Congenital and Acquired Aplastic Anemia

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HSCT Basics

- Restoration of a properly functioning bone marrow
- Restoration of a properly functioning immune system

Neutropenia

<table>
<thead>
<tr>
<th>Table 9-9. Management of Neutropenic Patient*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Admit to hospital for persistent fever over 101°F.</td>
</tr>
<tr>
<td>2. Obtain appropriate cultures (blood, throat, urine, infected area) and sensitivity.</td>
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<tr>
<td>3. Administer parenteral antibiotics (Chapter 26)</td>
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<tr>
<td>a. If an organism is isolated, 10-14 days' intravenous treatment is required.</td>
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<td>b. If no organism is isolated, antibiotic is continued until fever or neutropenia is resolved.</td>
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<tr>
<td>4. Place on reverse isolation to prevent superinfection with antibiotic-resistant organisms.</td>
</tr>
<tr>
<td>5. Observe strict hand-washing procedures.</td>
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<tr>
<td>6. Wash skin carefully with a Peridex-containing solution before all skin puncture procedures.</td>
</tr>
<tr>
<td>7. Minimize manipulation of skin, oral mucosa, perineum, and rectum; rectal temperatures and enemas are contraindicated.</td>
</tr>
<tr>
<td>8. Treat mouth ulcerations and gingivitis with appropriate systemic antibiotics if secondary bacterial infection is found and 3% hydrogen peroxide-1% alum mouthwash, which usually produces symptomatic relief.</td>
</tr>
</tbody>
</table>
10. Diamond-Blackfan anemia, and severe neutropenia following chemotherapy (the starting dose is 5 µg/kg SC with dose modification according to the patient's absolute neutrophil count). |

*Neutrophil count less than 500 cells/mm³.

Thrombocytopenic Bleeding

Petechiae
Hemorrhagic "Wet Purpura"
Bruises and "Dry Purpura"

Transfusional Iron Overload

- 1 unit PRBCs contains ~ 200 to 250 mg of iron
- No physiologic mechanism exists for iron excretion
- With repeated blood transfusions, iron accumulates
- Signs of iron overload can be seen after 10-20 transfusions

Congenital: Inherited Bone Marrow Failure Syndromes
Inherited Bone Marrow Failure Syndromes

**Pancytopenias**
- Fanconi anemia (FA)
- Dyskeratosis congenita (DC)
- Shwachman Diamond syndrome (SDS)
- Cartilage hair hypoplasia
- Pearson syndrome
- Reticular dysgenesis
- Congenital amegakaryocytic thrombocytopenia
- Familial marrow dysfunction
- Down, Dubowitz, Seckel, or Noonan syndrome

**Single Cytopenias**
- Red blood cells
  - Diamond-Blackfan anemia (DBA)
  - Congenital dyserythropoietic anemia (CDA)
- White blood cells
  - Severe congenital neutropenia (Kostmann)
- Platelets
  - Thrombocytopenia with absent radii (TAR)
  - Leukoerythroblastosis
  - Osteopetrosis

**Fanconi Anemia**

**Clinical presentation**
- Short stature, microcephaly, elfin face
- Thumb and radial anomalies
  - Thenar hypoplasia
  - Clinodactyly of 5th digit
  - Syndactyly
  - Hyperextensible thumbs
  - Absence or hypoplastic radius
- Renal and ureter abnormalities
- Hypogonadism
- Gastrointestinal abnormalities
- Hearing deficit
- No physical abnormalities

**Fanconi Anemia**

**Molecular Biology**
- G2M arrest
- Apoptosis
- Increased DNA breaks
- DNA repair defect
- Chromosomal instability

**Fanconi Anemia**

**Endocrinopathies**
- GH deficiency (not all patients)
- Hypothyroidism
- Glucose intolerance
- Premature menopause
- Decreased fertility, hypogonadism

