WHO classification of Acute Myeloid Leukemia (AML).

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BHS Training course
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- Definition and classification of AML
- Laboratory diagnosis of AML
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- AML with recurrent genetic abnormalities
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The WHO classification (2008)

- Based on morphology, cytochemistry, immunophenotype and genetic characteristics of the proliferating cells and on clinical findings
- Myeloid, lymphoid and histiocytic/dendritic cell neoplasms
- Precursor and mature cell neoplasms
- AML: precursor myeloid neoplasm
Hematopoiesis

Hoffbrand et al. 2006

Myeloid

- CFU<sub>GEMM</sub> Mixed myeloid progenitor cell
- BFU<sub>E</sub> Erythroid progenitors
- CFU<sub>E</sub> Megakaryocyte progenitor
- CFU<sub>GM</sub> Granulocyte monocyte progenitor
- CFU<sub>M</sub>
- CFU<sub>G</sub>

Lymphoid

- CFU<sub>basa</sub>
- CFU<sub>Eo</sub> Eosinophil progenitor

Pluripotent stem cell

Bone marrow

Blood

- Red cells
- Platelets
- Monocytes
- Neutrophils
- Eosinophils
- Basophils
- Lymphocytes
- NK cell
Definition and classification of AML

- **Clonal expansion of myeloid blasts** in bone marrow, peripheral blood or other tissue
- One or more myeloid lineages involved

- **FAB classification (1976)** – 8 entities (M0 – M7)
  - Based on morphology and cytochemistry
  - AML: ≥ 30 % blasts in blood or bone marrow
Definition and classification of AML

- **WHO 2001**: 16 entities
  - Also *immunophenotype and cytogenetic data* used
  - AML: ≥ 20 % blasts in blood or bone marrow
  - If t(8;21), inv(16), t(16;16) or t(15;17): AML also with < 20 % blasts
  - Acute promyelocytic leukemia: promyelocytes
  - Acute erythroid leukemia:
    - erythroblasts ≥ 50 % of nucleated cells
    - and ≥ 20 % blasts on non-erythroid cells

- **WHO 2008**: 23 entities
  - Also *molecular data* used
Acute leukemia: high number of blasts

Normal BM

Acute leukemia
Laboratory diagnosis of AML

- **Morphology:**
  - BM smears:
    - % blasts, % promonocytes,
    - Cell lineage blasts (granules, Auer rods),
    - Maturation
    - Dysplasia
  - BM biopsy:
    - Cellularity, fibrosis

- **Cytochemistry:**
  - BM smears
    - Cell lineage: myeloperoxidase ($\geq 3\%$), esterases
Laboratory diagnosis of AML

- **Immunophenotyping**: cell suspensions
  - **Cell lineage and maturation stage**: antigen expression reflects normal myeloid differentiation
    - Progenitor cells (Pr): CD34, CD38, HLA-Dr
    - Myeloid cells (My): myeloperoxidase, CD13, CD117, CD33
    - Granulocytes (Gr): CD15, CD65
    - Monocytes (Mo): CD11b, CD11c, CD14, CD4, CD64, CD36, CD68
    - Megakaryocytes (platelet): CD61, CD41, CD42b
    - Erythroid cells (ery): CD235a, CD36, CD71
  - Often aberrant antigen expression (residual disease)
Laboratory diagnosis of AML

- Cytogenetics and molecular methods:
  - Cell suspensions or biopsies
  - Specific entities
  - Prognostic significance
Acute Myeloid Leukaemia and Related Precursor Neoplasms

Acute myeloid leukaemia with recurrent genetic abnormalities

Acute myeloid leukaemia with myelodysplasia-related changes

Therapy-related myeloid neoplasms

Acute myeloid leukaemia, not otherwise specified

Myeloid sarcoma

Myeloid proliferations related to Down syndrome

Blastic plasmacytoid dendritic cell neoplasm
AML, not otherwise specified (NOS)

