Aplastic Anemia (AA) and Pure Red Cell Aplasia (PRCA)

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September 22, 2018

Aplastic Anemia (AA)

Dr. Paul Ehrlich (1888) described AA as a non-malignant hematologic disorder characterized by an injured, markedly hypocellular, and thus ineffective bone marrow


Aplastic Anemia: Epidemiology

- Rare: 2 cases per 1 mn per year in the US and Europe
- 3 times higher in Asia
- Male / Female ratio: 1:1
- Affects all age groups
  - 1st peak at age 20
  - 2nd peak at age 60

Congenital Aplastic Anemia

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Etiology</th>
<th>Diagnostic testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fanconi’s anemia</td>
<td>Mutations in at least 10 different genes</td>
<td>Clastogenic assay using diepoxybutane (DEB) or mitomycin to assay for increased chromosomal breakage</td>
</tr>
<tr>
<td>Dyskeratosis congenita</td>
<td>Mutations in different genes including DKC1 (stabilizes telomerase complex), TERC (telomerase RNA component), TERT (telomerase reverse transcriptase)</td>
<td>Telomere length measurement, sequence analysis of DKC1, TERC, TERT</td>
</tr>
</tbody>
</table>

Skeletal Defects in Fanconi’s Anemia

Dyskeratosis Congenita

Panel A: nail dystrophy
Panel B: tongue leukoplakia
Panel C: reticulated hypopigmentation of the neck

Etiology of Acquired AA

- Idiopathic
- Cytotoxic drugs and radiation
- Drug reaction
  - Anti-seizure agents
  - Antibiotics: chloramphenicol
  - Nonsteroidal anti-inflammatory drugs
  - Anti-thyroid medications
  - Gold
  - Arsenicals
- Toxic chemicals
  - Benzene
  - Solvents
  - Glue vapors

- Viral infections
  - Epstein-Barr virus
  - Seronegative hepatitis (30%)
  - HIV
  - Other herpes viruses
- Immune disorders
  - Eosinophilic fasciitis
  - SLE
  - Graft-versus-host disease
- Miscellaneous
  - PNH
  - Thymoma
  - Pregnancy
  - Anorexia nervosa

Types of Stem Cell Injury in Aplastic Anemia

Hematopoiesis
51 y.o. teacher’s assistant
Referred in 4/17 after she presented with fatigue and pancytopenia, bruising, petechia and "wet purpura"

NEJM, March 28, 2002

Pancytopenia
Reduction in all of the three major cell lineages present in the peripheral blood (white blood cells, red blood cells, platelets)

<table>
<thead>
<tr>
<th>Normal range</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cell count (WBC)</td>
<td>4-10 x1000/µL</td>
</tr>
<tr>
<td>Absolute neutrophil count (ANC)</td>
<td>1-10 x1000/µL</td>
</tr>
<tr>
<td>Hemoglobin (Hgb)</td>
<td>12-18 g/dL</td>
</tr>
<tr>
<td>Platelets (Plts)</td>
<td>140-440 x1000/µL</td>
</tr>
<tr>
<td>Absolute reticulocyte count</td>
<td>0.0230-0.1400 x10⁶ cells/uL</td>
</tr>
</tbody>
</table>

Causes of Pancytopenia
• Non clonal (non malignant)
  – Toxins (alcohol)
  – Nutritional deficiencies (B12)
  – Infections (HIV, tick-born anaplasmosis)
  – Immune (aplastic anemia)
• Clonal (malignancy): most common
  – Acute myeloid leukemia (AML)
  – Myelodysplastic syndrome (MDS)

Diagnostic Test for Patients with Suspected AA
• CBC, Reticulocyte count, blood smear, LFTs
• Flow cytometry for PNH
• Vitamins: B12 (MMA, homocysteine) and folate
• Viral studies: Hepatitis A/B/C, EBV, CMV, HIV, Parvo B19
• Rheumatology work up: ANA and anti-ds DNA
• Younger patients (< 40 years old)
  – Blood chromosomal breakage analysis to r/out Fanconi’s anemia: Diepoxybutane test (DEB test)
  – Blood leukocyte telomere length

