiated from early polycythemia vera (PV).

**Interesting feature(s) of submitted case**
A possible role of the anti-neoplastic therapy received eleven years before cannot be completely excluded. Whether the presence of JAK2 V617F-positive CHIP carries a risk of eventual progression to PV or other type of myeloproliferative neoplasm is presently unknown and will require additional studies.

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<tr>
<th>Submitted by</th>
<th>Dr. Wayne X Tam</th>
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<tbody>
<tr>
<td>Institute</td>
<td>Weill Cornell Medicine</td>
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<td>Country</td>
<td>New York</td>
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<td>USA</td>
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**Panel Diagnosis**
### Clinical History

A 48 years old female. Pancytopenia was developed in July 2015. Her past medical history was unremarkable. There was pallor and petechia in physical examination. Organomegaly and lymphadenopathy were not detected. There were no prior drug use or chemical exposure. Her wbc was 2900 x10^9/L, neutrophil was 600 x10^9/L, hemoglobin was 7 gr/dL, platelet count was 10000x10^9/L and absolute reticulocyte count was 0,0258 x10^12/L (0,02 - 0,16) at the time of referral. Her nutritional parameters were within normal ranges. She was evaluated in terms of infectious and rheumatological diseases. Her viral markers including parvovirus were unremarkable. She had no rheumatological symptoms and her serological tests were negative. Paroxysmal nocturnal hemoglobinuria (PNH) was excluded with both flow cytometry and FLAER. After this evaluation, a bone marrow biopsy was performed. The bone marrow was consistent with aplastic-hypoplastic anemia. Cytogenetic was normal. Flow cytometry was not diagnostic. It was considered as aplastic anemia and cyclosporine (CsA) treatment was performed. During 6 months of CsA treatment blood count and patient's transfusion demand did not changed. Second bone marrow biopsy was performed in February 2016. Bone marrow cellularity was strikingly increased compared to the previous biopsy without striking dysplasia. Monosomy 7 was detected in cytogenetic analysis (12 of 20 metaphases). At this point patient was considered unresponsive to CsA and treatment was terminated. At these days CT was performed due to the complaints of abdominal discomfort which revealed thickening of terminal ilum wall. Colonoscopy and the biopsy did not reveal any intestinal pathology. During this evaluation, anti gliadin IgA positivity was detected. Despite there was not enough evidence for celiac disease, gluten free diet was started. G-CSF for supporting the bone marrow to overcome neutropenia was also started at the same time. Under gluten free diet patient did not need transfusion for two months. Her blood count slightly elevated but remained in the limits of pancytopenia. Following this two months’ treatment, platelet count decreased. A third biopsy performed in July 2016 which revealed hyper cellular bone marrow with decreased megakaryocytes and myeloid precursors, increased erythroid precursors without striking dysplasia and lymphocytes. Patient has quitted gluten free diet. Since October 2016 patient did not need any transfusion. In October 2016 patient started to receive regular platelet transfusions but still did not need erythrocyte transfusion. The fourth biopsy revealed similar findings with the previous but more striking decrease of megakaryocytes. The cytogenetics did not reveal any additional abnormality other than monosomy 7 (9 of 14 metaphases). Danazol treatment with G-CSF support was started. To exclude celiac disease duodenal biopsy was performed which showed normal histopathology. The patient was considered as CCUS with monosomy 7. Patient is still under treatment of danazol 400 mg/day and G-CSF twice a week. The demand of transfusion was disappeared. Her wbc count was 4240 x10^9/L, neutrophil count was 1500 x10^9/L, hemoglobin 11.1 gr/dL and platelet count was 25000 x10^9/L as of January 2017.

### Morphological findings

1- Diagnosis September 2015 (19488-15): Hypoplastic bone marrow with %15 cellularity decreased all three hematopoetic series and increased lymphocytes, polytypic plasma cells

2- Following CsA treatment February 2016 (3156-16) : Increased cellularity with %40 predominance of erythroid precursors. No striking dysplasia. Minimal erythroid dysplasia, decreased megakaryocytes and myeloid precursors.