- AML
  → Without recurrent genetic abnormalities
  → Without MDS related changes
  → Not therapy-related
- Classification reflects the cell lineage involved and the degree of maturation present as evaluated by morphology, cytochemistry and immunophenotype of the leukemic cells
- Is comparable with the FAB classification
- 25 – 30 % of AML
- Number of cases will be reduced when more recurrent genetic abnormalities are defined
AML, not otherwise specified

<table>
<thead>
<tr>
<th>WHO</th>
<th>FAB</th>
<th>Morphology/cytochemistry/immunophenotype</th>
<th>% AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal differentiation</td>
<td>M0</td>
<td>no differentiation, MPO neg (LM), myeloid antigens</td>
<td>1 - 5</td>
</tr>
<tr>
<td>No maturation</td>
<td>M1</td>
<td>blasts ≥ 90% NEC, MPO+ (LM)</td>
<td>5 - 10</td>
</tr>
<tr>
<td>With maturation</td>
<td>M2</td>
<td>≥ 10% neutro, &lt; 20% mono in BM</td>
<td>10</td>
</tr>
<tr>
<td>Myelomonocytic</td>
<td>M4</td>
<td>≥ 20% blast + promono, ≥ 20% neutro, ≥ 20 mono</td>
<td>5 - 10</td>
</tr>
<tr>
<td>Monoblastic</td>
<td>M5A</td>
<td>≥ 80% mono, &lt; 20% neutro, majority of monoblasts</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Monocytic</td>
<td>M5B</td>
<td>≥ 80% mono, &lt; 20% neutro, majority of promonocytes</td>
<td></td>
</tr>
<tr>
<td>Erythroid/Myeloid</td>
<td>M6</td>
<td>≥ 50% erythroblasts, ≥ 20% myeloblasts in NEC</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Pure erytroid</td>
<td>M6</td>
<td>≥ 80% erythroblasts</td>
<td></td>
</tr>
<tr>
<td>Megakaryoblastic</td>
<td>M7</td>
<td>≥ 20% blasts of which ≥ 50% megakaryoblasts</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Basophilic</td>
<td></td>
<td>differentiation to basophils, toluidin blue positif</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>
AML, “not otherwise specified”

Minimal differentiation

No maturation

granules

Auer rod

myeloperoxidase
AML, “not otherwise specified”

With maturation

NCA esterase

Myelomonocytair

neutrophils

monocytes

neutrophils
AML, “not otherwise otherwise specified”

Monoblastic

myeloperoxidase

Alfa-naphtylesterase

myeloblasts

Pure erythroid

Myeloid/erytroid

Megakaryoblastic
AML with recurrent genetic abnormalities

- Genetic abnormalities with **prognostic significance**
- **Balanced translocations or inversions**
  - Fusion gene required but usually not sufficient for leukemogenesis
  - Detection by RT-PCR (high sensitivity)
  - Sometimes characteristic morphology or phenotype
  - t(8;21), inv(16), t(16;16), t(15;17): AML also with < 20% of blasts
  - Therapy-related AML/MDS are excluded
- **Gene mutations:**
  - FLT3, KIT, WT1, NMP1, CEBPA,
  - Also in AML with normal karyotype
## AML with balanced translocations or inversions