Paroxysmal Nocturnal hemoglobinuria (PNH)
• Acquired mutations in the PIG-A gene
• Deficiency of GPI-anchor proteins
• Peripheral blood flow cytometry to detect surface proteins (CD59, CD55, FLAER)
• FLAER is a fluorescently labeled variant of aerolysin that binds directly to the GPI anchor

51 y.o. teacher’s assistant
Referred in 4/17 after she presented with fatigue and pancytopenia, bruising, petechia and "wet purpura"

She received RBC and Plt transfusions and prophylactic antibiotics (acyclovir, levofloxacin; voriconazole not started due to insurance issues)

Work up: normal / negative
  – Normal vitamin B12, serum folate, iron panel
  – Negative: EBV, CMV, HIV, hepatitis B/C, parvovirus B19
  – Blood flow for paroxysmal nocturnal hemoglobinuria (PNH)
Causes of Pancytopenia

- Non clonal (non malignant)
  - Toxins (alcohol)
  - Nutritional deficiencies (B12)
  - Infections (tick-born anaplasmosis)
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  - Acute myeloid leukemia (AML)
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Bone marrow examination

51 y.o. teacher's assistant

- Bone marrow aspiration / biopsy done on 4/14/17
  - Hypocellular marrow with < 5% cellularity
  - Karyotype was normal
- Diagnosis:
  - Very severe aplastic anemia
  - Alloimmunized to platelet transfusions

Bone Marrow Findings

Normal
Patient: Marrow Aplasia

Aplastic Anemia: Pancytopenia in Association with Bone Marrow Hypoplasia / Aplasia

<table>
<thead>
<tr>
<th>Classification</th>
<th>Criteria</th>
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<tr>
<td>Severe (SAA)</td>
<td>BM cellularity &lt; 30% and ≥ 2 of the following:</td>
</tr>
<tr>
<td></td>
<td>• Peripheral blood neutrophil count &lt; 0.5x10^9/L</td>
</tr>
<tr>
<td></td>
<td>• Peripheral blood platelet count &lt; 20x10^9/L</td>
</tr>
<tr>
<td></td>
<td>• Transfusion dependence with peripheral blood reticulocyte count &lt; 60x10^9/L</td>
</tr>
<tr>
<td>Very severe (vSAA)</td>
<td>As above but peripheral blood neutrophil count must be &lt; 0.2x10^9/L</td>
</tr>
<tr>
<td>Non severe or moderate (MAA)</td>
<td>Hypocellular marrow with depression of at least 2/3 hematopoietic lineages in peripheral blood not meeting criteria for severe aplastic anemia</td>
</tr>
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</table>

Aplastic Anemia (AA): Treatment

- Non severe (moderate) AA may be observed or treated with supportive care and mild immunosuppression
- SAA or vSAA require treatment as unless patients successfully treated, over 70% may die within one year

Progress of Immunosuppressive Therapies (IST) for Severe Aplastic Anemia

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<th>Era</th>
<th>Treatment</th>
<th>Response</th>
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<tr>
<td>1960s</td>
<td>corticosteroids</td>
<td>~10%</td>
</tr>
<tr>
<td>1970s</td>
<td>ATG</td>
<td>40-50%</td>
</tr>
<tr>
<td>1980s</td>
<td>ATG+CSA</td>
<td>60-70%</td>
</tr>
<tr>
<td>1960s - 10% survival in 1 year</td>
<td>2010 - 90% survival in 1 year</td>
<td></td>
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IST, bone marrow transplant, supportive care
Supportive Care

- Blood product transfusions
  - Irradiated products
  - Single donor psoralen treated platelets
- Severe neutropenia
  - Antibiotics / antifungals
- IST: Acyclovir / inhaled pentamidine
- Iron chelation

