Laboratory Diagnosis of Chronic Leukemias

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What Do you see and report?

- Anemia
- WBC
- PLT
- Lymphocytes
- Prolymphocytes

The leukemia cells found in the blood smear are characteristically small, mature lymphocytes with a narrow border of cytoplasm and a dense nucleus lacking discernable nucleoli and having partially aggregated chromatin, which is highly suspicious to be CLL.

Comment: Immunophenotyping for definitive Dx should be considered.

Lazarchick, J. ASH Image Bank 2001;2001:100175
What do you see & report?

- Anemia
- WBC
- PLT
- Lymphocyte
- Prolymphocytes
- Smudge cells

Maslak, P. ASH Image Bank 2003;2003:100935
Prolymph. Vs Lymph.
### CLL - blood count

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC x $10^9$/L</td>
<td>150</td>
<td>[4-11]</td>
</tr>
<tr>
<td>Hb g/L</td>
<td>98</td>
<td>[120-160]</td>
</tr>
<tr>
<td>MCV fl</td>
<td>87</td>
<td>[79-98]</td>
</tr>
<tr>
<td>Platelets x $10^9$/L</td>
<td>48</td>
<td>[150-450]</td>
</tr>
<tr>
<td>Neuts x $10^9$/L</td>
<td>1.5</td>
<td>[2-7.5]</td>
</tr>
<tr>
<td>Lymphs x $10^9$/L</td>
<td>130</td>
<td>[1.5-4]</td>
</tr>
<tr>
<td>Monos x $10^9$/L</td>
<td>0.5</td>
<td>[0.2-0.8]</td>
</tr>
<tr>
<td>Eos x $10^9$/L</td>
<td>-</td>
<td>[0-0.7]</td>
</tr>
<tr>
<td>Basos x $10^9$/L</td>
<td>-</td>
<td>[0-0.1]</td>
</tr>
<tr>
<td>Smudge Cells x $10^9$/L</td>
<td>28</td>
<td>[0]</td>
</tr>
</tbody>
</table>

**Film Comment:** lymphocytosis with smudge cells: appearances suggest CLL
CLL – new definition

- The requirement for a diagnosis of CLL was modified from a *chronic absolute lymphocytosis 5.000/μl* to an *absolute count of 5.000/μl monoclonal B cells with a CLL immunophenotype in the peripheral blood (PB)*, if there is an absence of disease-related symptoms or cytopenias, or tissue involvement other than BM.

- The B cell count is a continuous variable, and the best thresholds vary around $10-11 \times 10^3/\mu l$ in different studies.
Small Lymphocytic Lymphoma, SLL

A diagnosis of small lymphocytic lymphoma (SLL) is made when lymphadenopathy or splenomegaly because of infiltrating CLL cells is found, with <5000 /µl CLL type cells in the blood.
Diagnosis

- However, in the absence of lymphadenopathy or organomegaly (as defined by physical examination and CT scans), cytopenias, or disease-related symptoms, the presence of fewer than 5000 B-lymphocytes per μL blood is defined as “monoclonal B-lymphocytosis” (MBL).

- **MBL** seems to progress to frank CLL at a rate of 1-2% per year.
Chronic lymphocytic leukemia

- Immunophenotype
  - B-cells (CD19+, dim CD20+)
  - Clonal (dim kappa or lambda)
  - Co-express CD5 and CD23
  - No FMC7
Chronic lymphocytic leukemia

**Prognosis**

- **Heterogenous clinical course**
  - Some patients do not need definitive therapy for years
  - Others have progressive clinical disease despite therapy

- **Markers of poor prognosis**
  - Increased prolymphocytes
    - 10-55%--CLL/PL, CLL with increased prolymphocytes
  - Cytogenetics
  - Zap 70 expression
    - Surrogate marker for whether or not cell of origin is pre or post germinal center
## Mutational status of IgVH genes in CLL

