Aplastic Anemia: Current Thinking on the Disease, Diagnosis, and Non-Transplant Treatment
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Objectives

- To review a brief history/epidemiology of aplastic anemia
- To review what makes the diagnosis of Aplastic Anemia
- To discuss non-transplant treatment options for newly diagnosed AA
- To discuss non-transplant treatment options for relapsed or refractory AA

Textbook Definition of Aplastic anemia (AA)

- deficiency of all types of blood cells caused by failure of bone marrow development.
- Diagnosis of exclusion
- Most cases are autoimmune and acquired

Wintrobe’s Clinical Hematology

HISTORY & EPIDEMIOLOGY

From where we come…

History

- 1888 Earliest case description of AA by Dr. Ehrlich
- 1899 Anti-lymphocyte serum (immunosuppression) describe by Dr. Metchnikoff
- 1904 Aplastic Anemia coined by Dr. Chauffard
- 1972 First successful bone marrow transplant for AA
- Later 1970s immunosuppressive regimens progressed

Epidemiology

- Precise estimates of number of patients with AA are confounded by imprecision in diagnosis
  - In Europe, Israel, USA ~2-4 cases per 1 million people
  - Higher in Asia ~5-8 cases per 1 million people
- Two age groups for presentation
  - Ages 15-25 years
  - Age >60 years
- Acquired and inherited cases
  - Most acquired are idiopathic
  - Drug or toxins cause are minority
CAUSES & PATHOGENESIS

Drug/Toxin Causes

- Carbamazepine
- Phenytion
- Hydantoins
- Sulfonamidse
- Chloramphenicol
- Phenybutazone
- Indomethacin
- Methimazole
- Propylthiouracil
- Gold
- Arsenicals
- Benzene

What causes acquired AA? (Pathophysiology)

- T cell immune attack at the CD34 progenitor cell

APLASTIC ANEMIA: Pathophysiology

- High quality HSC
- Low quality HSC
- Progenitors

Aplastic Anemia is Bone Marrow Failure

- The bone marrow is the spongy stem cell tissue that produces the blood:
  - Red cells
  - White cells (neutrophils)
  - Platelets

- When all three cell lines are low → Pancytopenia
Bone marrow failure: Overlapping diseases

- Aplastic anemia (acquired)
- Paroxysmal nocturnal hemoglobinuria
- Inherited syndromes
- Hypoplastic MDS
- Myelodysplastic syndrome
- Large granular lymphocyte leukemia
- Pure Red Cell Aplasia
- Myeloproliferative disorders
- (AML)

Aplastic Anemia: Differential Dx

- Congenital disorders
  - Fanconi: all patients < 40yo (DEB, MMC)
  - Others: careful history
- PNH - flow cytometry, LDH
- Hypoplastic MDS – morphology, cytogenetics, CD34 count

Patient History
- Duration of cytopenias (Are pediatric records available?)
- Medications (prescribed and over-the-counter supplements)
- Immunization records
- Exposures

Family History
- Constitutional abnormalities
- Malignancies

Physical examination
- Height (in context of mean parental height)
- Limb abnormalities
- Skin and nail abnormalities (café au lait spots, nail dystrophy, pale patches)

Laboratory
- Peripheral blood
  - Beta-HCG (Consider even if intercourse is not explicitly stated)
  - Complete blood count with differential
  - Reticulocyte counts
  - Chemistries
  - Transaminases and bilirubin, Hepatitis serologies
  - FLAER assay for PNH
  - Chromosome breakage tests, Telomere length, Mutational analysis
- Bone marrow
  - Aspirate and biopsy
  - Flow cytometry (including quantitative CD34)
  - Cytogenetics

Classification of AA: Camitta Criteria

<table>
<thead>
<tr>
<th>Peripheral Blood Cytopenias</th>
<th>Non-severe (Mild) aplastic anemia (not meeting criteria for severe disease)</th>
<th>Severe aplastic anemia (any 2 of 3)</th>
<th>Very-severe aplastic anemia (meets criteria for severe disease and absolute neutrophils &lt; 200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow cellularity</td>
<td>&lt; 25%</td>
<td>&lt; 25%</td>
<td>&lt; 25%</td>
</tr>
<tr>
<td>Absolute neutrophil count</td>
<td>&lt; 500 / μl</td>
<td>&lt; 200 / μl</td>
<td></td>
</tr>
<tr>
<td>Platelet count</td>
<td>&lt; 20,000 / μl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reticulocyte count</td>
<td>&lt; 1.0% corrected or &lt; 60,000 / μl</td>
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</table>

