Severe Aplastic Anaemia and Infectious Diseases
Working Parties

Bruno Rotoli Memorial
Joint Educational Course

29th Sept – 1st Oct 2014, Naples, Italy

The differential diagnosis of inherited aplastic anemias
Carlo Dufour
Contents

• Differential diagnosis & diagnostic algorithm of inherited Bone Marrow Failure Syndromes (IBMFS)

• **Single lineage**
  - Blackfan Diamond Anemia
  - Severe Congenital Neutropenia
  - Congenital Amegacaryocytic Thrombocytopenia
  - Thrombocytopenia Absent Radii (TAR)

• **More than one lineage**
  - Fanconi Anemia
  - Pearson Syndrome
  - Schwachman Diamond Syndrome
  - Variants of Dyskeratosis Congenita with neonatal onset
Algorythm of most common Bone Marrow Failure Syndromes

Single lineage
- Blackfan Diamond Anemia
- Severe Congenital Neutropenia
- Congenital Amegacaryocytic Thrombocytopenia
- Thrombocytopenia Absent Radii (TAR)

More than one lineage
- Fanconi Anemia
  - Pearson Syndrome
  - Schwachman Diamond Syndrome
  - Variants of Dyskeratosis Congenita with neonatal onset
Differential diagnosis, diagnostic algorithm

If FBC shows:
Cytopenia of one, two or three lineage

Bone marrow aspiration
(morphology, cytogenetics and if possible colony assays & immunophenotype)
and
Bone marrow trephine biopsy

Normal total cellularity, but reduced single lineage

Reduced total cellularity
Normal total cellularity, but a reduced single lineage

\[\downarrow\text{Erythroid line}\]

Blackfan-Diamond Anemia (BDA)
Blackfan-Diamond Anemia

- Disorder of ribosomal synthesis.
- 14 genes identified so far
  - 11 ribosomal
- About 35% of patients are gene orphan (Italian Registry)
- Macrocytic/normocytic anemia at birth or within first 6 mos birth (ca 60%)
- Reticulocytopenia.
- Elevated red cell ADA.
- Normocellular bone marrow with selective erythroid precursor deficiency.
Blackfan-Diamond Anemia (BDA)
Blackfan-Diamond Anemia

- Malformations co-exist in 50% of patients (Italian Registry)
  - Upper limb
  - heart
  - cranio-facial
  - uro-genital
  - mental retardation
  - low stature
Differential Diagnosis

- Transient Erythroblastopenia of Childhood (TEC)

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<th>In active phase</th>
<th>In remission</th>
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<td>DBA</td>
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<td>Previous normal blood counts</td>
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<tr>
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<tr>
<td>Macrocytosis</td>
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<td>Hb F ↑</td>
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<td>Permanent spontaneous remission</td>
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- Autoimmune and post-viral cytopenias

Culture studies ± autologus serum, ± T cells
Viral studies (serology and DNA/RNA)
Quanti/qualitative characterization of RNA by capillary electrophoresis with Agilent Bioanalyzer 2100 - NANO chip
**DIAGNOSTIC TEST**

Distinct 28S ribosomal subunit
Distinct 18S ribosomal subunit

Fluorescence

Time (seconds)

Marker

5S and 5.8S subunits, tRNAs, and small RNA fragments about 100bp
18S/28S Subunit ratio test

Am J Hematol. 2014 Jul 15. doi: [Epub ahead of print]
Exploiting pre-rRNA processing in Diamond Blackfan anemia gene discovery and diagnosis. Farrar JE, Quarello P, et al.
Normal global hematopoietic cellularity, but a reduced single lineage

maturation arrest of myelopoiesis at the promyelocyte/myelocyte stage

Blackfan-Diamond Anemia (BDA)

Severe Congenital Neutropenia (SCN)
Severe Congenital Neutropenia (SCN)
Severe Congenital Neutropenia (SCN)

- Genes associated with maturative block
  
  ELA-2  
  HAX-1  
  G6PC3  
  WAS (exon 9)  
  JAGN1

- Gene associated with no block + physical abnormalities
  
  VPS45,  
  CSF3R

- Genes associated with immune deficiency
  
  GFI1  
  WAS  
  CXCR4 (WHIM)  
  COH (Cohen)  
  BTK X linked, CD40 L (Iper IgM)
Normal global hematopoietic cellularity, but a reduced single lineage

