206 Myelodysplastic Syndromes (MDS), Aplastic Anemia, and Other Bone Marrow Failure States

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Myelodysplastic syndromes (MDS) are a group of clonal hematopoietic stem cell diseases characterized by cytopenias, dysplasia, ineffective hematopoiesis, and an increased risk of acute leukemia in ~30% of cases. MDS shows overlapping clinical features with aplastic anemia, paroxysmal nocturnal hemoglobinuria (PNH), and certain lymphomas, such as T-cell large granular lymphocytic leukemia. Therefore, when working up a potential case of MDS, other causes of cytopenias, namely nutritional deficiencies or excess, medications, viral infections, and lymphoproliferative disorders need to be considered in the differential diagnosis. Using case presentations the presenters will illustrate typical cases of MDS and aplastic anemia, as well as disease conditions that mimic MDS or aplastic anemia. This session will offer a systematic approach to the work-up of patients with cytopenias integrating clinical, laboratory, immunophenotypic, and genetic features into final diagnosis. The course is designed for practicing pathologists, pathology trainees, and technologists interested in hematopathology and it will provide a complete review of the current WHO classification of MDS, as well as the underlying pathogenesis of aplastic anemia, with particular attention to mimickers of these disease entities.

- Describe the diagnostic criteria delineated by the current World Health Organization in the subclassification of myelodysplastic syndromes, and focus on differential diagnostic considerations mimicking myelodysplasia. Outline a systemic approach to ancillary studies needed in the distinction of the different entities.
- Describe the diagnostic criteria for aplastic anemia and guidelines for adequacy of a specimen for interpretation, as well as outline a systemic approach to ancillary studies needed in the distinction of aplastic anemia and other bone marrow failures mimicking aplastic anemia.
- Upon completion of this course, participants should be able to develop a systematic diagnostic algorithm for pancytopenia and isolated cytopenias integrating clinical, laboratory, morphologic, immunophenotypic, and genetic features.

**FACULTY:**

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Entire Pathology Team  
Hematopathology  
Hematopathology  

1.0 CME/CMLE Credit

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Myelodysplastic Syndromes (MDS)

- Cytopenia(s)
- Dysplasia in one or more myeloid lines
- Ineffective hematopoiesis
- Increased risk of development of acute myeloid leukemia (~30% of cases)
Pathophysiology of MDS

• Genetic alterations
  – Isolated del(5q) affects the RPS14 (encodes a component of 40S ribosomal unit) and SPARC (tumor suppressor gene) genes
  – Patients with chromosomal abnormalities of ch5 and ch7 often acquire additional mutations in the RAS or TP53 genes or promoter methylation changes in CDKN2B

• Epigenetic alterations
  – DNA methylation – regulates gene transcription by methylation of cytosines in CpG islands – silencing of tumor suppressor genes

Pathophysiology of MDS, cont’d

  – Histone deacetylation leading to reduced transcriptional activity

• BM microenvironment
  – Stromal cells take part in the regulation of hematopoiesis by secreting cytokines
  – Angiogenesis – mediated by macrophages which produce proinflammatory cytokines and VEGF – these are elevated in high-risk MDS as compared to low-risk MDS

Myelodysplastic Syndromes (MDS)

• Incidence has increased from 3.3 per 100,000 in 2001 to 3.8 per 100,000 in 2006

• The increase has been attributed to
  – Enhanced awareness
  – Aging population
  – Availability of more effective therapies, making hematologists more likely to pursue the diagnosis
Case #1

- 69-year old female with pancytopenia:
  - WBC: 2.2 (4.5-11 K/cumm)
  - Hemoglobin: 10.6 (13.0-17.0 g/dl)
  - Platelets: 103 (140-400 K/cumm)
Erythroid precursor with nuclear budding
Metamyelocyte
Myeloblast
Megakaryocyte

Ring sideroblast

Ring sideroblasts
Cytogenetics

- Fluorescence in situ hybridization for MDS panel:
  - -7/del7q (D7S486): negative
  - -5/del5q (EGR1): negative
  - del20q (D20s108): negative
  - 12p13 (ETV6): negative
  - +8 (CEP8): negative
  - del13q (D13S319): negative
- Normal conventional karyotype

Diagnosis

Hypercellular marrow for age with multilineage dysplasia; no excess blasts seen

Comment: Dyspoiesis is a morphologic change that can be due to myelotoxic / myelosuppressive drugs, nutritional deficiency, chronic viral infection, severe or sustained inflammatory conditions, or primary myelodysplastic syndrome (MDS). Diagnosis of MDS, specifically, refractory cytopenia with multilineage dysplasia may be considered if all secondary causes are excluded.