<table>
<thead>
<tr>
<th>WHO entity</th>
<th>Fusion gene</th>
<th>FAB</th>
<th>Prognosis</th>
<th>% AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(8;21)(q22;q22)</td>
<td>RUNX1- RUNX1T1</td>
<td>M2</td>
<td>Favourable</td>
<td>5</td>
</tr>
<tr>
<td>inv(16)(p13.1q22)</td>
<td>CBFB-MYH11</td>
<td>M4-eo</td>
<td>Favourable</td>
<td>5 - 8</td>
</tr>
<tr>
<td>or t(16;16)(p13.1;q22)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(15;17)(q22;q12)</td>
<td>PML-RARA</td>
<td>M3, M3v</td>
<td>Favourable</td>
<td>5 - 8</td>
</tr>
<tr>
<td>t(9;11)(p22;q23)</td>
<td>MLLT3-MLL</td>
<td>M4, M5</td>
<td>Intermediate</td>
<td>2</td>
</tr>
<tr>
<td>t(6;9)(p23;q34)</td>
<td>DEK-NUP214</td>
<td>M2, M4</td>
<td>Poor</td>
<td>0.7 - 1.8</td>
</tr>
<tr>
<td>inv(3)(q21q26.2)</td>
<td>RNP-EVI1</td>
<td>M1, M7</td>
<td>Poor</td>
<td>1 - 2</td>
</tr>
<tr>
<td>or t(3;3)(q21;q26.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(1;22)(p13;q13)</td>
<td>RMB15-MKL1</td>
<td>M7</td>
<td>Poor</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>
AML with balanced translocations or inversions

- **Core binding factor (CBF) leukemias:**
  - Heterodimeric transcription factor consisting of CBFalpha (RUNX1, chr 8) and CBFbeta (CBFB, chr 16))
  - Function of CBF is suppressed by t(8; 21) and inv(16) or t(16;16): impaired myeloid differentiation
  - Inv(16) and t(16;16): myelomonocytic leukemia with increase of abnormal eosinophils (M4-eo)

- **Acute promyelocytic leukemia:**
  - t(15;17)(q22; q12): retinoic acid receptor alfa (RARA, chr 17)) fuses with promyelocytic leukemia gen (PML): abnormal promyelocytes
  - Often associated with DIC
AML with recurrent translocations or inversions

- t(15;17) FAB- M3 hypergranular
- Inv(16) M4-eo
- Abnormal eosinophils
- Immature granules
- NCA esterase
- Auer rods
- FAB- M3v microgranular
- Acute promyelocytic leukemia

- MPO

- Immature granules
- Auer rods
AML with recurrent gene mutations

- Gene mutations with prognostic significance
- **FLT3 mutations:**
  - Constitutive activation of tyrosine kinase receptor: increased signal transduction
  - 20–40% AML
    - AML with t(6;9), acute promyelocytic leukemia
    - 30% AML with normal karyotype
  - 75–80% internal tandem duplications (FLT3-ITD)
  - FLT3-ITD+: poor prognosis
- **KIT mutations:**
  - Gain-of-function mutations of this tyrosine kinase: poor prognosis in CBF leukemias
- **WT1 mutations:**
  - Poor prognosis in AML with normal karyotype
AML with recurrent gene mutations

- AML with mutated MPN1: provisional entity
  - Nucleophosmin 1: nucleocytoplasmic protein involved in
    - control of cell proliferation and apoptosis
    - maintenance of genomic stability
  - 27 – 35 % AML in adults
  - 45 - 64 % AML with normal karyotype
  - 5 – 15 % show chromosomal aberrations
  - Usually mutually exclusive of the recurrent balanced translocations and inversions
  - Myelomonocytic and monocytic AML
  - If normal karyotype, NMP1+, FLT3-ITD neg: favourable prognosis
AML with recurrent gene mutations

- **AML with mutated CEBPA: provisional entity**
  - CCAAT/enhancer-binding protein – alfa
  - Transcription factor: control of proliferation and differentiation of myeloid progenitors
  - 6 – 15 % AML
  - 70 % CEBPA+ AML has normal karyotype
  - 30% CEBPA+ AML is also FLT3-ITD+
  - Most often: AML without or with maturation
  - Usually biallelic mutations (2/3)
  - If normal karyotype, biallelic CEBPA+, FLT3-ITD neg: favourable prognosis
Frequencies and distributions of \textit{NPM1}, \textit{CEBPA}, \textit{RUNX1}, \textit{MLL}-PTD and \textit{FLT3}-ITD mutations in \textit{normal-karyotype AML}