- Blackfan-Diamond Anemia (BDA)
- Severe Congenital Neutropenia (SCN)
- ↓/absent megakaryocytes
- Congenital Amegakaryocytic Thrombocytopenia (CAMT)
  - Thrombocytopenia absent radii (TAR)
Congenital Amegacaryocytic Thrombocytopenia (CAMT)

- Severe thrombocytopenia in neonatal period associated with bleeding

- Normocellular bone marrow with selective deficiency/absence of megacaryocytes

- Normal MPV

- Progressive evolution to pancytopenia and AML.

- High TPOserum level.

- Mutation in the gene of thrombopoietin receptor- c-mpl -that impairs mega and other lineages development.
Thrombocytopenia absent radii
TAR

- Moderate/severe thrombocytopenia in neonatal period
- Not marked bleeding tendency
- Thrombocytopenia tends to improve over time
- No tendency to develop leukemia and aplastic anemia
Normal global hematopoietic cellularity, but reduced single lineage

- ↓ Erythroid line
- maturation arrest of myelopoiesis at the promyelocyte/myelocyte stage
- ↓/absent mega karyocytes

Blackfan-Diamond Anemia (BDA)
Severe Congenital Neutropenia (SCN)
Congenital Amegakaryocytic Thrombocytopenia (CAMT)

Thrombocytopenia absent radii

Memo!
A single lineage disease can, over time, become a global marrow failure (DBA, CAMT, Pearson, SDS)
Sites of neonatal hematopoiesis

Normal hematological values in the neonate

Diagnostic difficulties and limits in the neonate

Algorithm of most common Bone Marrow Failure Syndromes

Single lineage
Blackfan Diamond Anemia
Severe Congenital Neutropenia
Congenital Amegacaryocytic Thrombocytopenia
Thrombocytopenia Absent Radii (TAR)

More than one lineage
Fanconi Anemia
   Pearson Syndrome
   Schwachman Diamond Syndrome
   Variants of Dyskeratosis Congenita with neonatal onset
If FBC shows:
Cytopenia
Of one, two, three lineages

Bone marrow aspiration
(morphology, cytogenetics, immunophenotype, colony assays)
± Bone marrow trephyne biopsy

Normal total cellularity, but reduced single lineage

Reduced total cellularity
TOTAL reduced hematopoietic cellularity

- no major dysplasia
  + normal cytogenetics
  + blasts <5%
  + somatic abnormalities
    • Fanconi Anemia (FA)
    • Pearson Syndrome (PS)
    • Shwachman Diamond Syndrome (SDS)
    • Variants of DKC with neonatal onset (Hoyerdaal-Hreidarsson Syndrome, Revesz Syndrome, Clericuzio Syndrome)
    • Other genetic thrombocytopenias

- Somatic abnormalities
  • CAMT in aplastic phase
  • Reticular dysgenesis
  • Asymptomatic DC

- dysplasia
  +/- abnormal cytogenetics
  +/- ALIP
  +/- fibrosis
  + blasts >5%
  + increased CD 34+
Fanconi Anemia

- Progressive pancytopenia that usually develops in the first decade of life +/- somatic malformations

- **Somatic malformations** diagnostic suspect,
  - esophagus,
  - GI-tract, genitourinary tract,
  - upper limbs,
  - hands/thumb,
  - VACTERL complex (vertebral, anal atresia, cardiac, tracheo-esophageal fistula, renal, limb)

- DNA-repair deficiency disease, cancer proneness, bone marrow failure
- Diagnosis: Chromosomal fragility test (DEB/MMC)
- *NGS*
16 known FA genes

NEW GENES: RAD51C (FANCO), SLX4 (FANCP) and ERCC4 (FANCQ)
Complementation analysis of FA patients

![Graph showing cell survival (%) against MMC (mM) for Control, Fanconi, Fanconi+FA-G, and Fanconi+FA-A categories.]
Pearson Syndrome

- Macrocytic anemia in first months of life, neutropenia and thrombocytopenia,

- Characteristic bone marrow morphology: vacuolated myeloid and erythroid precursors, ringed sideroblasts.