**Fanconi Anemia**

**Diagnostics**
- Chromosome breaks in lymphocytes (Di-epoxybutane, mitomycin C)
- Flow cytometry (G2/M arrest)
- Fibroblast cultures (chromosome breaks)
- Complementation analysis
- Gene mutation cloning
Aberrations during DNA-replication phase
Broken chromatids in metaphase
Misrepair leads to chromatid interchange figures, quadriradials
Arrest/delay in late S- or G2 phase

Disease Course
- Heterogeneous
- If no treatment
  - Development of pancytopenia; transfusion dependent in 5 to 10 years
- Cytogenetic abnormalities
- MDS, AML (M6)
- Solid tumors: squamous cell carcinoma (head and neck), GU, liver
- Median age of survival 20 yrs; 25% live beyond 31 yrs

Treatment Approaches
- HLA identical BMT
- If no HLA-identical donor
  - Yearly checks
  - Bone marrow morphology, cytogenetics
  - Await development of cytopenias
  - G-CSF
- Androgens ??
- Alternative donor BMT

Overall Survival after BMT
Horowitz MM et al. 1995

BMT in Fanconi Anemia
- Future directions:
  - Avoid irradiation (reduce cancer risk)
  - Improve immunosuppression (reduce GVHD)
  - Avoid previous treatment with androgens, heavy transfusion history
  - Avoid infections (pre-treatment with Voriconazole for 4 wks)
Dyskeratosis Congenita

- Underrecognized in pediatrics
- Physical findings with increasing age
  - Lacey reticulated pigmentation
  - Dysplastic nails
  - Oral leukoplakia
- Autosomal recessive, autosomal dominant, X-linked

Dyskeratosis Congenita

- Short telomere length in all leukocyte subsets (flowcytometry with fluorescence in-situ hybridization FISH)
- Marrow failure 94% at age 40y
- Malignancy 35%
- Treatment similar to Fanconi anemia:
  - HLA-matched stem cell transplant
  - Increased risk of pulmonary and liver fibrosis
  - GCSF?
  - Androgens?

Dyskeratosis Congenita

- SBDS gene mutation 7p12-q11 (95% of patients)
- Chromosome 7 (75%)
  - 40% Isochromosome 7 (7q(i(7q)))
  - Rare in AML, MDS, ALL
  - No progression to MDS or AML
  - 60% Other chromosome 7 abnormalities
    - Monosomy 7
    - Monosomy 7+ i(7q)
    - Del 7q
    - Translocation 7
- Chromosome 20
  - Del(20q)(q12)
  - Rare in SDS, 4% MDS
  - Spontaneous resolution

Shwachman Diamond Syndrome

- Classical triad
  - Exocrine pancreas insufficiency in early infancy
  - Neutropenia (early infancy, skin infections)
  - Metaphyseal dysostosis
- Malnourishment, short stature, developmental delay, protuberant abdomen

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  - Del(20q)(q12)
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SDS Survival

- Probability
- Age in Years
- No Cx
- Aplastic
- Leukemia
- MDS

Nathan & Oski's 2003

Shwachman Diamond Syndrome

- Pancreatic enzyme replacement
- GCSF
- Hematopoietic stem cell transplantation
  - Bone marrow stroma defect?
  - Increased cardiac toxicity

Congenital Amegakaryocytic Thrombocytopenia

- Thrombocytopenia (median age 7 days)
- Develop aplastic anemia (age 5 yrs) 91%, AML (age 17 years) 55%
- No characteristic physical abnormalities
- Absent or abnormal megakaryocytes
- MPL mutation (thrombopoietin receptor)
- Autosomal recessive
- Hematopoietic stem cell transplant (aplastic anemia protocol)

Inherited Bone Marrow Failure Syndromes

- Diamond Blackfan Anemia
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Diamond Blackfan Anemia compared to Transient Erythroblastopenia of Childhood