Killick S et al. BJH. Volume 172, Issue 2 January 2016 Pages 187-207

Algorithm for Initial Management of SAA

Stop suspected drug; treat infection

<40 with matched sibling
- Sibling HSCT

>40 or no matched sibling
- Response at 6 months
- Horse ATG plus cyclosporine
- Immunosuppressive therapy (IST)
- Stop cyclosporine
- Long-term follow-up
- HLA typing of the patient and siblings at the time of SAA diagnosis

Phillip Scheinberg et al. ASH 2016;2016:489-520

Bone marrow rather than peripheral stem cells are used due to lower incidence of Graft-versus-Host Disease

Andrea Bacigalupo Blood 2017;129:1428-1436

First-line IST for SAA (EBMT 2001-2010)

Age 1-19 years; n = 870
- 55%

Age 21-40 years; n = 636
- 76%

Age >40 years; n = 226
- 55%

Horse vs Rabbit ATG: Overall Survival


Clonal Evolution of SAA to PNH and MDS and AML after IST

- Clinically relevant PNH: 15-25%
- “PNH-type” cells: up to 67%
- MDS/AML: 5-15% (observation period of 5 to 11.3 years)

Seishi Ogawa Blood 2016;128:337-347

Testing for PNH by blood flow cytometry:
- At the time of diagnosis
- Every 3-6 months during the 1st 2 years
- Yearly after

Nikolai Podoltsev, MD, PhD
Management of Relapsed/Refractory SAA

- Bone marrow aspiration / biopsy done on 4/14/17
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  - Karyotype was normal
- Diagnosis:
  - Very severe aplastic anemia
  - Alloimmunized to platelet transfusions
- Treatment started on 5/12/17
  - Clinical trial at the NIH
  - ATG + Cyclosporine + Eltrombopag

**Eltrombopag (EPAG): Non-peptide TPO Receptor Agonist**

- Orally administered
- FDA approved for chronic immune thrombocytopenic purpura (2008)

**Inflammatory cytokines are elevated in aplastic anemia**

**TPO and EPAG Bind C-MPL at Distinct Sites**

**First-line IST for SAA (EBMT 2001-2010)**

- Age 1-20 years; n = 870 - 52%
- Age 21-40 years; n = 636 - 69%
- Age >40 years; n = 256 - 85%

**51 y.o. teacher's assistant**

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Aplastic Anemia / Pure Red Cell Aplasia

Model of IFNγ-Mediated Bone Marrow Failure: Signaling Inhibition by TPO:IFNγ Heteromers in Human HSPCs

HSPC: Hematopoietic stem / progenitor cell
Alvarado LJ, ASH 2017

Management of Relapsed/Refractory SAA

IST + Eltrombopag for Initial Treatment of SAA


ATG + IST (CsA + Eltrombopag) in Treatment-naive SAA

In treatment-naive SAA, clonal evolution rates similar to IST without EPAG, but longer follow up required to establish late events

Robust Count Recovery among EPAG Responders when Compared to Historic IST Controls

Eltrombopag was FDA approved for patients with SAA who have had an insufficient response to IST in 8/2014.

Desmond et al, Blood, March 20, 2014
*Thomas Winkler, ASH 2017

Phillip Schenberg et al. ASH 2016;2016:489-520

Median Time to Count Recovery

- Neutrophils (ANC) > 500/uL: 48 days
- Red blood cell transfusion independence: 39 days
- Platelets transfusion independence: 32 days

Towersky DM et al, NEJM, May 28, 2016

51 y.o. teacher’s assistant

- CBC normalized by 7/20/17
  - Only 2 RBC transfusions since she was discharged from the NIH (last on 6/8/17)
  - Platelets increased to > 100 on 6/29/17
  - ANC to > 1000 on 7/20/17
- Follow up at NIH on 11/14/17: normal labs and marrow
  - Eltrombopag stopped
  - CSA dose decreased from 200 mg twice a day to 100 mg daily with plans to continue CSA for 18 months