<table>
<thead>
<tr>
<th></th>
<th>Unmutated</th>
<th>Mutated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence</td>
<td>45%</td>
<td>55%</td>
</tr>
<tr>
<td>M:F ratio</td>
<td>11:1</td>
<td>1.1:1</td>
</tr>
<tr>
<td>Morphology</td>
<td>Atypical</td>
<td>Typical</td>
</tr>
<tr>
<td>Trisomy 12</td>
<td>Frequent</td>
<td>Infrequent</td>
</tr>
<tr>
<td>Abnormal 13q14</td>
<td>Rare</td>
<td>Common</td>
</tr>
<tr>
<td>Stage (BiNet)</td>
<td>B&amp;C (2/3)</td>
<td>Stage A (2/3)</td>
</tr>
<tr>
<td>Disease course</td>
<td>Progressive</td>
<td>Stable</td>
</tr>
<tr>
<td>CD38/ZAP70</td>
<td>Expressed</td>
<td>Negative</td>
</tr>
<tr>
<td>Therapy</td>
<td>Required</td>
<td>Not needed</td>
</tr>
<tr>
<td>Response</td>
<td>Poor</td>
<td>Normal</td>
</tr>
<tr>
<td>Survival</td>
<td>Short</td>
<td>Long</td>
</tr>
</tbody>
</table>
CLL-Mixed Type (CLL/PL)

10% < Prolymphocytes < 55%

B. Poopak
What do you see & report?

- Anemia
- WBC
- PLT
- Lymphocyte
- Prolymphocytes

B.Poopak
Morphology Comparison

CLL

PLL

AML

B. Poopak
What do you see & report?

Abnormal Lymphoid cells with hairy projections seen which is suspicious to be Hairy cell leukemia. TRAP & Immunophenotyping for definitive Dx should be considered.
What morphologic alterations are seen in this blood smear field?

The hairy lymph. comes from a person suffering from active infection (without any malignant disease). The granules identify it as a T or NK cell.
Hairy cells
Hairy cells
TRAP Positive Hairy Cells
TRAP positive
Tartrate resistant acid phosphatase
Acid phosphatase staining after incubation with tartrate
Immunophenotype

Clonal B cells that express: CD103, CD25 and CD11c
What do you see?

Medium to large in size
Pale abundant cytoplasm
Prominent fine or coarse cytoplasmic vacuoles

Cytotoxic cells
Granule contents
  Granzyme
  Perforin
Large granular lymphocyte leukemia

- ~2-3% of cases of small lymphocytic leukemias
- Persistent (>6 months) increase in large granular T lymphocytes (2-10X10^9/liter)
- Indolent clinical course
  - Unexplained cytopenias
    - Anemia
    - Neutropenia
  - Splenomegaly
- Associated with autoimmune disease
  - Rheumatoid arthritis
Immunophenotype

- Mature T cells
  - Decreased CD5 expression
  - CD16 expression
- CD8+/CD4-
- CD57+/-
- CD56-/+
**Morphology**

Small to intermediate sized lymphoid cells with non-granular pale, basophilic cytoplasm. Round, oval or irregular nuclei. May have prominent nucleoli. Some have prolymphocytic morphology.
T cell prolymphocytic leukemia (T-PLL)

- 2% of small lymphocytic leukemias in adults over 30 years
- Present with very high or rapidly rising WBC
  - Often over 100 (median 171)
  - Anemia and thrombocytopenia
- Hepatosplenomegaly, lymphadenopathy
- Skin infiltration
- Effusions
  - 21% pleural or pericardial
T-PLL

- Immunophenotype
  - Mature T cells
  - CD4+>CD4+/CD8+>CD8+

- Genetics
  - Inversion 14(q11q32) (80% of patients)
  - Trisomy 8 (70-80% of patients)

- Prognosis
  - Poor
  - Median survival 7.5 months
What do you see?
What morphologic alterations are seen in this blood smear field?
What morphologic alterations are seen in this blood smear field?
Sezary syndrome