Peripheral Blood and Bone Marrow Findings in MDS

<table>
<thead>
<tr>
<th>Disease</th>
<th>Blood</th>
<th>Bone marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractory cytopenia with unilineage dysplasia (RCUD)</td>
<td>Uni- or bicytopenia, &lt;1% blasts</td>
<td>Unilineage dysplasia, &lt;5% blasts, &lt;15% ring sideroblasts</td>
</tr>
<tr>
<td>Refractory anemia with ring sideroblasts (RARS)</td>
<td>Anemia, &lt;1% blasts</td>
<td>≥15% ring sideroblasts, dyserythropoiesis only, &lt;5% blasts</td>
</tr>
<tr>
<td>Refractory cytopenia with multilineage dysplasia (RCMD)</td>
<td>Cytopenias, &lt;1% blasts, no Auer rods</td>
<td>Dysplasia (&gt;10% of cells) in at least two lineages, &lt;5% blasts, no Auer rods</td>
</tr>
<tr>
<td>Refractory anemia with excess blasts – 1 (RAEB-1)</td>
<td>Cytopenia(s), &lt;5% blasts, no Auer rods</td>
<td>Uni- or multilineage dysplasia, 5-9% blasts, no Auer rods</td>
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</tbody>
</table>

Modified: 2008 WHO Classification of Tumors of Hematopoietic and Lymphoid Tissue
Peripheral Blood and Bone Marrow Findings in MDS, cont’d

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<tr>
<th>Disease</th>
<th>Blood</th>
<th>Bone marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractory anemia with excess</td>
<td>Cytopenia(s), 5-19% blasts, +/- Auer rods</td>
<td>Uni- or multilineage dysplasia, 10-19% blasts, +/- Auer rods</td>
</tr>
<tr>
<td>blasts – 2 (RAEB-2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myelodysplastic syndrome –</td>
<td>Cytopenias, ≤1% blasts</td>
<td>Unequivocal dysplasia &lt;10% of cells and cytogenetic abnormality, &lt;5% blasts</td>
</tr>
<tr>
<td>unclassified (MDS-U)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDS with isolated del(5q)</td>
<td>Anemia, usually normal or ↑ platelet count,</td>
<td>Normal to ↑ mgs with hypolobation, &lt;5% blasts, no Auer rods, isolated del(5q) by cytogenetics</td>
</tr>
<tr>
<td></td>
<td>&lt;1% blasts</td>
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Modified: 2008 WHO Classification of Tumors of Hematopoietic and Lymphoid Tissue

MDS associated with isolated del(5q)

Blast % in the Subclassification of MDS

- If BM myeloblast % is <5% but there are 2-4% myeloblasts in PB → RAEB – 1
- If BM myeloblast % is <10% and myeloblasts in PB <5%, but Auer rods present → RAEB – 2
- If BM or PB ≥20% → AML with myelodysplasia-related changes
WHO Criteria for AML with Myelodysplasia-Related Features

- \( \geq 20\% \) blood or marrow blasts
- AND
- Previous history of MDS and/or
- MDS-related cytogenetic abnormality and/or
- Multilineage dysplasia (>10% of cells)
- AND absence of
- Prior cytotoxic therapy for an unrelated disease
- AML-related cytogenetic abnormality
Erythroid precursors

Mitotic figure

Blasts

EML with myelodysplasia-related changes

Work-up of a Suspected MDS

- Correlate with CBC (cytopenia or cytosis?)
- Perform an iron stain on aspirate, core or clot section
  - Ringed sideroblasts can be found in
    - Sideroblastic anemia
    - Alcoholism
    - Hyposplenism
    - Lead poisoning
    - Copper deficiency
    - Zinc intoxication
    - Drugs (isoniazid, chloramphenicol)

From Color Atlas of Hematology, E. Glassy
Work-up of a Suspected MDS

- Check laboratory values for
  - Reticulocytes
  - Cooper
  - Vit. B12 and folate
- Correlate with clinical presentation (splenomegaly?, HIV status?, age, sudden or insidious onset of symptoms, medications: cotrimoxazole, zinc containing cold remedies)
- Perform flow cytometry to assess for clonal B-cell population or abnormal T-cells (hairy cell leukemia?, T-cell LGL leukemia?)

