Diagnosis of acquired and inherited Aplastic Anemia

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Aplastic Anemia – Educational Day
Thursday 3/11/2011
Barcelona
 Definitions in Aplastic Anemia

- Pancytopenia
- Persistent, unexplained marrow aplasia
  - Hematopoiesis replaced by fat cells
- No specific marker
  - Diagnosis by exclusion
- Distinction between
  - Diagnostic criteria
  - Criteria for severity
Diagnostic steps in AA

- Confirm the suspicion of diagnosis and exclude other bone marrow failure diseases
- Define the severity of the disease
- Characterize the AA
Aplastic Anemia Diagnosis

Can be difficult due to overlapping with other entities in particular bone marrow failures

Neil Young et al. Blood, online 2006
Peripheral Blood

Full blood counts:
- Pancytopenia
- Anemia is accompanied, by reticulocytopenia
- Macrocytosis is common
- Lymphocyte count is usually preserved
- Early stages isolated cytopenia, particularly thrombocytopenia
- Monocytopenia

Careful examination of the blood film to exclude:
- dysplastic neutrophils
- abnormal platelets
- blasts and other abnormal cells, such as hairy cells, LGL

Fetal hemoglobin (pre-transfusion in children)
*Prognostic factor in pediatric MDS*
Bone Marrow Examinations

Biopsy

A trephine is crucial to assess:
- overall cellularity
- topography of hemopoietic cells
- to exclude an abnormal infiltrate

**Tangential biopsies:** subcortical marrow normally is hypocellular

Bone marrow cellularity is age dependant

### Table 1: Characterization of patients

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number of cases</th>
<th>Male/female</th>
<th>Bone marrow cellularity (%)^a^</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–9</td>
<td>9</td>
<td>6/3</td>
<td>60.0 ± 20.0^b^</td>
</tr>
<tr>
<td>10–19</td>
<td>13</td>
<td>4/9</td>
<td>56.5 ± 4.4</td>
</tr>
<tr>
<td>20–29</td>
<td>12</td>
<td>7/5</td>
<td>54.6 ± 4.6</td>
</tr>
<tr>
<td>30–39</td>
<td>11</td>
<td>4/7</td>
<td>54.6 ± 4.6</td>
</tr>
<tr>
<td>40–49</td>
<td>10</td>
<td>6/4</td>
<td>54.6 ± 18.2</td>
</tr>
<tr>
<td>50–59</td>
<td>9</td>
<td>9/0</td>
<td>52.4 ± 9.5</td>
</tr>
<tr>
<td>60–69</td>
<td>12</td>
<td>6/6</td>
<td>58.3 ± 8.3</td>
</tr>
<tr>
<td>70–79</td>
<td>13</td>
<td>9/4</td>
<td>56.5 ± 8.7</td>
</tr>
<tr>
<td>80–100</td>
<td>11</td>
<td>3/8</td>
<td>41.2 ± 5.9</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>54/46</td>
<td></td>
</tr>
</tbody>
</table>

^a^ Bone marrow cellularity was measured by the image analyzing system and determined by the percentage of cellular marrow, represented by the formula: (area of hematopoietic cells)/(total area of bone marrow examined) × 100 (%).

^b^ Values presented as mean ± S.E.M.

Proliferation stable

Apoptosis: ↑ ageing

**Ogawa et al. Mechanisms of Ageing and Develop 117 (2000) 57-68**
Normal cellularity

Hypocellular bone marrow

Cellularity <10%
Bone Marrow Examinations in AA

- Required:
  - bone marrow aspirate
    - dry-tap: suspicion of a diagnosis other than aplastic anemia
  - trephine biopsy should be done
- Bone marrow
  - hypocellularity (<25%) (rather than aplastic)
    - “hot spots” with dominating erythropoiesis
    - dyserythropoiesis
  - few or no megakaryocytes
  - mast cells
  - lymphoid hyperplasia
  - plasma cells
  - macrophages
Megakaryocytes few or absent
Normal morphology
Increase of spindle shaped mast cells
Worse response to immunosuppressive treatment

Ozdemir et al; J Clin Path 2004,57,107
T lymphoide hyperplasia

CD20

CD3

CD34

MPO
Aplastic anemia in association with a lymphoproliferative neoplasm

Fig. 1. Bone marrow histology of case 1, a 74-year-old patient with AA and a lymphoplasmacytic lymphoma. Hypocellular bone marrow with focal, lymphoplasmacytic infiltration.