- Circulating cutaneous T cell lymphoma
  - Related to Mycosis fungoides which involves the skin (plaque or nodule/tumor)
- Erythroderma, lymphadenopathy
- Rare, disease of adults
- Morphology
  - Small to intermediate in size
  - Cerebroform nuclei
  - >1000 cells/microliter
- Immunophenotype
  - Mature T cells
  - CD7-
  - CD4+/CD8-
What do you see and report?
Adult T cell leukemia/lymphoma

ATLL

- Uncommon in the US but much more common in regions of the world where HTLV-1 is endemic
  - HTLV-1-human T cell lymphotrophic virus
    - Blood borne virus
    - Japan
      - ATLL occurs in approximately 2.5% of HTLV-1 carriers
    - Caribbean
    - Central Africa

- Presentation
  - Acute (leukemic phase)
  - Lymphomatous
  - Chronic
  - Smoldering
Presentation

- Acute Variant
  - Leukemic presentation
    - Elevated WBC
    - Skin rash
    - Generalized lymphadenopathy
    - Hypercalcemia
    - +/- lytic bone lesions

- Morphology
  - Medium to large sized cells
  - Marked nuclear irregularities
    - Floret cells
  - Coarse chromatin
  - Variably prominent nucleoli
What do you see?
What do you see in BMA smear?
Which tests do you request for patient?
## Diagnostic Evaluation of Multiple Myeloma

<table>
<thead>
<tr>
<th>Test</th>
<th>Finding(s) With Myeloma</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBC with differential counts</td>
<td>↓ Hgb, ↓ WBC, ↓ platelets</td>
</tr>
<tr>
<td>Electrolytes</td>
<td>↑ Creat, ↑ Ca+, ↑ Uric acid, ↓ Alb</td>
</tr>
<tr>
<td>Serum electrophoresis with quantitative immunoglobulins</td>
<td>↑ M protein in serum, may have ↓ levels of normal antibodies</td>
</tr>
<tr>
<td>Immunofixation</td>
<td>Identifies light/heavy chain types M protein</td>
</tr>
<tr>
<td>β₂-microglobulin</td>
<td>↑ Levels (measure of tumor burden)</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>↑ Levels (marker for myeloma growth factor)</td>
</tr>
<tr>
<td>24-hour urine protein electrophoresis</td>
<td>↑ Monoclonal protein (<em>Bence Jones</em>)</td>
</tr>
<tr>
<td>Bone marrow biopsy</td>
<td>≥10% plasma cells</td>
</tr>
<tr>
<td>Skeletal imaging</td>
<td>Osteolytic lesions, osteoporosis</td>
</tr>
<tr>
<td>Serum free light chain</td>
<td>↑ Free light chains</td>
</tr>
<tr>
<td>MRI</td>
<td>Evaluation of involvement of disease</td>
</tr>
</tbody>
</table>

Alb = albumin; CBC = complete blood count; Creat = creatinine; Hgb = hemoglobin; MRI = magnetic resonance imaging; WBC = white blood cell

### Diagnosing Multiple Myeloma

#### Three Diagnostic Criteria Required for a Positive Diagnosis of Multiple Myeloma

1. **Monoclonal plasma cells present in the bone marrow ≥10%**
   - Presence of a documented plasmacytoma

2. **Presence of M component in serum and/or urine**

3. **One or more of the following (CRAB criteria):**
   - **Calcium elevation** (serum calcium >11.5 mg/dL)
   - **Renal insufficiency** (serum creatinine >2 mg/dL)
   - **Anemia** (hemoglobin <10 g/dL or 2 g/dL < normal)
   - **Bone disease** (lytic lesions or osteopenia)

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*Monoclonal M spike on electrophoresis IgG >3.5 g/dL, IgA >2 g/dL, light chain >1 g/dL in 24-hour urine sample.*

Immunofixation-Polyclonal Pattern
Immunofixation-
Monoclonal IgG-Kappa
Immunofixation-
Monoclonal IgM-Kappa
Immunofixation-
Monoclonal IgA-Kappa
Urine-Immunofixation
Monoclonal Lambda
MULTIPLE MYELOMA-case