Studies with Eltrombopag

- European (RACE) Phase 3 Trial for SAA:
  - hATG + CSA vs hATG + CSA + Eltrombopag
- Global (SOAR) Phase 2 Trial for SAA:
  - CSA + Eltrombopag
- NIH Extension Trial (cohort 3) for SAA:
  - hATG + CsA + Eltrombopag (day 1 – 6 months)

Karyotype

- Normal female karyotype
- Female karyotype with trisomy 8

In the past several years, important work showed that hematopoiesis is clonal in the majority of idiopathic acquired aplastic anemia patients

Recurring Cytogenetic Abnormalities in Acquired AA

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<th>Prognostic impact</th>
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<td>13%</td>
<td>Favorable response to IST</td>
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<tr>
<td>Monosomy 7/del(7q)</td>
<td>2.0 - 13.3%</td>
<td>Higher risk of progression to MDS or AML</td>
</tr>
<tr>
<td>Trisomy 8</td>
<td>1.3 - 6.7%</td>
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<td>Del(13q)</td>
<td>0.4% - 1.8%</td>
<td>Favorable response to IST, possibly better survival</td>
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<td>Trisomy 6</td>
<td>2.4%</td>
<td>Unknown</td>
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<td>Trisomy 15</td>
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6pUPD: uniparental disomy of the 6p arm; IST: immunosuppressive therapy

Adapted from Shalis et al. EJH 2018; doi: 10.1111/ajh.13153

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6pUPD: uniparental disomy of the 6p arm; IST: immunosuppressive therapy

### Frequently Mutated Genes in Acquired AA

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<tr>
<th>Mutated gene</th>
<th>Incidence</th>
<th>Prognostic impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCOR / BCORL</td>
<td>4.0 - 9.3%</td>
<td>Favorable response to IST, improved PFS and OS</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>5.3 - 8.4%</td>
<td>Poor response to IST, inferior PFS and OS</td>
</tr>
<tr>
<td>ASXL1</td>
<td>6.2 - 8.6%</td>
<td>Poor response to IST, inferior PFS and OS</td>
</tr>
<tr>
<td>PIGA</td>
<td>7.5 - 40%</td>
<td>Favorable response to IST, improved PFS and OS</td>
</tr>
<tr>
<td>TERT</td>
<td>5.6%</td>
<td>Unknown</td>
</tr>
<tr>
<td>AS160</td>
<td>3.3%</td>
<td>Unknown</td>
</tr>
<tr>
<td>JAK2 and JAK3</td>
<td>1.8%</td>
<td>Poor response to IST, inferior PFS</td>
</tr>
<tr>
<td>RUNX1</td>
<td>1.5%</td>
<td>Poor response to IST, inferior PFS and OS</td>
</tr>
<tr>
<td>TP53</td>
<td>1.5%</td>
<td>Poor response to IST, inferior OS</td>
</tr>
<tr>
<td>C3MD1</td>
<td>1.0%</td>
<td>Poor response to IST, inferior OS</td>
</tr>
<tr>
<td>TERT</td>
<td>0.5% - 0.7%</td>
<td>Unknown</td>
</tr>
<tr>
<td>SRSF2</td>
<td>0.6%</td>
<td>Unknown</td>
</tr>
<tr>
<td>U2AF1</td>
<td>0.6%</td>
<td>Unknown</td>
</tr>
<tr>
<td>ERBB2</td>
<td>0.6%</td>
<td>Unknown</td>
</tr>
<tr>
<td>MFL</td>
<td>0.6%</td>
<td>Unknown</td>
</tr>
<tr>
<td>TERT</td>
<td>0.5%</td>
<td>Unknown</td>
</tr>
<tr>
<td>TERF1 or TERF2</td>
<td>N/A</td>
<td>Unknown</td>
</tr>
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IST, immunosuppressive therapy; OS: overall survival; PFS: progression-free survival