Medinger et al. Leuk Res. 2011 Sep 29. [Epub ahead of print]
Hematopoiesis in 3 dimensions: bone marrow architecture visualized by confocal microscopy

Takaku et al. Blood 2010 (116);15
## Severity of the Disease

Definition of disease severity based on peripheral values and bone marrow findings

<table>
<thead>
<tr>
<th>Condition</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe AA</td>
<td>BM cellularity &lt;25%, or 25–50% with &lt;30% residual haemopoietic cells*</td>
</tr>
<tr>
<td>(Camitta <em>et al</em>, 1975)</td>
<td>2/3 of the following:</td>
</tr>
<tr>
<td></td>
<td>Neutrophil count &lt;$0.5 \times 10^9/l</td>
</tr>
<tr>
<td></td>
<td>Platelet count &lt;$20 \times 10^9/l</td>
</tr>
<tr>
<td></td>
<td>Reticulocyte count &lt;$20 \times 10^9/l</td>
</tr>
<tr>
<td>Very severe AA</td>
<td>As for severe AA but neutrophils &lt;0.2 \times 10^9/l</td>
</tr>
<tr>
<td>(Bacigalupo <em>et al</em>, 1988)</td>
<td>Patients not fulfilling the criteria for severe or very severe aplastic anaemia</td>
</tr>
<tr>
<td>Non-severe AA</td>
<td></td>
</tr>
</tbody>
</table>

*Cellularity should be determined by comparison with normal controls (Tuzuner & Bennett, 1994).

The separation between AA and hypoplastic MDS can be problematic

29% of MDS and 13% of AML can be hypocellular

*Tuzuner & Bennett. Leuk Research 1994;18:645-7*

The identification of hypoplastic myeloid neoplasms is compounded by the lack of clear cut diagnostic criteria

*Bennett & Orazi. Haematologica 2009 Feb; 94(2):264-843-70*
## Distinction between Aplastic Anemia and hypoplastic MDS

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>AA</th>
<th>hypoplastic MDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>dyserythropoiesis</td>
<td>sometimes</td>
<td>yes</td>
</tr>
<tr>
<td>abnormal neutrophil</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>dysplastic megakaryocytes</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>fibrosis</td>
<td>no</td>
<td>occasional</td>
</tr>
<tr>
<td>increased blasts</td>
<td>no</td>
<td>Sometimes (ALIPS)</td>
</tr>
<tr>
<td>CD34+ cells in BM</td>
<td>&lt; 1.0%</td>
<td>sometimes increased</td>
</tr>
<tr>
<td>clonality</td>
<td>possible</td>
<td>sometimes</td>
</tr>
<tr>
<td>splenomegaly</td>
<td>absent</td>
<td>occasional</td>
</tr>
</tbody>
</table>

*References*


Bennett & Orazi. *Haematologica* 2009 Feb; 94(2):264-843-70

Hypoplastic MDS

Aplastic Anemia
Cytogenetic investigations

- Due to hypocellular bone marrow frequently insufficient metaphases
- FISH for chromosomes 5 and 7 should be considered
- An abnormal cytogenetic clone does not imply the diagnosis of MDS or AML
- Cytogenetic abnormalities can be present in up to 12% of typical AA patients

Socie et al. Seminars in Hematol 2000;37:91-100
Gupta V et al. BJH 2006;34:95-99
Cytogenetic investigations

Most frequent abnormalities include:

- trisomy 8
- trisomy 6
- 5q-
- anomalies of chromosome 7 and 13

Abnormal cytogenetic clones:

- often are small
- may arise during the course of the disease
- may be transient and disappear spontaneously/after IS

Socie et al. Seminars in Hematol 2000;37:91-100
Gupta V et al. BJH 2006;34:95-99
Cytokine signature profiles in acquired aplastic anemia and myelodysplastic syndromes