3- June 2016 (15077.16) : Increased cellularity with %70 predominance of erythroid precursors with minimal dysplasia. Minimal erythroid dysplasia, decreased megakaryocytes and myeloid precursors.
<table>
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<tr>
<th>Date</th>
<th>Description</th>
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<tbody>
<tr>
<td>October 2016 (21573-16)</td>
<td>Increased cellularity with 70% predominance of erythroid precursors with minimal dysplasia. Minimal erythroid dysplasia, decreased megakaryocytic</td>
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<tr>
<td><strong>Immunophenotype</strong></td>
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<tr>
<td>September 2015 (19488-15)</td>
<td>MPO, Lysozyme, Glicoforin A, CD3, CD20, CD4, CD8, Lambda, Kappa, CD61</td>
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<tr>
<td>June 2016 (15077.16)</td>
<td>Hypoplastic bone marrow with 15% cellularity decreased all three hematopoetic series and increased lymphocytes, plasma cells</td>
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<tr>
<td>June 2016 (15077.16)</td>
<td>Increased cellularity with 70% predominance of erythroid precursors with minimal dysplasia. Increased T lymphocytes, polyploid plasma cells, predominance of erythrocyte precursors. Decreased megakaryocytes and myeloid precursors. MPO, Lysozyme, Glicoforin A, CD3, CD20, CD138, CD4, CD8, Lambda Kappa.</td>
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<tr>
<td>October 2016 (21573-16)</td>
<td>Increased cellularity with 70% predominance of erythroid precursors with minimal dysplasia. MPO, Lysozyme, Glicoforin A, CD3, CD20, CD138, CD4, CD8, Lambda Kappa, CD34, CD117, CD61</td>
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<tr>
<td><strong>Cytogenetics</strong></td>
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<tr>
<td>September 2015 (19488-15)</td>
<td>Diagnosis: No abnormality</td>
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<tr>
<td>February 2016 (3156-16)</td>
<td>Following CsA treatment: Monosomy 7</td>
</tr>
<tr>
<td>June 2016 (15077.16)</td>
<td>Gluten free diet and GCS-F: Monosomy 7</td>
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<tr>
<td>October 2016 (21573-16)</td>
<td>Followed GCS-F: Monosomy 7</td>
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<tr>
<td><strong>Molecular studies</strong></td>
<td>not performed</td>
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<tr>
<td><strong>Proposed diagnosis</strong></td>
<td>Clonal Cytopenia of Undetermined Significance (CCUS) with monosomy 7 appeared following the immunosuppressive treatment with cyclosporine, presented as aplastic-hypoplastic anemia</td>
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<tr>
<td><strong>Interesting feature(s) of submitted case</strong></td>
<td>This is an interesting and a very well followed up case with its clinical and pathological features, also with its response to the therapeutics. First presented with rapid development of aplastic-hypoplastic anemia and developed clonal haematopoiesis following the immunosuppressive treatment, without striking dysplastic morphological features. This case represents the evolution of a clonal cytopenia of undetermined significance (CCUS) with a high risk genetic abnormality (monosomy 7) and responsive to the supportive hypoplastic anemia treatment.</td>
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**Submitted by** Prof. ISINSU KUZU

**Institute** Ankara University Faculty of Medicine, Department of Medical Pathology

**Country** TURKEY

**Co authors** Co AUTHORS:  
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3- GULSAH KAYGUSUZ (department of Pathology)  
4- GUNHAN GURMAN ( Department of Hematology)
Case 208
Workshop
II CHIP, ICUS and IDUS

Title
Role of Mutational Profiling in Cytopenic Patients with Minimal Bone Marrow Atypia: A Single Institutional Experience

Clinical History
Bone marrow biopsy #1, December 2015: A 65 year-old woman with a history of Hashimoto's thyroiditis and Sjogren syndrome was first noted to have anemia (Hgb: 9.1 g/dL) in April 2014. Esophagogastroduodenoscopy and colonoscopy showed no evidence of bleeding and no further workup was pursued. Over the subsequent year, she was intermittently treated with low dose prednisone and/or hydroxychloroquine for Sjogren symptoms. Hashimoto's thyroiditis was controlled with levothyroxine. In July 2015, routine blood work revealed persistent macrocytic anemia (Hgb: 10.4 g/dL, MCV 109 fL) and new onset thrombocytopenia (PLT: 73 x10^3/µL) with a normal white blood cell count (WBC: 5.4 x10^3/µL) and differential. Following an episode of fevers, chills, fatigue, and a 10 lb weight loss, prednisone dosing was temporarily increased to 60 mg daily, but was decreased to 30 mg daily secondary to side effects. Laboratory workup for hemolysis and vitamin deficiency was unrevealing. Given persistent cytopenias, the patient was referred to hematology in December 2015. A bone marrow biopsy is detailed below (Dec 2015).

