New Frontiers in Pathology
Case 13

Riccardo Valdez, M.D.
• 78-year-old man developed *C. difficile* colitis following antibiotic treatment for dental extraction.

• CBC showed WBC count of 17.0 K/uL with increase to 60 K/uL during nine-day hospital admission.

  -- Absolute neutrophil count = 55.6 K/ul
  -- Absolute monocyte count = 0.8 K/ul
  -- Absolute lymphocyte count = 2.7 K/ul
  -- Absolute eosinophil count = 0.9 K/ul
  -- Absolute basophil count = 0.2 K/ul

• Normal hemoglobin level and platelet count.
Causes of Neutrophilia

Acute shift from marginalizing to circulating pool or chronic stimulation of proliferative pool by cytokines?

- Infection
  - Primarily bacterial
- Medications
  - Epinephrine
  - Corticosteroids
  - GM-CSF
- Acute stress
  - Trauma/burns
  - Seizure
  - Exercise
- Pregnancy

- Systemic inflammation
  - Collagen vascular diseases
  - Gout
  - Juvenile rheumatoid arthritis
- Leukocyte adhesion defect
- Neoplasm
  - Carcinomas
  - Marrow metastasis
  - Certain lymphomas (e.g. CHL)
  - Myeloid neoplasms
Clues to Cause of Neutrophilia

- **Reactive**
  - Usually <30 K/uL
  - Toxic granulation, Döhle bodies, and vacuoles
  - Slight thrombocytosis (<500 K/uL)
  - Bands > metamyelocytes > myelocytes
  - Resolves spontaneously or following treatment

- **Neoplastic**
  - Persistent, unexplained
  - Splenomegaly
  - Marked thrombocytosis (>600 K/uL)
  - Basophilia
  - Myelocytes > metamyelocytes
  - Absence of ‘toxic’ changes
Clinical Course

- Leukocytosis attributed to acute infection.
  - CBC normal two months prior.
- Infectious colitis resolved with vancomycin treatment.
- Neutrophil count remained variably elevated over next several months (range 10-20 K/uL)
  - Referred to a hematologist.
Causes of Neutrophilia

- Infection
  - Primarily bacterial
- Medications
  - Epinephrine
  - Corticosteroids
  - GM-CSF
- Acute stress
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  - Exercise
- Pregnancy

- Systemic inflammation
  - Collagen vascular diseases
  - Gout
  - Juvenile rheumatoid arthritis
- Leukocyte adhesion defect
- Neoplasm
  - Carcinomas, especially gastric
  - Marrow metastasis
  - Certain lymphomas (CHL)
  - Myeloid neoplasms

**Chronic Myeloid Leukemia?**
• Myeloproliferative neoplasm with neutrophils as the major proliferative component
  • Sometimes still referred to as Chronic Granulocytic Leukemia (CGL)

• Worldwide incidence: 1-2 cases per 100,000 with slight male predominance; increases with age (>2.5 cases/100,000 in older adults)

• Predisposing factors largely unknown

• Triphasic disease with most patients presenting in chronic phase (CP)

• Symptoms include: fatigue, malaise, weight loss, night sweats, anemia, palpable splenomegaly in 50%
  • Nearly half of newly diagnosed patients asymptomatic and disease only discovered when routine examination shows abnormal CBC
Chronic Myeloid Leukemia

- Median WBC is 80 K/uL due to neutrophils with variable left-shift
  - High proportion of myelocytes
  - Blasts present but less than 2%

- Absolute basophilia and eosinophilia common

- Absolute monocytopoiesis may be present, but proportion usually less than 3%

- Platelet count can be normal or increased
  - Marked thrombocytosis uncommon in CP
• Hematopoietic stem cell disorder characterized by chromosomal translocation t(9;22)(q34;q11.2), which results in formation of Philadelphia chromosome and BCR/ABL fusion gene

• Most cases can be diagnosed on basis of peripheral blood findings combined with the detection of the Philadelphia chromosome and/or BCR-ABL1 by cytogenetic or molecular testing

• Effectively treated with tyrosine kinase inhibitors
CBC at time of referral to hematology:

WBC 14.5 K/uL (ANC 11.9 K/uL)
Hgb 11.9 g/dl
Platelets 172 K/uL

Slight granulocytic left-shift without circulating blasts.

No evidence of infection.

No organomegaly.

Negative for BCR-ABL1 fusion by RT-PCR
Clinical Course

• Definitive etiology of neutrophilia not identified (leukemoid reaction).

• Findings not diagnostic of chronic myeloid leukemia.

• Followed in hematology clinic over next several months for persistent unexplained neutrophilia.

• WBC and absolute neutrophil count remained variably increased.