- **Serum free light chains**
  - Free kappa: 16.2 mg/L
  - Free lambda: 3754.1 mg/L
  - Ratio: 0.0

- **Interpretation**
  - Free lambda light chain monoclonal gammopathy

- **Radiology**
  - Diffuse osteolytic lesions in thoracic and lumbar regions with several compression fractures
Serum Free Light Chain Assays:
Polyclonal Ab to sequestered Light Chain epitopes
What do you see?
Characteristic features of WM

- Retinal hemorrhage from hyperviscosity
- BM showing increased numbers of lymphoid and plasmacytoid cells
- BM showing IgM in cytoplasm of lymphoid cells with immunofluorescence

The serum protein electrophoretic pattern is characterized by a tall, narrow peak (*bottom center*) or dense band.
Lymphoplasmacytic Lymphoma and Waldenstrom Macroglobulinemia

- The definition of **WM** and its relationship to **LPL** have also been problematic.
- The 2008 classification adopted the approach of the second International Workshop on WM, which defined WM as the presence of an *IgM monoclonal gammopathy of any concentration associated with BM involvement by LPL*. Therefore, **LPL and WM are not synonymous**, with WM now defined as a subset of LPL.
- The presence of even a large IgM paraprotein in the absence of a LPL is no longer considered WM, and LPL in the absence an IgM paraprotein is not WM.
The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications

Elias Campo, Steven H. Swerdlow, Nancy L. Harris, Stefano Pileri, Harald Stein and Elaine S. Jaffe
WHO classification of tumors of hematopoietic and lymphoid tissues

- Mature B-cell neoplasms
- Mature T-cell and NK-cell neoplasms
- Hodgkin lymphoma
- Histiocytic and dendritic cell neoplasms
- Post-transplantation lymphoproliferative disorders (PTLDs)
<table>
<thead>
<tr>
<th>WHO classification of tumors of hematopoietic and lymphoid tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mature B-cell neoplasms</strong></td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia/small lymphocytic lymphoma</td>
</tr>
<tr>
<td>B-cell prolymphocytic leukemia</td>
</tr>
<tr>
<td>Splenic marginal zone lymphoma</td>
</tr>
<tr>
<td>Hairy cell leukemia</td>
</tr>
<tr>
<td>Splenic lymphoma/leukemia, unclassifiable*</td>
</tr>
<tr>
<td>Splenic diffuse red pulp small B-cell lymphoma*</td>
</tr>
<tr>
<td>Hairy cell leukemia variant*</td>
</tr>
<tr>
<td><strong>Lymphoplasmacytic lymphoma</strong></td>
</tr>
<tr>
<td>Waldenström macroglobulinemia</td>
</tr>
<tr>
<td>Heavy chain diseases</td>
</tr>
<tr>
<td>α Heavy chain disease</td>
</tr>
<tr>
<td>γ Heavy chain disease</td>
</tr>
<tr>
<td>μ Heavy chain disease</td>
</tr>
<tr>
<td><strong>Plasma cell myeloma</strong></td>
</tr>
<tr>
<td>Solitary plasmacytoma of bone</td>
</tr>
<tr>
<td>Extrasosseous plasmacytoma</td>
</tr>
<tr>
<td>Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)</td>
</tr>
<tr>
<td><strong>Nodal marginal zone lymphoma</strong></td>
</tr>
<tr>
<td>Pediatric nodal marginal zone lymphoma*</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
</tr>
<tr>
<td>Pediatric follicular lymphoma*</td>
</tr>
<tr>
<td>Primary cutaneous follicle centre lymphoma</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
</tr>
</tbody>
</table>
Mature B-cell neoplasms, cont