Bone marrow biopsy #2, April 2016: Given the undetermined significance of the findings from the initial bone marrow biopsy, the patient received no specific therapy. She continued on low dose prednisone (5 mg daily) for rheumatologic symptoms. In April 2016, the patient was admitted to the inpatient service for diffuse tender lymphadenopathy and diarrhea. A CBC showed 10% circulating blasts; the subsequent bone marrow biopsy is detailed below (April 2016). The patient expired less than 1 week following this biopsy.

Morphological findings
Dec 2015: H&E stained sections of the core biopsy show a variably cellular marrow (30%) with mild atypia. Megakaryocytes are normal in number with focal clustering and mild dyspoiesis (occasional monolobated and hyperlobated, hyperchromatic forms). The aspirate smear is technically suboptimal secondary to hemodilution; therefore, maturing myeloid and erythroid forms are poorly represented. Rare erythroid precursors show mild atypia (occasional blebbing). There is no expansion of blasts. The degree of dysplasia in each lineage is deemed insufficient to support a diagnosis of myelodysplastic syndrome (MDS) (<10% dysplasia).
April 2016: H&E stained sections show hypercellular marrow (80%) with frank multilineage dysplasia. Blasts with irregular nuclei, somewhat condensed chromatin, prominent nucleoli and scant cytoplasm account for 50% of cellularity. Megakaryocytes are cytologically atypical (hypo- and abnormal lobation, micromegakaryocytes, senescent forms). The aspirate smear reveals that myeloid precursors are decreased and left shifted with cytologic atypia (hypolobation, hypogranulation). Erythroid precursors are left shifted with cytologic atypia (nuclear irregularity, multinucleation, megaloblastoid change).

Immunophenotype
Dec 2015: No discrete population of blasts.
April 2016: Blasts (accounting for 50% of cellularity) show following dominant immunophenotype: CD4(var)+ CD7(var)+ CD15(var)+ CD33(var)+ CD34(var)+ HLA-DR+.

Cytogenetics
Dec 2015: 47,XX,+8 [20]
April 2016: Targeted massively parallel sequencing revealed all 4 previously identified disease associated mutations: DNMT3A (41%), SRSF2 (50%), ETV6 (13%), and SETBP1 (13%). Additionally, 3 new disease associated mutations were identified: FLT3-ITD (57 base pairs, 19% allele frequency), PHF6 p.? (c.835-1G>A, 6% allele frequency), and PHF6 p.R225* (c.673C>T, 12% allele frequency).

Proposed diagnosis
Dec 2015: Clonal cytopenia of undetermined significance (CCUS) April 2016: Acute myeloid leukemia with myelodysplasia-related changes (AML-MRC)

Interesting feature(s) of submitted case
Our patient presented in Dec 2015 with unexplained cytopenias. The concurrent bone marrow biopsy showed mild atypia, most pronounced in megakaryocytes, in <10% of each lineage without an expansion of blasts. Mutational profiling revealed disease associated mutations in 4 genes which are commonly mutated in myeloid neoplasms with mutant allele frequencies ranging from 29 - 52%. Cytogenetic studies showed a gain of chromosome 8 in all 20 cells evaluated. Thus, our patient showed clonal hematopoiesis and cytopenias, in the absence of diagnostic dysplasia or an expansion of blasts. Based on 2016 WHO criteria, these findings are insufficient to support a diagnosis of MDS. They are, however, compatible with the non-WHO classification of “clonal cytopenias of undetermined significance (CCUS).” In less than 5 months, our patient progressed to overt acute myeloid leukemia with myelodysplasia-related changes. Mutational profiling identified persistence of the four previously identified mutations, and the emergence of new mutations in FLT3-ITD and PHF6 at allele frequencies ranging from 6 - 19%.