Work-up of a Suspected MDS

- Correlate with cytogenetics
  - Fluorescence in situ hybridization for MDS panel (fast turn-around-time 24-48hrs):
    - -7/del7q (D7S486)
    - -5/del5q (EGR1)
    - del20q (D20s108)
    - 12p13 (ETV6)
    - +8 (CEP8)
    - del13q (D13S319)

Case #2

- 31-year old male who was transferred to the hematology/oncology service with chief complaint of pancytopenia, fatigue, and dyspnea on exertion:
- Complete blood count:
  - WBC: 2.9 (4.5-11 K/cumm)
  - Hemoglobin: 7.7 (13.0-17.0 g/dl)
  - Platelets: 62 (140-400 K/cumm)
Diagnosis

Hypercellular marrow with erythroid hyperplasia and megaloblastic changes; no excess blasts seen

Review of laboratory data revealed a B12 deficiency of 77 (211-911 pg/ml).

Comment: Dyspoiesis is a morphologic change that can be due to myelotoxic / myelosuppressive drugs, nutritional deficiency, chronic viral infection, severe or sustained inflammatory conditions, or primary myelodysplastic syndrome (MDS). Diagnosis of MDS may be considered if all secondary causes are excluded.
Conditions Mimicking MDS Clinically and Morphologically

- Viral infections (HIV, Parvovirus B19)
- Vit. B12 and folate deficiency
- Toxic agents (lead, chemotherapy, arsenic, zinc)
- Copper deficiency
- T-cell large granular lymphocytic leukemia
- Hairy cell leukemia
- Medication (e.g. Cotrimoxazole)

Case #3

- 66-year old male with long-standing anemia
- Complete blood count:
  - WBC: 14.6 (4.5-11 K/cumm)
  - Hemoglobin: 7.7 (13.0-17.0 g/dl)
  - Platelets: 274 (140-400 K/cumm)

Patient #3
Patient #3

Leder

Erythroid cluster

H&E
Diagnosis

Hypercellular marrow with erythroid hyperplasia and atypia, including frequent (>15%) ringed sideroblasts.

Review of laboratory data revealed a copper deficiency of 0.55 (0.75-1.45 pg/ml). Vit. B12 and folate levels were normal at 882 pg/ml and 18.9 mcg/L, respectively.
Diagnosis (without clinical or laboratory information)
Hypercellular marrow with erythroid hyperplasia and atypia, including frequent (>15%) ringed sideroblasts

Comment: Dyspoiesis is a morphologic change that can be due to myelotoxic/myelosuppressive drugs, nutritional deficiency, chronic viral infection, severe or sustained inflammatory conditions, or primary myelodysplastic syndrome (MDS). Diagnosis of MDS, specifically refractory anemia with ringed sideroblasts may be considered if all secondary causes are excluded.

Differential Diagnoses of Ringed Sideroblasts
- Myelodysplastic syndrome
- Sideroblastic anemia
- Alcoholism
- Hyposplenism
- Lead poisoning
- Copper deficiency
- Zinc intoxication
- Drugs (isoniazid, chloramphenicol)

Conditions Mimicking MDS Clinically and Morphologically
- Viral infections (HIV, Parvovirus B19)
- Vit. B12 and folate deficiency
- Toxic agents (lead, chemotherapy, arsenic, zinc)
- Copper deficiency
- T-cell large granular lymphocytic leukemia
- Hairy cell leukemia
- Medications (e.g. Cotrimoxazole)
HIV-Infection Related BM Changes

- Plasmacytosis
- Lymphocytosis
- Lymphohistiocytic infiltrate
- Dysplastic features are not clonal!