<p>| Diffuse large B-cell lymphoma (DLBCL), NOS |
| T-cell/histiocyte rich large B-cell lymphoma |
| Primary DLBCL of the CNS |
| Primary cutaneous DLBCL, leg type |
| EBV-positive DLBCL of the elderly* |
| DLBCL associated with chronic inflammation |
| Lymphomatoid granulomatosis |
| Primary mediastinal (thymic) large B-cell lymphoma |
| Intravascular large B-cell lymphoma |
| ALK-positive large B-cell lymphoma |
| Plasmablastic lymphoma |
| Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease |
| Primary effusion lymphoma |
| Burkitt lymphoma |
| B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma |
| B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma |</p>
<table>
<thead>
<tr>
<th>Mature T-cell and NK-cell neoplasms</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-cell prolymphocytic leukemia</td>
</tr>
<tr>
<td>T-cell large granular lymphocytic leukemia</td>
</tr>
<tr>
<td>Chronic lymphoproliferative disorder of NK cells*</td>
</tr>
<tr>
<td>Aggressive NK-cell leukemia</td>
</tr>
<tr>
<td>Systemic EBV-positive T-cell lymphoproliferative disease of childhood</td>
</tr>
<tr>
<td>Hydroa vacciniforme-like lymphoma</td>
</tr>
<tr>
<td>Adult T-cell leukemia/lymphoma</td>
</tr>
<tr>
<td>Extranodal NK/T-cell lymphoma, nasal type</td>
</tr>
<tr>
<td>Enteropathy-associated T-cell lymphoma</td>
</tr>
<tr>
<td>Hepatosplenic T-cell lymphoma</td>
</tr>
<tr>
<td>Subcutaneous panniculitis-like T-cell lymphoma</td>
</tr>
<tr>
<td>Mycosis fungoides</td>
</tr>
<tr>
<td>Sézary syndrome</td>
</tr>
<tr>
<td>Primary cutaneous CD30+ T-cell lymphoproliferative disorders</td>
</tr>
<tr>
<td>Lymphomatoid papulosis</td>
</tr>
<tr>
<td>Primary cutaneous anaplastic large cell lymphoma</td>
</tr>
<tr>
<td>Primary cutaneous γδ T-cell lymphoma</td>
</tr>
<tr>
<td>Primary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma*</td>
</tr>
<tr>
<td>Primary cutaneous CD4+ small/medium T-cell lymphoma*</td>
</tr>
<tr>
<td>Peripheral T-cell lymphoma, NOS</td>
</tr>
<tr>
<td>Angioimmunoblastic T-cell lymphoma</td>
</tr>
<tr>
<td>Anaplastic large cell lymphoma, ALK-positive</td>
</tr>
<tr>
<td>Anaplastic large cell lymphoma, ALK-negative*</td>
</tr>
</tbody>
</table>
Post-transplantation lymphoproliferative disorders (PTLDs)

- Early lesions
  - Plasmacytic hyperplasia
  - Infectious mononucleosis–like PTLD
- Polymorphic PTLD
- Monomorphous PTLD (B- and T/NK-cell types)†
- Classical Hodgkin lymphoma type PTLD†
What do you see?

CML
What morphologic alterations are seen in this blood smear field?

Left shift following G-CSF
Left shift following G-CSF
Toxic Granulation
Dohle body
White Blood Cell Differential Count at the Time of Diagnosis in 90 Cases of pH-Chromosome–Positive Chronic Myelogenous Leukemia

<table>
<thead>
<tr>
<th>Percent of Total Leukocytes (Mean Values)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloblasts</td>
<td>3</td>
</tr>
<tr>
<td>Promyelocytes</td>
<td>4</td>
</tr>
<tr>
<td><strong>Myelocytes</strong></td>
<td>12</td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td>7</td>
</tr>
<tr>
<td>Band forms</td>
<td>14</td>
</tr>
<tr>
<td><strong>Segmented forms</strong></td>
<td>38</td>
</tr>
<tr>
<td>Basophils</td>
<td>3 - Flow cytometry using <strong>anti-CD203c</strong> provides very accurate assessment of the basophil frequency</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>2</td>
</tr>
<tr>
<td>Nrbc</td>
<td>0.5</td>
</tr>
<tr>
<td>Monocytes</td>
<td>8</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>8- The total absolute lymphocyte count is increased (mean: approximately 15 x 10⁹/L) in patients with CML at the time of diagnosis as a result of the balanced increase in T-helper and T-suppressor cells. B lymphocytes are not increased</td>
</tr>
</tbody>
</table>