<table>
<thead>
<tr>
<th></th>
<th>DBA</th>
<th>TEC</th>
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<tbody>
<tr>
<td>Diagnosis (median)</td>
<td>2.5 mo</td>
<td>23 mo</td>
</tr>
<tr>
<td>Age &gt; 1 yr</td>
<td>12%</td>
<td>83%</td>
</tr>
<tr>
<td>Etiology</td>
<td>Inherited</td>
<td>Acquired</td>
</tr>
<tr>
<td>Antecedent History</td>
<td>None</td>
<td>Viral illness</td>
</tr>
<tr>
<td>Abnormal physical findings</td>
<td>24%</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>Red cell adenosine deaminase (ADA)</td>
<td>Increased</td>
<td>Normal</td>
</tr>
<tr>
<td>MCV increased (at dx)</td>
<td>80%</td>
<td>8%</td>
</tr>
<tr>
<td>Hb F increased (at dx)</td>
<td>100%</td>
<td>25%</td>
</tr>
<tr>
<td>i Antigen increased (at dx)</td>
<td>100%</td>
<td>20%</td>
</tr>
</tbody>
</table>
Clinical Features

- Abnormal or triphalangeal thumbs
- Flat thenars

Diamond Blackfan Anemia

- Autosomal dominant
- RPS19, RPS24, RPS17 mutation - 30%
- Encode for ribosomal proteins
- Precise mechanism not elucidated
- Red cell transfusion therapy
- 79% steroid responsive
- 17% steroid non-responsive
- 25% improve ("spontaneous remission")
- Steroids: cushingoid features, fractures, cataracts

Severe Congenital Neutropenia

- Early onset neutropenia (<0.5 x 10^9/L)
  - Exclude cyclic neutropenia
  - Pyogenic infections (fever, gingivitis)
  - Marrow maturation arrest at promyelocytes
  - No characteristic physical abnormalities
- Accelerated apoptosis in promyelocytes
- ELA2 mutation in 50% - autosomal dominant
- Other: WAS, GFI1
- Kostmann syndrome, HAX1 – autosomal recessive

Diamond Blackfan Anemia

- HLA-identical stem cell transplantation
  - Actuarial survival 72 ± 10%
- Alternative donor transplantation
  - Actuarial survival 19 ± 11%
- Cancer up to 50%, median age 23 years
- MDS, Leukemia, Osteosarcoma

Severe Congenital Neutropenia

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  - No characteristic physical abnormalities
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- Other: WAS, GFI1
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Severe Congenital Neutropenia

- >95% respond to GCSF
- 50% reduction in infections

- 21% MDS/AML at 10 years
- Role of GCSF unclear
  - GCSF-unresponsive patients may have higher potential for MDS/AML (40% vs. 10%)
- Consider stem cell transplant for unresponsive patients

Thrombocytopenia and Absent Radii

- Bilateral absence of radii with presence of thumbs
- Shortened humeri and clavicles
- Purpura, petechiae

- Thrombocytopenia 80% <50 x 10^9/L
- Leukemoid reaction
- Absent, decreased, or immature megakaryocytes

Bone Marrow Failure is a Risk Factor for Clonal Evolution

<table>
<thead>
<tr>
<th>FA</th>
<th>DC</th>
<th>DBA</th>
<th>SDS</th>
<th>SCN</th>
<th>CAMT</th>
<th>TAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia MDS</td>
<td>35%</td>
<td>5%</td>
<td>20%</td>
<td>71%</td>
<td>55%</td>
<td>53%</td>
</tr>
<tr>
<td>Solid tumors</td>
<td>Head &amp; neck, gyn, brain</td>
<td>Head &amp; neck</td>
<td>Osteosarcoma</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age at cancer median, range</td>
<td>16 y (0.1-48)</td>
<td>28 y (1.2-44)</td>
<td>23 y (2.4-3)</td>
<td>14 y (2.2-6)</td>
<td>12 y (1.6-17)</td>
<td>5.3 y (0.6-7)</td>
</tr>
<tr>
<td>Cancer by age 40-50y</td>
<td>85%</td>
<td>35%</td>
<td>52%</td>
<td>71%</td>
<td>55%</td>
<td>53%</td>
</tr>
</tbody>
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Acquired Aplastic Anemia