Hairy Cell Leukemia

- Splenomegaly, cytopenias (mono-cytopenia) and “hairy cells” in blood
- BM with interstitial infiltrate of CD20+ B-cells causing fibrosis
T-Cell Large Granular Lymphocytic Leukemia

T-Cell Large Granular Lymphocytic Leukemia

T-Cell Large Granular Lymphocytic Leukemia
Conclusions

• Diagnosis of MDS can be established if MDS-associated clone is present:
  – Unbalanced: +8, -7/del(7), -5/del(5), del(20q), -Y, i(17q) or t(17p), -13/del(13q), der(11q), del(11q), del(12p) or t(12p), del(9q), idic(X)(q13)
  – Balanced: t(11;16)(q23;p13.3), t(3;21)(q26.2;q22.1), t(1;3)(p36.3;q21.2), t(2;11)(p21;q23), inv(3)(q21q26.2), t(6;9)(p23;q34)

Conclusions (cont’d)

• If no clinical history, laboratory data, or cytogenetics available, but dysplastic features present and no increase in blasts sign out as:
  – Dyspoiesis is a morphologic change that can be due to myelotoxic / myelosuppressive drugs, nutritional deficiency, chronic viral infection, severe or sustained inflammatory conditions, or primary myelodysplastic syndrome (MDS). Diagnosis of MDS may be considered if all secondary causes are excluded and established if an appropriate cytogenetic abnormality is identified.
Conclusions (cont’d)

- If dysplastic features and increase in blasts:
  - Refractory anemia with excess blasts - 1 (RAEB-1)
  - Refractory anemia with excess blasts – 2 (RAEB-2)
  - Acute myeloid leukemia with myelodysplasia-related changes

References


Bone Marrow Failure Syndromes

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The Children's Hospital of Philadelphia
Penn Medicine
Bone Marrow Failure

- Definition
- Criteria
- Classification
- Work up
- Differential Diagnosis including important diagnoses to exclude
- Inherited bone marrow failure syndromes that should be considered
- Other Important tests to consider

Bone Marrow Failure

- Definition:
  - Inability of the marrow to produce circulating mature cells.
  - This may result from:
    - Reduction in number of progenitors and subsequently paucity of differentiated precursors in the periphery:
      - Aplastic anemia
    - Diamond Blackfan anemia
    - Increased number of differentiated precursors but reduction of mature products in the periphery
      - Ineffective hematopoiesis (ie myelodysplastic syndrome)

Aplastic Anemia - Peripheral Blood
Criteria for Diagnosis of Aplastic Anemia

Must have two of the following in the peripheral blood:
- ANC <0.5 x 10^9/L
- Platelet count <20 x 10^9/L
- Reticulocyte count <20 x 10^9/L

Bone marrow must show:
- Biopsy with <25% of normal cellularity for age OR
- 25-50% of normal cellularity for age, with <30% of the cells being hematopoietic

Bone Marrow Pathology, 3rd Edition, Volume 1. Foucar 2010
pg 138
What defines a hypocellular marrow?

- Pancytopenia and a hypocellular/aplastic marrow
  - Hypocellular is considered <25% of the NORMAL cellularity for AGE.
    - Newborn: 80-100% cellularity
    - 1-3 month: 80-100% cellularity
    - Child (>1 year): 50-80% cellularity
    - Adult (30-70 years): 40-70% cellularity
    - Adult (>70 years): ~25% cellularity

- Requires bone marrow biopsy and aspirate
  - Core biopsy is recommended > 2 cm for accurate determination of cellularity
  - Cellularity can be variable

Subcortical marrow is hypocellular

Adapted from Feuer, Bone Marrow Pathology, 3rd Edition, ASCP Press 2010 pg 32

Classification of Aplastic Anemia Based on Absolute Neutrophil Count (ANC)

- Non-severe
  - >500/μL
- Severe
  - 200-500/μL
- Very severe
  - <200/μL
Classification of Aplastic Anemia