Decision to treat

- Based on disease severity
  - Severe and Very Severe AA require prompt therapy
  - Moderate AA does not necessarily

Natural History of Untreated Disease
Non-severe (Moderate) Aplastic Anemia

- **36 yo Asian male with pancytopenia and fatigue**
  - Counts last normal 6 years ago
  - ANC 85000 (no infections)
  - Hemoglobin 8.9-10.3 (no transfusions)
  - Platelets 51 (no spontaneous bleeding, maybe increased bruising)
  - Bone marrow biopsy 25% cellular, normal cytogenetics, no dysplasia
  - will monitor as all counts safe (monitoring for 5 years and NO change)
  - 1/3 will improve
  - 1/3 will stay moderate (Counts abnormal but may not need treatment)
  - 1/3 will progress to severe aplastic anemia
  - Can consider low dose immunosuppression (like cyclosporine alone)

Severe Aplastic Anemia

- **18 yo male 5’4” presented with non-traumatic retinal hemorrhages and petechiae in palate**
  - WBC 2200 ANC 330
  - Hb 3.6 Plts 10
  - Peripheral smear: no blasts
  - Retic (corrected) 0.7%
  - Biopsy: hypocellular <5%
  - Karyotype normal
  - Therapy started within 1 week of making diagnosis

Inherited versus Acquired

**Acquired Aplastic Anemia**
- Loss of hematopoietic stem cells via immune attack or toxin exposure
- Treated with immunosuppressive therapy or bone marrow transplant
- Can evolve to MDS/AML

**Inherited Aplastic Anemia**
- Genetic disorder
- May only be single lineage cytopenias
- Associated with other syndromic features or phenotypic abnormalities
- Less likely to respond to immunosuppressive therapy
- Can evolve to MDS/AML

Rule out a Congenital Syndrome

- May be seen at time of progression to AML/clonal evolution
- Consider in patients age <30-40 with pancytopenia
  - May have therapeutic implications
- History and physical
  - Family with cytopenias, premature graying, pulmonary fibrosis
  - Short stature, physical abnormalities
- Differential of Aplastic Anemia
  - Fanconi anemia, Dyskeratosis congenita, Diamond-Blackfan anemia; Scwachman-Diamond; congenital Amegakaryocytoses Thrombocytopenia, Thrombocytopenia with absent radii

VERY Severe Aplastic Anemia

- **58 yo female presented with shortness of breath, pale, easy bruising**
  - WBC 1200 ANC 180
  - Hb 7.6 Plts 18
  - Peripheral smear: no blasts
  - Retic (corrected) 0.3%
  - Biopsy: hypocellular <5%
  - Karyotype normal
  - Therapy started within 1 week of making diagnosis

<table>
<thead>
<tr>
<th>MDS</th>
<th>Aplastic Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity</td>
<td>Increased or normal* (15% hypoplastic MDS)</td>
</tr>
<tr>
<td>CD34 count</td>
<td>Normal or increased</td>
</tr>
<tr>
<td>Dyserythropoiesis</td>
<td>Common</td>
</tr>
<tr>
<td>Ringed sideroblasts</td>
<td>Common</td>
</tr>
<tr>
<td>Myelodysplasia or blasts</td>
<td>Common</td>
</tr>
<tr>
<td>Dysplastic megakaryocytes</td>
<td>Common</td>
</tr>
<tr>
<td>PNH population</td>
<td>Rare</td>
</tr>
<tr>
<td>Abnormal karyotype</td>
<td>Common</td>
</tr>
</tbody>
</table>
Useful tests

**Karyotype**
- Karyotype abnormal
  - 19% AA
  - 54% hMDS

**CD34%**
- Normal or increased percentage of CD34+ cells more likely to be hMDS
- Low marrow CD34+ cells more likely AA


**Why it matters to us…**

Time to Response with PNH clone present is shorter

Overall Survival is Shorter if hMDS compared to AA

Telomeres

- Telomeres: regions of repetitive nucleotides at the ends of chromosomes that are there to protect the chromosomes from damage and breakdown.
- Telomere length testing very helpful in inherited AA DKC (very short)
- Reports suggest that telomeres are shorter (not very short) in up to one-third of patients with acquired SAA
- At NIH, telomere lengths measured in the white blood cells of 183 patients treated with IST
- Shorter telomeres not found to predict who would have improved blood counts at 6 months after IST
- Shorter lengths may be associated with late effects such as relapse or clonal evolution to MDS

Scheinberg et al. JAMA, 304 (2010), 1358-64.