- Deletion of mitochondrial DNA.

- Metabolic acidosis.

- Exocrine pancreas insufficiency, liver and renal failure
Vacuolated myeloid cells
Pearson Syndrome

- Macrocytic anemia in first months of life, neutropenia and thrombocytopenia,

- Characteristic bone marrow morphology: vacuolated myeloid and erythroid precursors, ringed sideroblasts.

- Metabolic acidosis.

- Exocrine pancreas insufficiency, diarrhoea/steatorrhoea.
- Liver and renal failure.

- Deletion of mitochondrial DNA.
Shwachman-Diamond Syndrome

- Neutropenia associated with severe infections (skin, lungs) in first months of life. May fluctuate

- Anemia (Macrocytic) and thrombocytopenia develop in up to 80% of pts.

- Exocrine pancreas insufficiency.

- Skeletal abnormalities (long bone metaphyses, costochondral junction).

- Others: liver, kidney, brain, immune system.

- Mutation analysis
  10% of patients are mutation orphan, monoallelic mutations, variants with no phenotypical consequences.
Hoyeraal-Hreidarsson Syndrome (DKC1)
- IUGR,
- microcephaly,
- cerebellar hypoplasia,
- progressive pancytopenia and immunodeficiency

Revesz Syndrome (*TNF2*)
- Bilateral exudative retinopathy
- CNS calcifications,
- cerebellar hypoplasia

Clericuzio Syndrome (Neutropenia with Poikylooderma (c16orf57))
- Poikylooderma
- Facial dysmorphisms
- Nail dysotrophy
- Neutropenia
Poichiloderma + nail dystrophy + facial dysmorphism
TOTAL reduced hematopoietic cellularity

- no major dysplasia
  - normal cyogenetics
  - blasts <5%

  + somatic abnormalities
  - Somatic abnormalities

  • Fanconi Anemia (FA)
  • Pearson Syndrome (PS)
  • Shwachman Diamond Syndrome (SDS)
  • Variants of DKC with neonatal onset

  • cAMT
  • Reticular dysenesis (hypoplasia thymus)
  • Asymptomatic DC.
TOTAL reduced hematopoietic cellularity

- no major dysplasia
- normal cyogenetics
- NO ALIP
- No fibrosis
- blasts <5%
- reduced CD34+

YES somatic abnormalities
- Fanconi Anemia (FA) (MMC or DEB test)
- Dyskeratosis Congenita (DC)
- TERC, TERt, TNF2, DKC1 gene mutations
- Pearson Syndrome (Mitochondrial DNA)
- Shwachman Diamond Syndrome
- SDS gene mutations
- Blackfan-Diamond Anemia
- (BDA gene mutations)
- Other genetic thrombocytopenias

No Somatic abnormalities
- Aplastic Anemia
- CAMT (cml gene mutations)
- Asymptomatic DC
- (TERC, TERt, TNF2, DKC1 gene mutations)
- FA (MMC or DEB test)

- dysplasia +/-
- abnormal cyogenetics +/-
- ALIP +/-
- fibrosis +
- blasts >5%
- increased CD 34+

Hypocellular congenital MDS
- Runx1, CeBPA, GATA1
Summary 2

Cytopenia

Bone marrow aspiration +/- trephine biopsy

single lineage reduced

- Erythroid line
- maturation arrest of myelopoiesis
- Megakaryocytes

DBA
- eADA

SCN

CAMT
Thrombocytopenia-absent radii

reduced hematopoietic cellularity

no major dysplasia + normal cytogenetic + blasts <5%

+ Somatic abnormalities
- Somatic abnormalities

- FA (DEB test)
- DKC Variants
- HHS
- PS
- SDS
- BDA
- Other genetic thrombocytopenias

- AA
- CAMT
- Reticular dysgenesis

dysplasia +/- abnormal cytogenetics +/- fibrosis + blasts >5% + increased CD 34+

Congenital hypocellular MDS
Take Home Message

- Diagnosis of IMFS may be challenging.
- NGS promising but not fully established at least in many European countries.
- Early diagnosis is important to address subsequent monitoring/treatment strategies.
- Follow-up supported/driven by ped-hem team.