**mean Hct: 31 %
mean WBC: 160 x 10⁹/L,
mean PLT: 442 x 10⁹/L at the time of diagnosis.**
Chronic Myeloid Leukemia and other Myeloproliferative Neoplasms (MPNs)

What do you request for patients with Dx of MPN?

1. CBC
2. BMA (?)
3. Cytochemistry (LAP Score)
4. Cytogenetics (Ph chromosome)
5. Molecular Genetics (*BCR-ABL*, *Jak2 Mutation*, *MPL Mutation*)
MPD - concepts

- **Neoplastic** (clonal) disorders of hemopoietic stem cells
- Over-production of all cell lines, with usually one line in particular
- Fibrosis is a secondary event
- Acute Myeloid Leukemia may occur
Hematopoietic Progenitors and MPNs

Genetic Mutation

Blood stem cell

Myeloid stem cell

Myeloblast

Lymphoid stem cell

Lymphoblast

Red blood cells
Platelets

White blood cells

National Cancer Institute
More Definitions

The type of disorder is often based on the predominant cell line that is affected, but because blood counts are often abnormal in more than one cell line, diagnoses based upon blood counts alone may be inaccurate.

Four Main MPNs:
1. Chronic Myelogenous Leukemia (CML)
2. Polycythemia Vera (PV)
3. Essential Thrombocytosis (ET)
4. Primary Myelofibrosis (PMF)

Additional MPNs:
1. Systemic Mastocytosis
2. Hypereosinophilic Syndrome
3. CMML
4. Chronic Neutrophilic Leukemia
5. Chronic Eosinophilic Leukemia
2008 WHO classification of chronic myeloid neoplasms

Myeloproliferative Neoplasms

- MDS
  - No cytosis

- MPN
  - Cytosis without dysplasia

- MDS/MPN
  - Cytosis with dysplasia or monocytosis

- Malignantly characterized MPN with eosinophilia
  - Eosinophilia and PDGFR/FGFR1 mutation
2008 WHO classification of chronic myeloid neoplasms

Myeloproliferative Neoplasms

- CMML
- JMML
- Atypical CML

MDS

MPN

- Chronic myelogenous leukemia (CML)
- Polycythemia vera (PV)
- Essential thrombocytopenia (ET)
- Primary myelofibrosis (PMF)

MDS/MPN

- Chronic neutrophilic leukemia (CNL)
- Chronic eosinophilic leukemia, not otherwise specified (CEL-NOS)
- Hypereosinophilic syndrome (HES)
- Systemic mastocytosis (SM)

Molecularly characterized MPN-eos
Clinical manifestations of myeloproliferative neoplasms

- Erythrocytosis
  - PV
- Thrombocytosis
  - ET, PV, PMF or CML
- Leukocytosis
  - CML, ET, PV or PMF

- Splenomegaly from extramedullary hematopoiesis
  - PMF, ET, PV or CML

- Bone marrow myeloproliferation

- Myelofibrosis
  - PMF, PV or CML
- Osteosclerosis
- Angiogenesis

- Anemia
  - PMF or CML

- Thrombocytopenia
  - PMF

- Cachexia
CML – Pathophysiology – the Philadelphia Chromosome
The Philadelphia chromosome results when a piece of chromosome #9 switches places with a piece of chromosome #22. The translocation forms an extra-long chromosome #22 (called der 9) and an extra-short chromosome #22, which is the Philadelphia chromosome that contains the abnormal, fused BCR-ABL gene.
Multiple Breakpoints in Bcr-Abl
BCR-ABL1 junctions are usually e13a2 (b2a2) or e14a2 (b3a2)

BCR-ABL1 fusion genes

BCR-ABL1 mRNAs

...so RQ-PCR can use CML-specific primers
Diagnostic Considerations in Chronic Myeloid Leukemia

Demonstrating the presence of the t(9;22) or its gene product is absolutely essential in diagnosing a patient with CML.