Ideal therapy for SAA

- Available to all patients
  - Not limited by age and donor status
- Low toxicity
  - Rapid hematopoietic reconstitution
  - Low risk for graft failure/GVHD/infections
- Reduces or eliminates risk of secondary clonal disease
  - MDS/Leukemia
  - PNH

Now we have the diagnosis, on to…

TREATMENT
Treatment in AA affected by

1. AGE
2. Availability of matched sibling donor

Young patients (<40 years Old)

Severe Aplastic Anemia

With HLA matched sibling

Hematopoietic Cell Transplant

Immunosuppressive Therapy

Response

Clinical follow up

Relapse

Assessment of late effects

Alternative donor transplantation versus repeat immunosuppression

Without HLA matched sibling

Older patients (>40 years Old) or No Suitable Donor

Severe Aplastic Anemia

Immunosuppressive Therapy

Response

Clinical follow up

Relapse

Alternative donor transplantation versus repeat immunosuppression

Assessment of late effects

Allogeneic BMT for SAA

• Type of graft and age are the 2 most important prognostic factors for survival

Data from CIBMTR

YEARS

Probability of Survival

0 20 40 60 80 100

0 1 2 3 4 5 6

P = 0.0001

HLA-identical sibling, ≥20y (N = 844)

HLA-identical sibling, >20y (N = 845)

Unrelated, >20y (N = 114)

Unrelated, ≥20y (N = 244)

Immunosuppressive therapy (IST)

• STANDARD OF CARE:
  • Anti-thymocyte globulin (ATG) (anti lymphocyte globulin ALG)
  • Polyclonal buffed serum from horse or rabbit that has been immunized with human T cells
    – HORSE >RABBIT in USA
  • Often ATG is usually combined with pill immunosuppressants as well- usually CYCLOSPORINE (CsA)
• Metrics to evaluate treatment: response, relapse, survival, clonal evolution (getting MDS, AML, PNH)

NON-TRANSPLANT TREATMENT OF AA
PREPARATION and use of ATG

Efforts to Improve Response

- Change type of ATG
  - Horse vs Rabbit
- Add more IST
  - Other pills or IV chemos

IST

- Antithymocyte globin (ATG) + Cyclosporine (CsA)
  - 60-70% response rate!!
  - Horse (71% response rate) versus Rabbit (43% response rate)
  - 2005-2010 hematologic response at 6 mos (blood counts) 120 patients (60 in each group)

2003 Report of Response rates

<table>
<thead>
<tr>
<th></th>
<th>ATG alone</th>
<th>ATG + CsA</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response Rate</td>
<td>41%</td>
<td>70%</td>
<td>0.015</td>
</tr>
<tr>
<td>Relapse Rate</td>
<td>45%</td>
<td>30%</td>
<td>0.4</td>
</tr>
<tr>
<td>Overall Survival</td>
<td>54%</td>
<td>58%</td>
<td>0.6</td>
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Clonal Evolution
The actuarial probability of malignant diseases was 18% at 11.3 years. It was 8% for MDS or leukemia. The interval from treatment of AA to the diagnosis of MDS or leukemia was 6.6 to 9.5 years.

Kinetics of Response to Therapy

Horse ATG + CsA

Response
- Cumulative incidence of relapse at 3 years = 28%
- Incidence of clonal evolution = 21%
  - Monosomy 7 (=MDS)
  - Leukemia
- Other studies
  - 35% relapse at 5 years
  - Clonal evolution to MDS or PNH as high as 10%

Overall Survival
- 96% at 3 years

Side Effects OF ATG
usually temporary

- Decrease in blood counts further
  - Increased need for transfusions
  - Increased risk of bleeding
  - Increased risk of infection
- Liver toxicity
  - Rise in transaminases (AST/ALT)
- Kidney Toxicity
  - Rise in creatinine

Table 2: Hematologic Response at 3 and 6 Months to Horse ATG and Rabbit ATG

<table>
<thead>
<tr>
<th>Response</th>
<th>Horse ATG (N=60)</th>
<th>Rabbit ATG (N=60)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 3 mos</td>
<td>45-74</td>
<td>20 (11)</td>
<td>0.002</td>
</tr>
<tr>
<td>At 6 mos</td>
<td>56-80</td>
<td>22 (17)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Serum sickness

- Reaction to the horse (or rabbit) proteins
  - Immune complex hypersensitivity reaction
- Flu-ish feeling when getting ATG
- High fevers, flushing, myalgias
- Usually within 4-10 days of ATG
- To reduce the incidence of this, methylprednisolone 1mg/kg should be administered with the ATG and then steroids continue and are tapered over the subsequent month