N=33    AA
N=57    MDS
N=48    Healthy controls

Hepatitis-associated Aplastic Anemia

- The onset of aplastic anemia occurs 2–3 months after an acute episode of hepatitis
- More common in young males

- In all new diagnosed AA should be performed:
  - Liver function test
  - Blood test for hepatitis A, B, C, EBV, CMV
  - HIV (so far not clear recognised as cause of AA)
### Hepatitis-associated Aplastic Anemia

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>HAA (n=214)</th>
<th>non-HAA (n=3702)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range) years</td>
<td>15 (2–73)</td>
<td>20 (1–94)</td>
<td>0.00001</td>
</tr>
<tr>
<td>Interval diagnosis-treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range) d</td>
<td>46 (1–3788)</td>
<td>62 (1–5477)</td>
<td>0.00001</td>
</tr>
<tr>
<td>Patient gender</td>
<td></td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>Male (%)</td>
<td>145 (68%)</td>
<td>2129 (58%)</td>
<td></td>
</tr>
<tr>
<td>Year of treatment</td>
<td></td>
<td></td>
<td>0.8</td>
</tr>
<tr>
<td>Median (range)</td>
<td>1998 (90–07)</td>
<td>1998 (90–06)</td>
<td></td>
</tr>
<tr>
<td>Severity</td>
<td></td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>vSAA</td>
<td>31 (41%)</td>
<td>459 (31%)</td>
<td></td>
</tr>
<tr>
<td>SAA</td>
<td>12 (16%)</td>
<td>337 (22%)</td>
<td></td>
</tr>
<tr>
<td>nSAA</td>
<td>19 (26%)</td>
<td>454 (31%)</td>
<td></td>
</tr>
<tr>
<td>Missing data</td>
<td>13 (17%)</td>
<td>235 (16%)</td>
<td></td>
</tr>
<tr>
<td>First line therapy</td>
<td></td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>IS/Transplant</td>
<td>75/139</td>
<td>1485/2217</td>
<td></td>
</tr>
<tr>
<td>Median follow up</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surviving patients in days (range)</td>
<td>1506 (244–6317)</td>
<td>1385 (172–6291)</td>
<td></td>
</tr>
</tbody>
</table>

N=3916 AA p

Reported 1990-2007

Prevalence in Europe 5% (n=214)

(Young NS, BJH 1986)
(Mary JY, Blood 1990)

Locasciulli et al. BJH 2010, 149:890-95
AA and Autoimmune Diseases

Associations of AA and other autoimmune disease (AID) have been shown in many case reports

Associated with:

- psoriasis
- rheumatoid arthritis
- autoimmune thyroiditis
- scleroderma
- diabetes type 1
- celiac disease
- systemic lupus erythematosus
- multiple sclerosis

- Sjögren’s syndrome
- autoimmune gastritis
- colitis ulcerosa
- Guillain-Barré
- ITP
- vasculitis
- antiphospholipid syndrome
# AA and Autoimmune Diseases

<table>
<thead>
<tr>
<th>Studies</th>
<th>Prevalence</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cesaro et al.</td>
<td>50/1251, 4%</td>
<td>Multicenter</td>
</tr>
<tr>
<td>Stalder et al.</td>
<td>13/243, 5.3% before AA</td>
<td>One center</td>
</tr>
<tr>
<td></td>
<td>12/243, 4.5% after AA</td>
<td></td>
</tr>
</tbody>
</table>

**Frequency of a concomitant AID is higher in older AA-patients**

**AID can occur at any time before or after the AA**

Age at diagnosis of AA was significantly younger in patients without AID compared to patients with AID (median 20 vs 52 years)
Flow cytometry is the Gold Standard method for Screening and Diagnosis of PNH.
- This is currently best achieved by analysis of GPI-linked antigens.
  - Monoclonal antibodies
  - Fluorescent aerolysin (FLAER)

The ability to detect small PNH clones has implications for defining the disease.
- About 50% are ‘aplastic’ with small clones and no hemolysis

PNH clone size measurements at presentation and serial monitoring should be performed (every 12 months)
Multiparameter flow cytometry analysis of peripheral blood in PNH

Classical AA patient with small PNH clone

PNH patient with large PNH clone
Is the disease an inherited bone marrow failure syndrome?