• Additional CBC and blood smear abnormalities, including rare circulating blasts manifested, over the next 18 months

• Bone marrow biopsy performed.
CBC at time of biopsy showed:
- WBC 88.3 K/μL
- HGB 11.9 g/dL
- MCV 94.1 fl
- PLT 81 K/μL

Leukocytosis due to absolute neutrophilia; normal monocyte, eosinophil, and basophil counts

Peripheral blood smear revealed:
- Granulocytic left-shift with 2% blasts
- Few nucleated red blood cells
- Blasts with Auer rods not found
- Dysgranulopoiesis
- Thrombocytopenia with few hypogranular platelets
Bone core nearly 100% cellular due to prominent myeloid expansion with left-shift
Aspirate smears confirmed increased myeloid-to-erythroid ratio with myeloid left-shift, but no increase in blasts. Dysmorphic changes in all three cell lines, most prominent in myeloid cells.
• Karyotypic analysis revealed: 46,XY,del(20)(q11.2q13.3)[15]/46, XY[5].

• Interphase FISH showed no abnormalities of BCR/ABL1, PDGFRA/FIP1L1, PDGFRB, or FGFR1.

• Molecular testing was negative for mutations in JAK2 V617F, JAK2 exon 12, MPL, and CALR.
Leukocytosis with abnormal blood and bone marrow morphology including trilineage dysplasia (most prominent in granulocytes), clonal cytogenetic abnormality → myeloid neoplasm, not leukemoid reaction

Less than 5% blasts in blood, no increase in bone marrow blasts, and no AML defining cytogenetic abnormalities → not AML; MDS?

No BCR/ABL by cytogenetics, FISH, or PCR → not CML

No myeloproliferative neoplasm (MPN) associated abnormalities by FISH and molecular testing → helps exclude PV, ET, PF

No monocytosis → not CMML
### Chronic myeloid neoplasms

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Chronic Neutrophilic Leukemia

• Rare MPN characterized by sustained neutrophilia, hypercellular bone marrow due to granulocytic proliferation (without significant dysplasia), and hepatosplenomegaly

• Diagnosis requires exclusion of reactive neutrophilia, other MPNs (no Philadelphia chromosome), and myelodysplastic/myeloproliferative overlap disorders

• Very rare; affects mostly older adults

• About 25% of reported cases associated with plasma cell myeloma or MGUS
  • Most likely represent leukemoid reaction resulting from synthesis of G-CSF by neoplastic plasma cells

• WBC usually >25 K/uL with substantial increase in bands
  • Otherwise no significant left-shift, and blasts not found in blood

• Neutrophils normal or show toxic granulation and Dohle bodies (no dysplasia)

• Strongly associated with CSF3R mutation, often together with SETBP1 or ASXL1 mutation
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• Rare hematopoietic stem cell neoplasm with overlapping features of myelodysplastic syndrome and myeloproliferative neoplasm.

• Overlap disorders have clinical and morphologic features of both MDS and MPN:
  • Peripheral cytopenias and/or dysplastic bone marrow changes (MDS)
  • Clonal proliferation with leukocytosis, thrombocytosis, and/or organomegaly (MPN)

• Current WHO classification recognizes the following in this group:
  • Chronic myelomonocytic leukemia (CMML)
  • Juvenile chronic myelomonocytic leukemia (JMML)
  • Atypical chronic myeloid leukemia (aCML)
  • MDS/MPN with ringed sideroblasts and thrombocytosis (MDS/MPN-RS-T)
  • MDS/MPN neoplasm, unclassifiable

• Neutrophil lineage principally involved in atypical CML (similar to typical CML)
• Incidence approximately 1–2% of BCR-ABL1-positive CML.

• Median age at diagnosis sixth and seventh decade of life with several studies demonstrating slight male predominance.
  • May occur in younger patients

• Median survival 15 months (range 12.4–37 months).

• Shorter survival associated with older age (>65 years), female gender, leukocytosis >50 K/uL, and the presence of circulating blasts.

• Natural history characterized by increasing leukemic cell burden, organomegaly, anemia, and bone marrow failure with transformation to acute myeloid leukemia (AML) in 30–40% of cases.
Atypical Chronic Myeloid Leukemia: Peripheral Blood Findings

- WBC count $\geq 13$ K/uL
  - Median values 24-96 K/uL
  - Count $>300$ K/uL reported

- Granulocytic left-shift
  - $>10\%$ metamyelocytes, myelocytes, and promyelocytes
  - Blasts usually $\leq 5\%$

- Basophilia not prominent

- Absolute monocytes may be increased but $<10\%$

- Moderate anemia common

- Variable thrombocytopenia

**KEY FEATURE:**

**DYSGRANULOPOIESIS**

- Pelgeroid change
- Other nuclear abnormalities
- Abnormal cytoplasm
Atypical Chronic Myeloid Leukemia:
Bone Marrow Findings

- Hypercellular due to myeloid expansion with left-shift (M:E >10)

- **Dysgranulopoiesis invariably present**

- Dyserythropoiesis in 40%

- Megakaryocytes normal or increased with dysmegakaryopoiesis
  - Micromegakaryocytes
  - Small megakaryocytes with hypolobated nuclei

- Blasts may be increased but <20%

- Slight marrow fibrosis may be present at diagnosis in some patients
Atypical Chronic Myeloid Leukemia: Genetic Findings

- Abnormal karyotype found in up to 88% of patients.
  - Most common: gain of chromosome 8 and deletion 20q
  - Abnormalities of chromosomes 5, 7, 12, 13, 14, 17, 19 common

- **NO BCR-ABL1 FUSION** (no Philadelphia chromosome).