<table>
<thead>
<tr>
<th>ACQUIRED (80-90%)</th>
<th>INHERITED (10-20%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic (~70%)</td>
<td>Fanconi anemia</td>
</tr>
<tr>
<td>Secondary (~10-20%)</td>
<td>Dyskeratosis congenita</td>
</tr>
<tr>
<td>Drugs/toxins</td>
<td>Shwachman-Diamond syndrome</td>
</tr>
<tr>
<td>Viruses (HBV, Hepatitis, EBV)</td>
<td>Amegakaryocytic thrombocytopenia</td>
</tr>
<tr>
<td>Autoimmune (SLE)</td>
<td>Familial aplastic anemias</td>
</tr>
<tr>
<td>Hepatitis (non-viral)</td>
<td>Pearson syndrome</td>
</tr>
<tr>
<td>Nutritional deficiencies (Vit B12/Folate)</td>
<td></td>
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<tr>
<td>PNH</td>
<td></td>
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<tr>
<td>Malignant infiltration (tumors)</td>
<td></td>
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<tr>
<td>Normal/infiltration (storage disorders)</td>
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<tr>
<td>Other (morena nervosa, pregnancy)</td>
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</tbody>
</table>

Pathophysiologic Mechanisms of Acquired Aplastic Anemia

- Acquired Aplastic Anemia
  - Iatrogenic - direct toxicity
  - Drugs/toxins
- Idiopathic Aplastic Anemia
  - Immune mediated
  - Progenitor/stem cell numbers are reduced due to cytotoxic T cells producing apoptosis inducing cytokines (TNFa, IFNg).
  - Destruction of hematopoietic stem cells.

Case #1:
55 year old male with cytopenias and splenomegaly
Diagnostic Algorithm for Aplastic Anemia

- Step 1: Establish criteria
- Step 2: Exclude Malignancy
- Step 3: Etiology
- Step 4: Prognostic Data

Hematopoietic Malignancies that Masquerade as Aplastic Anemia

Immuno histochemical Panel

- Acute Leukemia
  - **CD34**
  - Acute lymphoblastic leukemia (TdT)
  - Acute myeloid leukemia (MPO)
- Myelodysplastic syndrome
  - **CD34**
  - Prostaglandin E2

2 year old female with pancytopenia
No lymphadenopathy
History of viral illness and fever
PAS Stain

CD34 highlights clusters of blasts. CD79a, CD19 and TdT were also positive.

Diagnosis:
Hypocellular Precursor B Acute Lymphoblastic Leukemia (B-ALL)

Case #1:
55 year old male with cytopenias and splenomegaly
CD20 Immunohistochemistry

Hypocellular hairy cell leukemia

Positive for:
CD19, CD20, CD103, CD25, CD11c
Case #2

- 14 year old male
- Pancytopenia
- Mild immunodeficiency
- Small stature
- Family history:
  - Mother small stature and history of anemia
  - Father small(ish)
  - 3 brothers normal size

Diagnosis:

- Aplastic marrow, no leukemia seen.

- Recommendations:
  - Flow fish for telomere length
  - Fanconi anemia testing
  - PNH flow cytometry
Inherited Bone Marrow Failure Syndromes

- Primarily (but not always!!!) associated with pediatric and young adult
  - Reports of patients being diagnosed in 5th-8th decades
- Can affect single lineage or multilineage
- Associated with increased risk of developing hematopoietic and other malignancies
  - Important to identify these patients

Who Should Be Screened for Inherited Bone Marrow Failure Syndromes?

- Pediatric, adolescent or young adult
  - Primarily occur in this age group including young adult
- Any congenital abnormalities
  - Congenital abnormalities (including cardiac and renal) are important clues
  - 30-50% of IBMFS patients have NO associated abnormalities
- Family history of aplastic anemia, cytopenias, pulmonary fibrosis, AML or epithelial malignancies
- Pre-transplant workup for AA
  - IBMFS patients cannot undergo standard conditioning and undergo reduced intensity conditioning therapy pretransplant

Inherited Forms Associated with Multilineage Bone Marrow Failure

- Two disorders classically present with aplastic or hypoplastic bone marrow failure involving all hematopoietic lineages:
  - Fanconi anemia
  - Dyskeratosis congenita
- Both of these disorders are associated with a high risk of MDS and AML (in adults also epithelial neoplasms)
Fanconi Anemia

- All racial and ethnic groups are at risk
- 1 in 300 persons in US/Europe are heterozygotes
- Genetically heterogeneous disorder with different mutations of genes encoding diverse DNA repair proteins.
- 13 complement groups are known to date
- Function of FA proteins is to maintain chromosomal stability