**high suspicion**

**Family history**
- Other affected members
- History of anemia, macrocytosis, cytopenias
- Malignancies

**Clinical examination**

**Normal:**
- in ‘cryptic’ Dyskeratosis congenita
- may be normal in late onset of Fanconi anemia

**Unusual features:**
- should alert the possibility of a congenital form of aplastic anemia

- **Fanconi anemia**
  - Short stature, café au lait spots and skeletal anomalies
  - Presentation 3 - 14 years
  - Occasionally later presentation
  - (males up to 32 years in females up to 48 years)
  - Young & Alter, (1994)

- **Dyskeratosis congenita**
  - Leukoplakia, nail dystrophy and pigmentation of the skin
  - Presentation 7 years (6m to 26y)
  - Occasionally later presentation by failure to respond to IST
  - Dokal, (2000)
  - Walne & Dokal, (2009)
  - Vulliamy & Dokal (2006)
Screen for inherited disorders

- **Fanconi anemia:**
  - Positive chromosomal breakage test (MMC or DEB) that still represents the diagnostic golden standard.

- **Dyskeratosis congenita**
  - Screening: telomere length
  - Asymptomatic:
    - Frequent association with TERC, TERT mutation
      - (10% all idiopathic forms)
    - Rarely, with TINF2 gene mutation
  - Recognizable phenotype of DC:
    - TINF2, NHP2, NOP10, DKC1 mutation
Should we measure telomere length in patients with acquired AA?

Acquired AA:
about 15% will undergo evolution to a bone marrow malignancy typically MDS or AML
sometimes it occurs years after good hematologic recovery after IST

Rosenfeld S, et al. JAMA 2003; 289: 1130–1135

- Patients with shorter telomeres are at higher risk of malignant transformation
- Shorter average telomere lengths inversely correlated with monosomy 7 at diagnosis
- Clinical implications

Calado et al. Leukemia (2011), 1–8 online pub
Aplastic Anemia
Differential Diagnosis

- Hypocellular MDS
- Hypocellular ALL
- Hairy cell leukemia
- Lymphomas with hypocellular BM
- Mycobacterial infections
- Anorexia nervosa

- Aplastic single-lineage cytopenias
  - Pure red cell aplasia (PRCA)
    - Idiopathic
    - Thymoma
    - B19 parvovirus
  - Pure white cell aplasia
    - Idiopathic
    - T-LGL-leukemia
  - Amegakaryocytic thrombocytopenia (DD ITP)
Aplastic Anemia
Diagnosis Recommendations

All new patients presenting with aplastic anemia should be carefully assessed to:

- confirm the diagnosis and exclude other possible causes of pancytopenia with hypocellular bone marrow
- classify the disease severity using standard blood and bone marrow criteria
- document the presence of associated PNH and cytogenetic clones
- exclude a possible late onset inherited bone marrow failure disorder

The complete treatment algorithm for SAA, EBMT SAA-WP
Can we predict the truly refractory SAA?

In aplastic anemia, predictive markers of response to immunosuppressive therapy have not been well defined.

Some trials evaluated different parameters to predict response after IST but not to define refractoriness:

- The baseline **absolute reticulocyte count** and **absolute lymphocyte count** were defined as a simple predictors of response following IST.
  
  *Scheinberg P et al. Haematologica. 2009 Mar;94(3):348-54*

- Lower **white blood cell count** (< $2.0 \times 10^9$/L) was the most significant predictive marker of better response.
  

- Patients receiving G-CSF, **the lack of a neutrophil response by day 30** was associated with significantly lower response rate and survival.
  
  *Tichelli et al. Blood 2011,117: 4434-4441*
Thank you very much for your attention
Cytokines gene polymorphisms in acquired aplastic anemia

Some patients with acquired AA have polymorphisms which are linked to high production of proinflammatory cytokines, particularly TNF-a and IFN-c


IL-23R SNPs and serum IL-23 level have no apparent impact on susceptibility to AA