- \textit{NPM1}: 45–55%
- \textit{CEBPA}: 9–10%
- \textit{RUNX1}: 6%
- \textit{MLL}-PTD: 7%
- \textit{FLT3}-ITD: 30–33%
Prognostically relevant molecular and cytogenetic subgroups of AML (2009)

- Adverse
  - Other adverse 14%
  - MLL-PTD 6%
  - Inv(3)/t(3;3)/EVI-1 3%
  - FLT-ITD/NPM1 wt 12%
- Favourable
  - t(15;17)/PML-RARA 11%
  - t(8;21)/RUNX1-RUNX1T1 8%
  - Inv(16)/t(16;16)/CBFB-MYH11 5%
  - NPM1 mut/FLT3-ITD negative/WT1 wt 18%
  - CEBPA mut (biallelic)/FLT3-ITD negative 3%

Postgraduate Haematology, Hoffbrand et al
AML with myelodysplasia-related changes

- Are included in this entity:
  - AML arising from previous MDS or MDS/MPN
  - AML with MDS-related cytogenetic abnormality
  - AML with multilineage dysplasia

- Absence of recurrent genetic abnormalities of AML
- No prior cytotoxic or radiation therapy

- Multilineage dysplasia: dysplasia in $\geq 50\%$ BM cells in $\geq 2$ cell lines
- 24 – 35 % AML
- Poor prognosis (except AML with multilineage dysplasia?)
MDS-related cytogenetic abnormalities

<table>
<thead>
<tr>
<th>Complex</th>
<th>Unbalanced</th>
<th>Balanced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greater than three unrelated abnormalities (none of which would qualify a case to be categorized as AML with recurrent genetic abnormality)</td>
<td>–7 or del(7q)</td>
<td>t(11;16)(q23;p13.3)*</td>
</tr>
<tr>
<td></td>
<td>–5 or del(5q)</td>
<td>t(3;21)(q26.2;q22.1)*</td>
</tr>
<tr>
<td></td>
<td>i(17q) or t(17p)</td>
<td>t(1;3)(p36.3;q21.2)</td>
</tr>
<tr>
<td></td>
<td>–13 or del(13q)</td>
<td>t(2;11)(p21;q23)*</td>
</tr>
<tr>
<td></td>
<td>del(11q)</td>
<td>t(5;12)(q33;p12)</td>
</tr>
<tr>
<td></td>
<td>del(12p) or t(12p)</td>
<td>t(5;7)(q33;q11.2)</td>
</tr>
<tr>
<td></td>
<td>del(9q)</td>
<td>t(5;17)(q33;p13)</td>
</tr>
<tr>
<td></td>
<td>idic(X)(q13)</td>
<td>t(5;10)(q33;q21)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t(3;5)(q25;q34)</td>
</tr>
</tbody>
</table>

AML, acute myeloid leukaemia; t-AML, therapy-related AML.
* As long as t-AML is excluded.
AML with multilineage dysplasia

- Hypoplasia
- Dyserythropoiesis
- Micromegakaryocyte
Therapy-related myeloid neoplasms

- t – AML, t – MDS, t – MDS/MPN: unique syndrome
- Late complication of cytotoxic or radiation therapy
- 10 – 20 % of AML, MDS and MDS/MPN
- Most common: 5 – 10 years after therapy
- 20 – 30 %: 1 – 5 years after topoisomerase II inhibitors
- Abnormal karyotype in 90 %; often complex karyotypes with unbalanced aberrations (chromosomes 5 and 7)
- Balanced translocations and inversions of AML may be present
- Poor prognosis
WHO classification of AML

- After cytotoxic therapy:
  - Yes: 15%
  - No

- Recurrent genetic abnormalities:
  - Yes: 30%
  - No

- MDS related changes:
  - Yes: 30%
  - No

- AML NOS:
  - Yes: 25%
  - No: 30%
Literature

- JW Vardiman et al, Blood 2009; 114, 937 – 951
- H Döhner et al, Blood 2010; 115, 453 – 474
- D Grimwade et al, Blood 2010; 116, 354 – 365
Questions?