Fanconi Anemia

Megaloblastic Changes
Fanconi Anemia

- Clinical findings may include developmental anomalies such as thumb and limb abnormalities, skin pigmentation abnormalities, and abnormalities of the GI, GU, and renal systems.
- Clinical features are highly variable
  - ~50% of people with FA don’t have any obvious developmental or physical abnormalities, however, most FA patients have a cytopenia or pancytopenia

Fanconi Anemia Test

- Best screening method:
- Chromosome Breakage test
- Genetic testing is usually performed if there is a positive chromosome breakage test
Dyskeratosis Congenita

- DC is a rare bone marrow failure syndrome
- Classic triad:
  - abnormal skin pigmentation
  - nail dystrophy
  - oral leukoplakia
- Pancytopenia is the hematologic hallmark of DC
- Median age ~10 years
- 50% of patients develop severe AA and ~90% develop a cytopenia by 40 years

Dyskeratosis Congenita

- Clinical course is variable
- Progressive immunodeficiency
- Hematologic abnormalities in first decade of life, usually after dermatological manifestations have developed
- Bone marrow failure is the principal cause of death in 71% of patients
  - Bone marrow examination is similar to Fanconi Anemia with a gradual aplasia and megaloblastic changes
- Increased risk of developing MDS and AML
- Epithelial malignancies later in life (>30)
Dyskeratosis Congenita

Age dependent Manifestations of Dyskeratosis Congenita

Case #2
Among Patients with BMF Telomere Length Measurement is a Sensitive Method to Identify Dyskeratosis Congenita

Dyskeratosis Congenita

Myelodysplastic Syndrome/ Acute Myeloid Leukemia (MDS/AML) in Dyskeratosis Congenita

http://www.expertreviews.org/
Alogorithm for Congenital Aplastic Anemias

Hypocellular/Aplastic Marrow

Fancconi Anemia
Screen: Chromosome Breakage Test
positive negative
Fancconi Anemia
Non Fancconi Anemia AA
Assess other BM if necessary negative
Acquired AA

Dyskeratosis Congenital
Telomere length assessment:
Flow-FISH
short normal
Assess other BM if necessary negative
Acquired AA

Case Presentation:
15 year old with pancytopenia

May 2005
Normocellular marrow, no blasts, no dysplasia, cytogenetics normal

October 2005
Aplastic Anemia vs. MDS
- Can be very difficult to distinguish
- Aplastic anemia
  - Can see compensatory cellularity
  - Can be associated with macrocytic anemia
  - Can show “stress” dyserythropoiesis
  - Cytogenetic abnormalities can also be seen
    - Monosomy 7 – associated with progression MDS/AML****
    - Trisomy 8 – usually respond to immunosuppressive therapy
- Factors that favor MDS
  - Increase in blasts
  - Dysplasia in non-erythroid lineages (myeloid and megakaryocytic)

Diagnosis: Variably cellular marrow with dyserythropoiesis, see note.
- Note: The marrow markedly hypocellular with areas of hypercellularity. Dyserythropoiesis is present. No megakaryocytic or myeloid dysplasia is seen. No blasts are present. These findings may represent an evolving aplastic anemia, but an underlying MDS cannot be excluded. However, given that the dysplasia is limited to the erythroid lineage and no blasts are present, an evolving aplastic anemia is favored.
December 2005

January 2006

Case #4
22 year old with fatigue and found to have pancytopenia

Bone Marrow Aspirate: Hypocellular with myeloid dysplasia and scattered blasts (3-5%)
Clusters of mononuclear cells with minimal maturation. Immunohistochemical panel is necessary to characterize cells.

CD34

Clusters of CD117 positive mononuclear cells.

CD117
Dilemma

- FISH showed monosomy 7
- Reason for consultation:
  - Aplastic anemia OR
  - Hypocellular MDS
- Factors that favor MDS
  - Myeloid dysplasia
  - Increase in blasts (~5-10%)
    - Blasts seen on aspirate
    - Confirmed CD34 and CD117
    - Flow cytometry showed 5% myeloid blasts

Diagnosis....

