Lab 2 A and B: Acute Leukemia, MDS, MPN

Hematology Unit
2017
Objectives

Laboratory Instructor will:

1. Review the definition and classification of acute leukemia MDS/MPN
2. Review the clinical, morphological and cytochemical features of these diseases
3. Review the immunophenotypic markers
4. Assist students during self study

Students will:

1. Study the case histories provided for MD Lab 2
2. Examine the pathological material related to each case using virtual microscopy
3. Answer the questions related to each case
Acute Leukemia: Pathophysiology

- Defined by the presence of $\geq 20\%$ blasts in the blood or bone marrow
- Develops when acquired defects result in clonal expansion without significant maturation beyond the blast stage
- Blasts rapidly accumulate in the marrow
- The expansion of blast cells compromises normal hematopoiesis resulting in bone marrow failure:
  - Anemia
  - Neutropenia
  - Thrombocytopenia
- Infiltration of lymph nodes by leukemic cells leading to lymphadenopathy is commonly seen in acute lymphoblastic leukemia
Acute Leukemia: Pathophysiology

Consequences of leukemic transformation:
- Increased proliferation
- Blocked differentiation
- Decreased cell death (decreased apoptosis)
- Bone marrow failure
- Infiltration of organs by leukemic cells
Acute Leukemia: Classification

- Acute leukemia is divided into two broad categories:
  - Acute lymphoblastic leukemia (ALL): consists of blasts of either T cells or B cells
  - Acute Myeloid leukemia (AML) consists of blasts with characteristics of myeloid cells (granulocytes, monocytes, megakaryocytes, erythrocytes)

- Further subclassification is according to World Health Organization (WHO) system which incorporates morphology, cytochemistry, flow cytometry, genetic markers and clinical features
AML Morphology

Auer Rod: Diagnostic of AML

Figure 13.4 Continued (g) Erythroid showing preponderance of erythroblasts; (h) megakaryoblastic showing cytoplasmic blebs on blasts.

Figure 13.4 Morphological examples of acute myeloid leukaemia. (a) Blast cells without differentiation show few granules but may show Auer rods, as in this case; (b) cells in differentiation show multiple cytoplasmic granules; or (c) M1 blast cells contain prominent granules or multiple Auer rods; (d) myelomonocytic blasts have some monocytoid differentiation; (e) monoblastic leukaemia in which >80% of blasts are monoblasts; (f) monocytic with <80% of blasts monoblasts.
AML: Cytochemistry

Myeloperoxidase (MPO): Specific for myeloid differentiation

Sudan Black: Positive in myeloid cells (Courtesy of: www.hmds.org.uk/aml.html)

Non-specific esterase (NSE): Positive in monocyte lineage
Acute Promyelocytic Leukemia (AML-M3)

Characterized by:
- Characteristic morphology
- t (15;17) chromosomal translocation
- Disseminated intravascular coagulation
  - Micrangiopathic blood picture (schistocytes)
  - Microvasculature thrombosis (tissue necrosis)

Figure 13.7 Generation of the t(15;17) translocation. The PML gene at 15q22 may break at one of three different breakpoint cluster regions (BCR-1, -2 and -3) and joins with exons 3-9 of the RARα gene at 17q12. Three different fusion mRNAs are generated (termed long (L), variable (V) or short (S)) and these give rise to fusion proteins of different size. In this diagram only the long version resulting from a break at BCR-1 is shown.
Acute Lymphoblastic leukemia

Fast Facts:
- The most common malignant disease of childhood. 75% of cases occur before age 6
- Eighty-five percent of the cases are of B-cell lineage, the rest are T-cells
- The chromosome number in leukemic cells is prognostic: higher is better (>50= hyperdiploidy)
- Overall, 85% of children are now expected to be cured of ALL; adults with ALL have worse prognosis

