Pancytopenia in a 38 year old male: ICUS to CCUS to MDS

SOCIETY FOR HEMATOPATHOLOGY CASE SH2017-0050
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Clinical Presentation

38 year old male who was found to have leukopenia and thrombocytopenia when hospitalized for a snowboarding accident

Follow-up CBC in November 2015 at hematologist’s office:
- WBC 1.4 K/µl, Hct 39%, plt 92 K/µl ANC 280/µl, MCV 80.8 fl

Outside bone marrow biopsy dated 11/18/15: MODERATELY HYPERCELLULAR MARROW WITH MILD ERYTHROID AND MEGAKARYOCYTIC HYPERPLASIA
- Mild erythroid and megakaryocytic atypia
- Peripheral blood flow cytometry: no significant blast population and no monotypic B-cell or aberrant T cell population
- Karyotype: 46, XY

• ICUS: idiopathic cytopenia of uncertain significance
Clinical Presentation

• Patient was referred to VUMC for persistent pancytopenia (8/24/16, 10 mo. later)
  • No fatigue, night sweats, or weight loss
  • CBC: WBC 2.3 K/ul, Hct 40%, plts 104 K/ul

• Bone marrow diagnosis:
  • NORMOCYLLULAR MARROW WITH TRILINEAGE HEMATOPOIESIS; MILD MEGAKARYOCYTIC
    DYSPLASIA, NO INCREASE IN BLASTS
  • Occasional hypolobated neutrophils in the peripheral blood
  • No increase in blasts by morphology, flow cytometry, or immunohistochemistry
  • Single interstitial lymphoid aggregate, mix of T and B cells by immunohistochemistry
8/24/16 bone marrow aspirate and peripheral smear
8/24/16 bone marrow biopsy and particle-H&E stain
Ancillary studies on 8/24/16 marrow

• Flow cytometry: 3.3% myeloblasts with a normal immunophenotype
• Karyotype: 46, XY
• FISH: Normal signal patterns using standard panel of MDS probes (5p15.2, 5q31, cen7, 7q31, cen8, 20q12)
• What next? The clinician called and said, “Look, I know this isn’t your usual protocol, but I would like to do NGS”.
• OnkoSight BRLI myeloid disease panel of 37 genes
  • SRSF2 p.Pro95Leu (43.15%)
  • RUNX1 p.Arg169Lysfs*44 (11.59%)
  • IDH1 p.Arg132Cys (42.26%)
  • NRAS p.Gly12Asp (11.87%)
  • BCOR p.Met1020Val—unclear variant (19.85%)
ICUS ➝ CCUS

- Bone marrow: Normocellular marrow with trilineage hematopoiesis; mild megakaryocytic dysplasia; no increase in blasts; multiple mutations present consistent with CCUS

- CCUS: Clonal cytopenias of undetermined significance
1/25/17 peripheral blood smear
CBC: WBC 0.9K/µl, Hct 38%, Plts 183 K/µl ANC 280/µl

Hypogranular neutrophils
1/25/17 bone marrow aspirate
1/25/17 bone marrow biopsy
• Bone marrow on 1/25/17, interval of 5 months
  • CBC: WBC 0.9K/µl, Hct 38%, Plts 183 K/µl ANC 280/µl
  • BONE MARROW: NORMOCYLLULAR MARROW WITH BILINEAGE DYSPLASIA; NO INCREASE IN BLASTS; CONSISTENT WITH MYELODYSPLASTIC SYNDROME
    • Best classified as MDS with multilineage dysplasia (MDS-ML)
    • Myeloid and megakaryocytic dysplasia
    • No increase in myeloblasts by flow cytometry
    • Normal karyotype, 46, XY
Progression of the patient’s MDS

• Bone marrow 6/1/17: NORMOCELLULAR MARROW WITH ERYTHROID HYPERPLASIA, BILINEAGE DYSPLASIA, AND INCREASED BLASTS (10% BY IHC), CONSISTENT WITH MYELODYSPLASIA WITH EXCESS BLASTS

• Bone marrow 7/4/17: NORMOCELLULAR MARROW WITH PERSISTENT INVOLVEMENT BY MYELODYSPLASTIC SYNDROME WITH EXCESS BLASTS-1 (9.2% BY FLOW CYTOMETRY)

• Bone marrow 7/24/17: NORMOCELLULAR MARROW WITH MILD TRILINEAGE DYSPLASIA, CONSISTENT WITH PERSISTENT MYELODYSPLASTIC SYNDROME; NO INCREASE IN BLASTS
Progression of cytopenias