- No PDGFRA, PDGFRB, or FGFR1 rearrangement.

- Driver mutations associated with MPNs (JAK2, CALR, and MPL) typically absent with exception of JAK2 V617F which has been reported in approximately 4–8% of aCML cases.
  - JAK2 V617F unlikely to be a driver mutation in the pathogenesis given low frequency.
Atypical Chronic Myeloid Leukemia: Genetic Findings

- Heterogeneous molecular fingerprint including recurrent somatic mutations affecting genes involved in growth factor signaling and epigenetic regulation of DNA methylation and histone modification.

- Most common mutations detected in aCML include: ASXL1 (20–70%), SETBP1 (25–30%), SRSF2 (40%), TET2 (43%), ETNK1 (9%).

- SETBP1 mutations show preference for MDS/MPN syndromes, especially those with del(20q), −7, and iso-chromosome i(17)(q10) abnormalities.

- Strong correlation of mutated SETBP1 with mutations involving ASXL1 (histone modification gene), suggesting potential cooperative role in leukemic progression.

- Multiple studies have shown mutated SETBP1 associated with a more adverse clinical profile (higher WBC count, lower hemoglobin level, thrombocytopenia), and worse overall prognosis in both aCML and CMML.

- Mutations in CSF3R (granulocyte-colony stimulating factor 3 receptor), an important cellular signaling pathway oncogene, rarely present in aCML (less than 10%)
  - More commonly associated with chronic neutrophilic leukemia.
WHO-defined Diagnostic Criteria for Atypical CML

- Peripheral leukocytosis >13 K/uL due to increased neutrophils and their precursors (accounting for greater than ≥10% of leukocytes).
- Dysgranulopoiesis; may include abnormal nuclear chromatin clumping.
- Minimal or absent absolute basophilia (basophils <2% of WBCs).
- Minimal or absent absolute monocytosis (monocytes <10% of WBCs).
- Hypercellular bone marrow with myeloid proliferation and granulocyte dysplasia, with or without dyserythropoiesis or dysmegakaryocytopenia.
- Less than 20% blasts in the blood or bone marrow.
- Absence of BCR-ABL1 fusion gene; no PDGFRA, PDGFRB, or FGFR1 rearrangements.
- WHO diagnostic criteria for BCR/ABL1-positive chronic myeloid leukemia, polycythemia vera, essential thrombocythemia, or primary myelofibrosis not met.

Department of Pathology
Atypical chronic myeloid leukemia, BCR/ABL1-negative

- Next-generation sequencing revealed a gain-of-function mutation in SRSF2 and loss-of-function mutation in ASXL1.

  - SRSF2 (Serine and arginine Rich Splicing Factor 2)
    - Multi-faceted role in oncogenesis of MPN/MDS neoplasms, influencing transcription, splicing, translation, and genomic stability.
    - Insufficient evidence to establish it as a primary driver mutation.
    - Mutation more frequently associated with ASXL1 and SETBP1 mutations in aCML, as compared to other MPN/MDS entities, including CMML.

  - ASXL1 (additional sex combs like 1)
    - One of the most frequently mutated genes in myeloid neoplasms.
    - ASXL1 protein involved in epigenetic regulation of gene expression.
    - Generally associated with aggressive disease and poor clinical outcome.
• No standard of care for the treatment of aCML at present time.
  • Not BCR/ABL1-positive, thus not responsive to tyrosine kinase inhibitors

• Challenges in management of this relatively rare MDS/MPN stem from its heterogeneous clinical and genetic features, high rate of transformation to AML, and absence of sufficient randomized clinical trial data to support treatment recommendations.

• Current treatment approach incorporates strategies used for MDS and MPN, including use of hematopoietic stem cell transplantation, conventional chemotherapy, and adjunctive agents for disease cytoreduction.
  • Hydroxyurea most commonly utilized to control leukocytosis and/or symptomatic splenomegaly.

• Patient being managed on hydroxurea and darbepoietin
  • Recent WBC 9.5 K/uL, ANC 7.1 K/uL, Hgb 8.5 g/dl, platelets 103 K/uL
Atypical chronic myeloid leukemia is a rare MPN/MDS overlap disorder which does not feature a BCR/ABL1 fusion (Philadelphia chromosome), despite what the disease name may suggest.

It is a disease of older adults and presents with leukocytosis due to neutrophilia and granulocytic left-shift.

Reactive conditions, non-hematopoietic malignancies, and other myeloid neoplasms must be excluded as the cause of neutrophilia.
  - BCR/ABL1 fusion testing is an important part of the work-up

Dysgranulopoiesis is an key clue and diagnostic feature in aCML.

Several genetic abnormalities are associated with aCML, but none entirely specific.

Genetic abnormalities are useful in confirming malignancy, providing prognostic information, and serving as basis of future therapies.


Thank you!

Questions?