- Consistent with hypocellular MDS with increase in blasts, best classified as refractory anemia with excess blasts (RAEB1)
- Recommended tests based on the patient's age
  - Telomere length testing
  - Fanconi testing
  - PNH

Follow up

- Seen by BMF group at CHOP
- Clinical exam
  - Lymphedema in the lower extremities
  - Hypotelorism
  - Long incisor teeth
  - Long tapered fingers
- Diagnosis: Emberger Syndrome
  - Can be associated with other findings specifically congenital deafness
  - AML preceded by MDS with monosomy 7
Case #5 with fatigue and bruising

History
- Diagnosed with acquired idiopathic aplastic anemia
- Placed on corticosteroids and ATG managed for 8 years
- Developed hemolysis and venous thrombosis
  - Coombs negative anemia
Diagnosis: PNH in setting of Aplastic Anemia

- PNH is an **ACQUIRED** clonal disorder of hematopoiesis
- Clone arises by somatic mutation in X-linked phosphatidylinositol glycan class A gene (PIG-A gene)
- Leads to deficiency in GPI anchor, deficiency of GPI linked proteins
- Results in hemolytic anemia, hypercoaguable state, and bone marrow failure.
Classes of PNH

- Classic PNH
  - Clinical evidence of intravascular hemolysis
  - No bone marrow abnormality that is clinically apparent

- PNH associated with another bone marrow failure disorder
  - Intravascular hemolysis with concomitant or previous bone marrow failure
  - Aplastic anemia
  - MDS

- Subclinical PNH
  - No clinical/laboratory evidence of hemolysis
  - Small populations of PNH clone detected by flow
Relationship of PNH with Aplastic Anemia (AA)

- Normal
- PNH

Hemolytic / Classical PNH

AA/ PNH

Cytopenia

Adapted from Neri & Lucattini 1989

Diagnostic Test for PNH
Flow for PNH White Blood Cells
Deficiency of GPI-Linked Proteins
on PNH Granulocytes

Flow Cytometry and Diagnosis of PNH
- High sensitivity flow cytometry
  - Sensitivity 0.01%
  - Traditional PNH flow cytometry sensitivity 1%
- Antibodies targeting GPI linked proteins (CD55 and CD59)
  - Ideally, at least 2 antibodies to 2 different GPI linked proteins on 2 cell lineages should be used to diagnosis PNH
  - Granulocytes/monocytes
  - RBCs
**FLAER Flow Cytometry**

- Newer technique is use of FLAER
  - (proaerolysin variant) that directly binds GPI-anchor and allows direct assessment of GPI anchor expression and is more accurate assessment of GPI deficit

**When to test for PNH**

- Recommended to test PNH at diagnosis of AA and then yearly
  - This is recommended for patients with inherited bone marrow failure syndromes too not just acquired AA
  - PNH clones may have a selective advantage in marrow failure
- Small clones can be seen in AA in up to 40-80% of cases
- Clone sizes are followed and not treated any differently than AA without a clone unless clinical symptoms develop and there is progression to classical PNH or paroxysmal nocturnal haemoglobinuria

**Aplastic Anaemia Patient With Increasing PNH Clone Sizes**

- Graph showing percentage of clone size over time with markers for biochemical haemolysis.
Significance of PNH clones

- AA associated with PNH clones may have a better prognosis and show a better response to immunosuppression.
- MDS has been associated with PNH clones (20% of cases)
- Prognosis of these clones in MDS is controversial although some have shown a better response other groups have not.

Summary

- Hypocellular/aplastic marrows require a vigorous work up
- Hypocellular presentations of malignancies should be excluded
  - ALL, AML, MDS, hairy cell leukemia, T cell LGL
  - Immunohistochemical panel CD34, CD20 and CD3
- Secondary causes of acquired AA should be ruled out
  - Clinical history, laboratory studies

Summary

- Inherited bone marrow failures should be considered
  - pediatric/young patients and those with family history and congenital anomalies
- Screening tests available
  - Fanconi – chromosome breakage test
  - Dyskeratosis Congenita – telomere length assessment FLOW-FISH

Prognostic data
- PNH clone
- Monitor clone in AA at diagnosis and yearly for progression to clinical PNH
Thank You!!!

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Selected References

Selected References
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