Side Effects of CsA
usually decrease when drug decreased/ stopped

- GI toxicity (nausea, diarrhea)
- High blood pressure
- Kidney toxicity
- Headaches
- Tremor (can limit driving rarely)
- Infections
- Thickening of gums in mouth
- Increased hirsutism
- Peripheral neuropathy

Addition of MORE IST

- CellCept (mycophenolate mofetil = MMF)
  - hATG + CsA + MMF in 104 pt study
    - Response rate 64% at 6 months
    - Relapse 37% at 4 years
    - Overall survival at 4 years 80%
    - Clonal Evolution 9% at 4 years
- Sirolimus
  - hATG + CsA + Sirolimus in 35 pts study (randomized to hATG + CsA)
    - Response rate (partials only) 51% at 6 months
    - Not as good as hATG + CsA = 62%
- BOTH additional added limited improvements in response over hATG + CsA and slightly more toxicity

Alemtuzumab

- Anti CD52 antibody
  - Immunosuppresses by selectively killing cells that have this cell surface marker

High dose cyclophosphamide
Another form of IST

- High dose = 50mg/kg daily for 4 days (similar to conditioning regimen for BMT)
- 66 patients studied at Hopkins
  - 44 treatment naïve (Response: 31/44 = 71%)
  - 23 refractory to standard ATG/CSA
- Median follow-up 63 months
- Median time to response: 5 (IQR, 2-10) months
- Study at NIH stopped due to toxicity from HiCY
  - Even moderate doses (30 mg/kg) of CY may be too toxic

High Dose Cy for SAA

<table>
<thead>
<tr>
<th>Overall survival</th>
<th>Failure-free survival</th>
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<td><img src="image" alt="Graph" /></td>
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n = 44
n = 23
Response to Immunosuppression

- PNH clone presence predicts:
  - Response to immunosuppressive therapy (IST)
  - Favorable prognosis in patients with aplastic anemia
- HLA DR15- predicts response to IST in hMDS
  - 8.5 x more likely to respond to IST in NIH study
- Shorter telomere lengths by PCR methods have been suggested to show same response rate but more clonal evolution or increased incidence of relapse

Supportive Care

- Central Venous Catheter
  - considered for all patients with AA, given the frequency of phlebotomy, transfusions, and administration of therapeutic medications (PICC, Hickman, Mediport)
- Blood transfusions
  - Irradiated – prevent transfusion associated GVHD
  - Leukofiltered – reduce viral infections and prevent alloimmunization
- Growth factors
  - May provide clinical benefit but do not induce disease remissions
- Infections
  - Granulocyte transfusions – controversial
  - Antibiotics – important

Relapsed or refractory?

- NON Transplant options
  - Repeat ATG– try rabbit if previously given horse– response rates reported ~60% after 2nd dosing
  - ELTROMBOPAG
- Transplant really must be considered
  - Sibling transplant
  - Alternative donor transplant- related or unrelated
- Clinical trials
  - Majority at present focus on transplant or combination with eltrombopag

Eltrombopag

- Thrombopoietic agonist that stimulates platelet production
  - PILL taken orally
  - Increases platelet production by increasing megakaryocytic differentiation and proliferation
- Previously FDA approved for low platelets in chronic ITP and Hepatitis C

Eltrombopag FDA approval 8/2014

- Phase II study of IST refractory SAA pts
- 11/25 patients (44%) had a hematologic response in at least one lineage at 12 weeks
- 9 became plt independent
- 6 with Hb increase (only 3 TI)
- 9 increase ANC
- Minimally toxic
- Unclear durability; ? Cytogenetic abnormalities

Eltrombopag phase II trial

- Clinical benefit but do not induce disease remissions
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Increased hematopoiesis
longer term follow up on total 43 patients

- No change in QoL metrics
- Toxicity profile favorable
- 8 pts developed clonal cytogenetic abnormalities during eltrombopag therapy

Summary for Aplastic Anemia

- Get correct diagnosis and move efficiently to treatment

- SAA can be treated with immunosuppressive therapy or transplant
  - BMT preferred for young patients with matched sibling donor
  - Horse ATG in combination with CsA preferred upfront therapy (over rabbit ATG)
    - Monitor for secondary PNH, MDS, leukemia post IST

- Options for refractory or relapse SAA
  - Eltrombopag
  - Clinical trial
  - Unrelated and mismatched BMT for SAA in setting of clinical trial at specialized center

THANK YOU!