### Management of Relapsed/Refractory SAA

- **Hematopoietic stem cell transplantation (HSCT) with histocompatible sibling, consider HSCT if suitable**
- **Non-HSCT options**
  - Echotoxine
  - Veltatherapy
  - Blood transfusion (1-2 units)
  - Granulocyte colony stimulating factor (G-CSF)
  - Experimental anti-tumor necrosis factor therapy (anti TNF-α)

### Danazol Treatment for Telomere Disease

- Telomeres are "mitotic clock"
- Genetic defects of telomere maintenance and repair cause bone marrow failure
- Telomere-mediated genome instability may lead to progression to MDS / AML
- Phase I/II study of danazol 800 mg po daily x 24 months for patients with telomere disease
  - Primary end point: Reduction of telomere attrition
    - qPCR and flow-FISH
  - N=27, stopped due to positive result in 12/12 evaluable pts
  - 11/12 (92%) had gain telomere length
  - Hematological responses in 19/24 (79%) pts at 3 months

**Phillip Scheinberg et al. ASH 2016;2016:489-520**
**AA: Conclusions**

- AA may be congenital or acquired
  - Telomeropathy and Fanconi anemia testing in younger pts
- AA based on neutrophil count may be
  - Very severe, severe or moderate
- Acquired SAA should be treated with
  - sibling HSCT (age < 40)
  - IST (ATG + CSA) + eltrombopag? (no donor or age > 40)
- Eltrombopag
  - Effective as single agent for refractory SAA
  - Together with IST high response rates among newly diagnosed pts
- Clonal evaluation may occur after IST / eltrombopag

**Etiology of Secondary PRCA**

- Thymoma (10% PRCA)
- Hematologic malignancies: chronic lymphocytic leukemia, large granular lymphocyte leukemia, multiple myeloma
- Solid tumors: stomach, breast, lung, renal cell
- Infections: HIV, EBV, viral hepatitis
- Collagen vascular diseases
- Drugs and chemicals
- Post-ABO incompatible bone marrow transplantation
- Autoimmune chronic hepatitis or hypothyroidism

**Pure Red Cell Aplasia**

- Severe anemia and otherwise normal counts
- Reticulocytopenia
- Absence of hemoglobin-containing cells in the otherwise normal marrow or maturation arrest at proerythroblast stage
- Acquired or congenital (Diamond-Blackfan anemia)
- Acquired anemia can be primary (idiopathic) or secondary (due to associated disease, infection or drug)

**3 Pathophysiologic Mechanisms of PRCA**

- Immune mediated
  - Primary PRCA (idiopathic)
  - Secondary PRCA (underlying disorder)
- Parvovirus B19 infection in a susceptible host (10%)
  - Directly cytotoxic to red blood cell precursors
  - Parvovirus B19–related PRCA manifests with anemia only in immunosuppressed patients or patients with shortened red cell survival
  - Test with blood PCR
- Myelodysplasia
  - Fifth disease, “Slapped Cheek” disease

**Hematopoiesis**

**PRCA Treatment**

- Discontinue suspected drugs; treat infection
- Thymomectomy (cure 30%)
- PRCA secondary to parvovirus B19 infection (10%) is treated with intravenous immunoglobulin
- In the absence of clear myelodysplasia or parvovirus B19 infection, PRCA is treated with sequential courses of immunosuppressive agents:
  - Prednisone (30%), CSA (75%), ATG (50%)
  - Oral cyclophosphamide (40%)
- 3 months trial with additional 3-6 months of treatment if there is a response
PRCA: Conclusions

- Isolated anemia with low reticulocyte count
- Marrow shows no RBC precursors, necessary to r/out lymphoma and MDS
- Blood PCR parvovirus B19 infection
- CT chest for thymoma
- Treatment of choice is immunosuppression