Diagnosis of acquired Aplastic Anemia and PNH

Alicia Rovó MD

Aplastic Anemia Diagnosis

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

Alicia Rovó | How do you diagnose AA and PNH?

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
Peripheral Blood

Full blood counts:
- Pancytopenia
- Anemia is accompanied, by reticulocytopenia
- Macrocystosis 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

Aplastic Anemia Diagnosis

- Required:
  - bone marrow aspirate
  - trephine biopsy should be done
Alicia Rovó | How do you diagnose AA and PNH?

Bone Marrow Examinations in AA

- **bone marrow aspirate**
  - dry-tap: suspicion of a diagnosis other than aplastic anemia

- fragments and trails are hypocellular
- prominent fat spaces
- variable amounts of residual hemopoietic cells
- megakaryocytes and granulocytic cells are reduced or absent
- megakaryocytes and granulocytic cells without dysplasia
Alicia Rovó | How do you diagnose AA and PNH?

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 dependent

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number of cases</th>
<th>Mean/min</th>
<th>Bone marrow cellularity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>9</td>
<td>6.0</td>
<td>62±1.8 (4)</td>
</tr>
<tr>
<td>10-20</td>
<td>12</td>
<td>7.0</td>
<td>76±1.4 (4)</td>
</tr>
<tr>
<td>20-30</td>
<td>16</td>
<td>8.0</td>
<td>74±1.2 (4)</td>
</tr>
<tr>
<td>30-40</td>
<td>10</td>
<td>9.0</td>
<td>80±1.3 (4)</td>
</tr>
<tr>
<td>40-50</td>
<td>5</td>
<td>9.0</td>
<td>82±1.4 (4)</td>
</tr>
<tr>
<td>50-60</td>
<td>13</td>
<td>9.0</td>
<td>84±1.5 (4)</td>
</tr>
<tr>
<td>60-70</td>
<td>15</td>
<td>9.0</td>
<td>86±1.5 (4)</td>
</tr>
<tr>
<td>70-80</td>
<td>15</td>
<td>9.0</td>
<td>88±1.6 (4)</td>
</tr>
</tbody>
</table>

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

Normal cellularity

Cellularity <10%

Hypocellular bone marrow

Proliferation stable
Apoptosis: ↑ ageing
Alicia Rovó | How do you diagnose AA and PNH?

**Bone Marrow Examinations in AA**

- 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
Alicia Rovó | How do you diagnose AA and PNH?

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

EBMT Severe Aplastic Anaemia and Complications QoL WP | Budapest, Hungary | 1-3 November 2012
Alicia Rovó | How do you diagnose AA and PNH?

Aplastic anemia in association with a lymphoproliferative neoplasm

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

Medinger et al. Leuk Res. 2012 Feb;36(2):250-1

Severity of the Disease

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

<table>
<thead>
<tr>
<th>Severe AA (SAA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least two of the following three criteria have to be fulfilled:</td>
</tr>
<tr>
<td>- Reticulocytes &lt;60x10^9/L (using an automated analyzer) or &lt; 20 x 10^9/L (manual count)*</td>
</tr>
<tr>
<td>- Platelets &lt; 20x10^9/L</td>
</tr>
<tr>
<td>- Neutrophil count &lt;0.5 x10^9/L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Very severe AA (vSAA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Same criteria of SAA have to be fulfilled; but the neutrophil count has to be &lt; 0.2 x10^9/L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-severe AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients not fulfilling the criteria for SAA and vSAA.</td>
</tr>
</tbody>
</table>

* The different values are because automated count may over-estimate the counting at low level of reticulocyte counts, i.e. it reads 50x10^9/L but in reality they are less.
Alicia Rovó | How do you diagnose AA and PNH?

### 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>

*Bennett et al. Sem Hemato 2000;37:15-29
Bennett & Orazi. Haematologica 2009 Feb; 94(2):264-843-70
Hama A et al. Rinsho Ketsueki 2011 Aug ;52(8) :653-8*
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

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
**PNH clone and SAA/PNH syndrome**

- 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)
Alicia Rovó | How do you diagnose AA and PNH?

Flow cytometry

- **Flow cytometry**

- **Multiparameter flow cytometry analysis of peripheral blood in PNH**

- **Classical AA patient with small PNH clone**

- **PNH patient with large PNH clone**
PNH clone and SAA/PNH-syndrome

- 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)

Characterization of the Aplastic Anemia

<table>
<thead>
<tr>
<th>Diagnostic tool</th>
<th>Provide information for</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver test</td>
<td>Association with viral hepatitis</td>
</tr>
<tr>
<td>Viral hepatitis studies (serological and DNA/RNA). RNA genome of RNA viruses such as HCV, HDV, HEV and HGV can be qualitatively detected by RT-PCR reaction and DNA of parvovirus B19 and TTv can be detected by Nested PCR. These studies along with EBV, CMV and other viral studies can be performed also on marrow.</td>
<td>Association with an autoimmune disease</td>
</tr>
<tr>
<td>Autoantibody screen (panel according to clinical presentation). Including anti-nuclear antibody and anti-DNA Antibody if systemic lupus erythematosus as underlying disease is suspected</td>
<td>Growing patterns can be useful in the differential diagnosis between marrow failures and MDS</td>
</tr>
<tr>
<td>In vitro colony assay – Results not well standardized. Not available in all centers</td>
<td>Ruled out vitamin deficiency. Ferritin and fibrinogen can be useful if hemophagocytosis syndrome is suspected</td>
</tr>
<tr>
<td>Vitamin B12, folate, iron status, fibrinogen</td>
<td></td>
</tr>
</tbody>
</table>
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

Response criteria of Aplastic Anemia

The response criteria of AA depend in part on the severity of the disease before treatment.

- **Complete remission:**
  - is defined in any case as a normalization of the blood values,
    - neutrophil count ≥1.5 x10^9/L,
    - platelet count ≥150 x10^9/L
    - hemoglobin ≥120 g/L

- **Partial remission:**
  - includes, transfusion independency
  - improvement of the severity degree of the AA,
  - or
    - neutrophil counts increasing ≥0.5 x10^9/L
    - and platelet count ≥20 x10^9/L
  - in case the values were lower before treatment.

- **Non response**
  - persistence of transfusion dependency
  - or values lower than those mentioned above
Alicia Rovó | How do you diagnose AA and PNH?

Diagnostic Challenge in Aplastic Anemia

- Identify AA patients at risk of failing IST
- Identify candidates for early HSCT
- Improving diagnostic standardization of inherited bone marrow failures