- **WBC**: 2.1, 2.9, 1.8, 1.5, 1.0, 1.6, 4.6
- **HCT**: 41, 12, 12, 12, 12, 12, 12
- **PLTS**: 125, 125, 125, 125, 125, 125, 125
- **ANC**: 1.6, 0.26, 0.28, 0.37, 0.57, 0.87, 1.2

**TREATMENT**
- Decitabine
- 6 cycles
- Pretransplant. Day 0=8/8/17
- Mismatched unrelated ablative PBSCT
Clinical significance of somatic mutation in unexplained blood cytopenia

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Study design:
- Learning cohort of 683 consecutive patients being worked up for unexplained cytopenias.
- Targeted NGS using a 40 gene panel was performed (Illumina TruSight Myeloid Sequencing Panel).
- Predictive values of mutation analysis were confirmed in an independent validation cohort of patients with a suspected diagnosis of myeloid neoplasm (190 patients).

Results:
- Standard work up of the cytopenic patients demonstrated that 60% of patients had a myeloid neoplasm, 22.5% had ICUS, 17.5% had other cause of cytopenia.
- 64% of patients with cytopenias had a somatic mutation in at least 1 of the 40 genes.
- Predictive value of mutations for myeloid neoplasm group
  - Spliceosome genes (SF3B1, SRSF2, U2AF1), JAK2, and RUNX1 mutations had highest predictive value for myeloid neoplasm (0.88-0.97).
  - Having 2 or more mutations had an odds ratio of 4.69 for having MDS or another myeloid neoplasm.
- Of the patients with original diagnosis of ICUS, 25% developed a myeloid neoplasm.
  - 36% of ICUS patients were found to have 1 or more mutation (CCUS).
  - Allele frequencies of 10% or greater were significant.
  - CCUS had a hazard ratio of 13.9 compared to ICUS for developing a myeloid neoplasm.
  - CCUS: 5 yr and 10 yr cumulative probability of progression to myeloid neoplasm: 82% and 95% compared to 9% for unmutated ICUS.
  - Highly predictive mutation pattern: Spliceosome gene mutations, or one of TET2, ASXL1, or DNMT3A with additional mutations.
Probability of progression to myeloid neoplasm of patients receiving a provisional diagnosis of ICUS, according to mutation status and pattern.

Luca Malcovati et al. Blood 2017;129:3371-3378
Our patient

• Highly predictive mutation pattern
  • Splicesome mutation: SRSF2
  • 2 or more mutations (5)
  • RUNX1, IDH1/IDH2, and BCOR in the list of most frequent co-occurring mutations associated with development of myeloid neoplasm

• Time interval between ICUS and MDS: approximately 15 months

• Initial NGS performed due to ICUS diagnosis changed treatment approach
  • Frequent monitoring by CBC and bone marrow biopsy
  • Treatment with Decitabine
  • Allo PBSCT: potential cure
Questions raised by these studies

• What is the mechanism by which spliceosome mutations lead to MDS?

• Should NGS be performed on all patients with unexplained cytopenias?
**How do spliceosome mutations cause MDS?**

- SRSF2 P95H mutation affects specificity of pre-mRNA binding
- Mis-splicing of known hematopoietic regulators, among other targets
- Specifically, EZH2 splicing is altered, introducing a premature termination codon and accelerated mRNA degradation
- EZH2 encodes a histone H3K27 methyltransferase commonly mutated in MDS
- SRSF2 and EZH2 mutations are mutually exclusive in mutational studies of MDS patients

*Eunhee Kim, Janine O. Ilagan, Yang Liang, et al. SRSF2 Mutations Contribute to Myelodysplasia by Mutant-Specific Effects on Exon Recognition*  
*Cancer Cell, Volume 27, Issue 5, 2015, 617–630*  
[http://dx.doi.org/10.1016/j.ccell.2015.04.006](http://dx.doi.org/10.1016/j.ccell.2015.04.006)
Should NGS be performed on all patients with unexplained cytopenias?

• Screening for other causes essential
  ◦ Nutritional
  ◦ Infectious
  ◦ Autoimmune

• Age cut-off?
  • Age range in the Malcovati study was 18-92 y.o., median 66 y.o., data not broken down by age
  • Previous study of 12,380 patients unselected for cancer or hematologic disease:
    • Genovese et al NEJM 371: 26, 2014
    • Whole exome sequencing of peripheral blood cell DNA
    • 10% incidence of somatic mutations in patients older than 65 years.
    • 1% incidence of somatic mutations in patients younger than 50 years.
Final panel diagnoses

• 8/24/16 bone marrow: Clonal cytopenia of undetermined significance (CCUS)

• 1/25/17 bone marrow: Myelodysplastic syndrome

• Subsequent bone marrow biopsies demonstrate progression to myelodysplastic syndrome with excess blasts-2.