Our patient's first bone marrow biopsy illustrates an increasingly common problem in hematopathology. With the increasing availability of high-throughput mutational testing, growing numbers of cytopenic patients without overt evidence of dysplasia are undergoing testing. Recent studies have shown that a significant subset of these patients harbor mutations in many of the same genes which are mutated in MDS(1–3). When cytopenic patients show clonal MDS-associated mutations, it is tempting to label them with presumptive MDS. However, other studies suggest that approximately 10% of individuals in the normal aging population (older than 70 years of age) with no evidence a hematologic malignancy have clonal hematopoiesis, termed “clonal hematopoiesis of indeterminate potential” (CHIP)(4–8). While patients with CHIP have a mildly increased risk of developing a hematologic neoplasm (approximately 1% per year), most of these patients will never develop MDS or AML. Given that the natural history of CHIP and CCUS are not fully understood, the 2016 WHO update states "the presence of MDS-associated somatic mutations alone is not considered diagnostic of MDS in this classification, even in a patient with unexplained cytopenia, where these mutations may be commonly found."(9) The identification of features that distinguish patients with CCUS at low risk of progression from those at high risk of progression would represent a major advance. While evidence is still accumulating, some studies have suggested that the number and combination of mutated genes, and mutant allele frequency may all have prognostic significance. Cargo et al showed that patients with 2 or more mutated genes at high allele frequency may be at higher risk for subsequent evolution to overt neoplasia(2). Moreover, multiple studies have shown that concurrent mutations in genes involved in RNA splicing and epigenetic modification are common in MDS and are likely early lesions in the evolution of MDS and AML(10–12). Our patient had 4 mutated genes at high mutant allele frequency, including a DNA-methylator (DNMT3A) and a member of the spliceosome (SRSF2). Each of these features may suggest our patient was at high risk of progression.
Also of interest is the emergence of additional mutations as our patient progressed from CCUS to AML. Mutations of genes involved in signaling pathways, including FLT3, are often acquired as late events in the progression of AML(7,12). PHF6, believed to play a role in transcriptional regulation, is also more commonly mutated in the mid-to-late stages of evolution to AML(7,10).

In view of the issues raised by this case, we sought to determine whether use of targeted next-generation sequencing is helpful in dealing with the challenging subgroup of cytopenic patients whose marrow findings are insufficient to allow a diagnosis of myelodysplasia. We performed a retrospective institutional study of patients with at least one documented cytopenia (Hgb: <10.5 g/dL, PLT: <125 x10^3/µL, WBC: <3.0 x10^3/µL) and with only mild evidence of dyspoiesis. Only clinically reported mutations characterized as pathogenic or disease-associated were analyzed. Patients were excluded if they had a history of an additional prior or concurrent hematologic neoplasm.

Patients with no mutations are classified as ICUS and patients with at least one mutation are classified as CCUS. Based on the previously described studies, we propose the following molecular features to help further subclassify patients as “high risk” CCUS: 1) At least two mutated genes from at least two functional classes (e.g. chromatin modification, DNA methylation, RNA splicing, transcription, etc) and 2) average mutant allele frequencies >35%. Both criteria must be met in order to be classified as “high risk.”

Between 2/2013 and 12/2016, 2749 patient samples were tested with our in-house clinical next-generation sequencing assay, which targets exomic regions in 68 genes associated with hematologic malignancies. Of 2749 patient samples, 29 were derived from cytopenic patients with concurrent bone marrow biopsies showing mild atypia deemed insufficient for a diagnosis of MDS.

Of these 29 patients, 21 (72%) had normal sequencing studies and were further classified as ICUS. 8 (28%) showed at least one disease-associated (including the patient presented in this case report) and are best classified as CCUS. Among those 8 CCUS patients, 4 (14%) had only a single mutated gene: 3 distinct DNMT3A mutations and one TET2 mutation. Conversely, 4 patients (14%) showed multiple mutations (average 3.8 mutations/specimen) with the following gene breakdown: 4 TET2 mutations, 3 ASXL1 mutations, 3 SRSF2 mutations, 2 DNMT3A mutations, 1 RUNX1 mutation, 1 SETBP1 mutation, 1 ETV6 mutation. The mutant allele frequencies in samples with multiple mutations were generally higher (avg. mutant allele frequency: 42%) than those with just a single mutation (avg. mutant allele frequency: 17%).

According to our proposed classification, four patients are classified as "high-risk" CCUS and four are classified as "low-risk". Unfortunately, the follow-up data on this small cohort of patients remains limited (median follow-up time 5.5 months), and definitive outcome conclusions are not possible. We will, however, continue to follow this small cohort of patients.

In summary, this case serves as an example of a patient with CCUS, with potentially “high-risk” mutational profiling, who rapidly progressed to AML. This case raises the possibility that mutational profiling may play a larger role in the evaluation of cytopenic patients in the future and may identify those patients at high risk of progression.

References

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<th>Dr Craig R Soderquist</th>
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**Panel Diagnosis**