Figure 17.3 Morphology, cytochemistry and immunophenotyping of acute lymphoblastic leukaemia (ALL). (a) Lymphoblasts show scanty cytoplasm without granules. (b) Lymphoblasts are large and heterogeneous with abundant cytoplasm. (c) Lymphoblasts are deeply basophilic with cytoplasmic vacuolation. (d) Indirect immunofluorescence reveals nuclear terminal deoxynucleotidyl transferase (TdT) (green) and membrane CD10 (orange). (Courtesy of Professor G. Janossy.)
Myeloproliferative Neoplasms

• Definition: Hematopoietic stem cell disorders characterized by proliferation of one or more of the myeloid lineages:
  – Granulocytic
  – Erythroid
  – Megakaryocytic
  – Mast cells
Myeloproliferative Neoplasms

• MPN’s typically present with:
  – Hypercellular bone marrow with maturation of cells
  – Increased numbers of peripheral blood neutrophils, red blood cells and/or platelets
  – Hepatosplenomegaly
  – Each has the potential to progress, resulting in marrow fibrosis and/or acute leukemia
Myeloproliferative Neoplasms

• Classification:
  – Chronic Myelogenous Leukemia
  – Polycythemia Vera
  – Primary Myelofibrosis
  – Essential Thrombocythemia
  – Mastocytosis
Primary Myelofibrosis

Above: Bone marrow biopsy showing loss of normal architecture hematopoietic cells surrounded by increased fibrous tissue (top right: sliver staining)

Right: Peripheral blood film showing tear-drop RBCs characteristic of this diagnosis and a normoblast (as part of leukoerythroblastic picture)
Essential Thrombocythemia (ET)

Bone marrow with increased Megakaryocytes in ET

**Figure 15.8** Peripheral blood film in essential thrombocythaemia showing increased numbers of platelets and a nucleated megakaryocytic fragment.
MDS - General

• Clonal disorder of marrow stem cell
• Occurs mainly in older patients
  – Median age is 68 years.
• Symptoms are secondary to cytopenias
• Hallmark is dysplastic features in the hematopoietic cells.
• Increased risk of blastic transformation
MDS - General

- Bone marrow is normocellular or hypercellular in >90%, but there is failure to produce mature cells.

- Prognostic variables:
  - Blast percent.
  - Number and degree of cytopenias.
  - Cytogenetic Abnormalities.
MDS: WHO Classification

- MDS with Single Lineage Dysplasia (Refractory cytopenia with single lineage Dysplasia)
- Refractory Anemia with ring sideroblasts (RARS)
- Refractory Cytopenia with Multilineage dysplasia (RCMD)
- Refractory anemia with excess blasts (RAEB-1, RAEB-2)
- MDS with isolated 5q del
- MDS Unclassified
Diagnosis of MDS

- Unequivocal evidence of dysplasia in one or more cell lineages
- Abnormality should involve ≥ 10% of the affected lineage
- Careful assessment of percentage of blasts
MDS: Morphological Features

Appearances of the peripheral blood and bone marrow.
(a) Multinucleate polychromatic erythroblasts.
(b) Perls’ stain showing iron overload in macrophages of a bone marrow fragment.
(c) Ring sideroblasts.
(d) White cells showing pseudo-Pelger cells, agranular myelocytes and neutrophils.
(e) Monocytoid cells and an agranular neutrophil.
(f) Mononuclear megakaryocyte.
MDS: Dysplastic Erythropoiesis

Nuclear irregularity, nuclear budding

Ring sideroblast
Dysplastic Megakaryocytes

Mononuclear or binucleated megakaryocytes some with hypogranular cytoplasm
Refractory Anemia with Excess Blasts (RAEB)

- Cytopenias with uni or multilineage dysplasia
  - **Type 1:**
    - <5% blasts in peripheral blood
    - 5-9% blasts in bone marrow
  - **Type 2:**
    - 5-19% blasts in peripheral blood
    - 10-19% blasts in bone marrow
- Risk of progression to AML:
  - Type 1 - 25%
  - Type 2 – 33%