Epidemiology

- Incidence 2 cases/10^6
  (International Aplastic Anemia and Agranulocytosis Study 1980-1984, Israel, Europe)
- Age 15-25 yrs, >60 yrs
- More common in Asia (4-7/10^6)
- No sex or racial differences
- Geographic variation is most likely due to environmental causes

Epidemiology

- Genetic association:
  - HLA-DR2, class II haplotype DRB*1501 is 2x more frequent than in normal population (cyclosporine-responsiveness)
    (Nakao S 1994; Nimer SD 1994; Nakao S 1992)

Etiology

- Determination of the actual cause in an individual patient is virtually impossible
- Idiopathic

Etiology – Secondary

Drugs, toxins, radiation

- Viruses
  - Non-A non-B hepatitis (0.07%; 2-5% of all pts with AA, up to 10% in Asia; male; <20 yrs old)
  - Epstein-Barr virus
  - Flavivirus
    - Arbovirus hemorrhagic fever; dengue
    - Lymphocyte activation, marrow-suppressive cytokine release
  - Cytomegalovirus, Human Herpesvirus 6
  - Graft failure in bone marrow transplant patients
  - Human Immunodeficiency virus (rare)
Etiology - Secondary

- Immune diseases
  - Eosinophilic fasciitis
  - Hypogammaglobulinemia
  - Thymoma
  - Post-transfusion GvHD in immunodeficiencies
- Paroxysmal nocturnal hemoglobinuria
- Myelodysplasia/Myelofibrosis/Osteopetrosis
- Pregnancy

Presentation and Evaluation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>Bleeding</td>
<td>41%</td>
</tr>
<tr>
<td>Anemia</td>
<td>27%</td>
</tr>
<tr>
<td>Infection</td>
<td>5%</td>
</tr>
<tr>
<td>Combination</td>
<td>20%</td>
</tr>
<tr>
<td>Routine examination</td>
<td>8%</td>
</tr>
</tbody>
</table>

Williams DM et al. 1973

Presentation and Evaluation

- CBC
  - Morphology, reticulocyte count
- Bone marrow aspirate and biopsy
  - Morphology, cytogenetics, culture
  - LDH, LFTs, renal parameters
  - Viral serologies
  - PNH studies
  - Chromosome breakage studies
  - HLA typing
  - Autoimmune disease evaluation

Guinan EC, 1997

Presentation and Evaluation

- Severe aplastic anemia
  - Bone marrow cellularity <25%
  - Two of three peripheral blood criteria
    - ANC < 500/cu.mm.
    - Platelets <20,000/cu.mm.
    - Reticulocytes <40,000/cu.mm.
  - No other hematologic disease
- Moderate aplastic anemia
  - Bone marrow cellularity <50%
  - 2 or 3 cytopenias for >6 weeks
    - ANC <1,500/cu.mm.
    - Platelets <100,000/cu.mm.
    - Reticulocytes <40,000/cu.mm.

Camitta BM et al. 1976, Khatib Z et al. 1994

Treatment and Outcomes

- Therapy
  - Bone marrow transplantation
  - Immunosuppression
- Other
  - Growth factors, androgens, steroids, splenectomy
  - New therapeutic approaches
Treatment Algorithm

Severe

<table>
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<tr>
<th>HLA-identical donor</th>
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<tr>
<td>Yes</td>
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<tr>
<td>No</td>
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Moderate

<table>
<thead>
<tr>
<th>Observation (?)</th>
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<tbody>
<tr>
<td>Yes</td>
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<tr>
<td>No</td>
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<thead>
<tr>
<th>HSCT</th>
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<tr>
<td>Non-responder</td>
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<tr>
<td>Responder</td>
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<table>
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<tr>
<th>Allogeneic BMT</th>
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<tr>
<td>Follow for relapse or late clonal disease</td>
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Bone Marrow Transplantation

Alternative donor

- Benesch M et al. 2004
  - Highly purified, positively selected CD34+ stem cells
  - 4 m unrelated, 4 mm unrelated, 1 mm related
  - 8 pts (89%) in CR at 47 months
  - 1 pt died of GVHD

- Woodard P et al. 2003
  - Positively selected CD34+ stem cells
  - 4 haploidentical, 1 unrelated
  - 3 successful, 1-2.5 years
  - 2 graft rejection with positive HLA crossmatches with the donors

Immunosuppressive Therapy

- **“Standard”**
  - Anti-thymocyte globulin (Prednisone)
    - 40 mg/kg/d x 4 days
  - Cyclosporine
    - Therapeutic level until transfusion-independence for 2 months
  - G-CSF or GM-CSF or no growth factor
    - Kojima S et al. 2000: no difference in survival, but difference in time to neutrophil recovery
Table 1. Results at median follow-up of 38 months

<table>
<thead>
<tr>
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<th>ATG/CSA (%)</th>
<th>Cy/CSA (%)</th>
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<tbody>
<tr>
<td>Overall response</td>
<td>13/16 (81)</td>
<td>8/15 (53)</td>
</tr>
<tr>
<td>CR</td>
<td>10 (63)</td>
<td>6 (40)</td>
</tr>
<tr>
<td>PRI</td>
<td>3 (18)</td>
<td>2 (13)</td>
</tr>
<tr>
<td>Relapse</td>
<td>6/13 (46)</td>
<td>2/8 (25)</td>
</tr>
<tr>
<td>Cytogenetic evolution</td>
<td>2/14 (14)</td>
<td>1/12 (8)</td>
</tr>
</tbody>
</table>

Overall responses are shown by type and overall percentage and do not differ between arms. Complete responses have been observed in 6 of 8 responders or 75% in the Cy arm and 10 of the 13 responders or 77% in the ATG arm (40% Cy and 63% ATG, overall complete response rates). No patients remain in the PRd response group. Relapse rates do not differ between arms ($P = .38$) with 6 among 13 responders and 2 among 8 responders relapsing in the ATG and Cy arms, respectively.
Late Clonal Disease

- Rosenfeld S et al. 2003
  - Chromosomal abnormalities 12/122 pts
  - Risk of PNH 10% at 2 yrs, remaining stable for 7 yrs
- Frickhofen et al. 2003
  - PNH 10% at 11 yrs (5 pts, 2 clinical sx)
  - MDS/Leukemia 8%, duration 6.6-9.5 yrs
  - Solid tumor 11%, duration 1-11 yrs

Pathophysiology

- Overlap of hematopoietic defect with paroxysmal nocturnal hemoglobinuria
- T-cell mediated, organ-specific attack of cytotoxic lymphocytes on CD34 hematopoietic stem and progenitor cells.

Paroxysmal nocturnal hemoglobinuria

- PNH
  - Absent or diminished surface expression of proteins with GPI (glycosylphosphatidylinositol) anchors
  - CD55, CD59, CD14, CD66, Campath-1
  - Acquired mutations in the X-linked PIG-A gene
  - Clinical PNH: Hemolysis, bone marrow failure, thromboses

Paroxysmal nocturnal hemoglobinuria

- Sugimori C et al. 2005
  - 122 patients, mean age 56 years
  - High-resolution two color flow cytometry to quantify CD55-CD59- granulocytes and RBCs
  - 68% PNH+ (0.005-23.1% GPI-AP+ cells)
  - Improved response rate in PNH+ pts, independent of magnitude of PNH+ clones (91% vs. 48%)
  - Increased failure-free survival at 5 yrs (64% vs. 12%)
  - No difference in overall survival

Figure 3. Response to immunosuppressive therapy.
New therapeutic approaches

- Anti-IL2 receptor antibody
- Mycophenolate mofetil
- Tacrolimus
- Rapamycin
- Eltrombopag
- Extracorporeal photopheresis
- Alternative bone marrow transplantation