**Karyotyping in CML**

1) Allows for the diagnosis of CML
2) Requires a bone marrow aspirate for optimal metaphases
3) Allows for evaluation of clonal evolution as well as additional chromosomal abnormalities in the non-Ph+ clones
4) Occasional cryptic and complex karyotypes can result in the missed identification of the t(9;22)
Diagnostic Considerations in Chronic Myeloid Leukemia

**Fluorescence in-situ hybridization (FISH) in CML:**

1) Allows for the diagnosis of CML
2) Does not require a bone marrow aspirate for optimal results
3) Allows for the identification of potential duplications of the Ph ch.
4) Allows for the identification of the loss of the der (9) ch.
5) Allows for the identification of cryptic translocations involving Bcr-Abl
FISH in CML

Red → Bcr probe
Green → Abl Probe
Yellow → fusion of Bcr and Abl
Diagnostic Considerations in Chronic Myeloid Leukemia

**Quantitative RT-PCR for Bcr-Abl in CML**

1) Allows for the diagnosis of CML
2) Does not require a bone marrow aspirate for optimal results
3) Can quantify the amount of disease
4) Allows for the identification of cryptic translocations involving Bcr-Abl
5) Many primers sets only detect the p190 and/or the p210 translocation and may miss the p230 or alternative translocations
Quantitative RT-PCR for Bcr-Abl in CML

Amount of Fluorescence

PCR Cycle Number

High Concentration
Moderate Concentration
Low Concentration
# Disease Diagnosis and Monitoring in CML

<table>
<thead>
<tr>
<th>Test</th>
<th>Target</th>
<th>Tissue</th>
<th>Sensitivity (%)*</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytogenetics</td>
<td>Ph chromosome</td>
<td>BM</td>
<td>1-10</td>
<td>▪ Confirm diagnosis of CML</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>▪ Evaluate karyotypic abnormalities other than Ph chromosome (ie, clonal evolution)</td>
</tr>
<tr>
<td>FISH</td>
<td>Juxtaposition of bcr and abl</td>
<td>PB/BM</td>
<td>0.5-5</td>
<td>▪ Confirm diagnosis of CML</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>▪ Routine monitoring of cytogenetic response in clinically stable patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>▪ Routine measurement of MRD</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>bcr-abl mRNA</td>
<td>PB/BM</td>
<td>0.0001-0.001</td>
<td>▪ Routine measurement of MRD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>▪ Determine the breakpoints of the fusion genes</td>
</tr>
</tbody>
</table>

*Number of leukemic cells detectable per 100 cells.

BM = bone marrow; FISH = fluorescence in situ hybridization; PB = peripheral blood; MRD = minimal residual disease; RT-PCR = reverse transcriptase polymerase chain reaction.
Please Compare what is your idea?

CML BMA at DX

The same patient – 36 months later under Imatinib Therapy
### Chronic Myeloid Leukemia - Diagnostic Criteria for the 3 Phases of the Disease

#### Chronic Myeloid Leukemia Classification

<table>
<thead>
<tr>
<th>Phase</th>
<th>Diagnostic Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML, chronic phase (CP-CML)</td>
<td>- A myeloproliferative disorder caused by the balanced translocation between the long arms of chromosome 9 and chromosome 22: t(9;22)(q34;q11)</td>
</tr>
<tr>
<td></td>
<td>- Not meeting criteria for accelerated or blastic phase</td>
</tr>
<tr>
<td>CML, accelerated phase (AP-CML)</td>
<td>Diagnose if one or more of the following is present:</td>
</tr>
<tr>
<td></td>
<td>• Blasts 10% to 19% of peripheral blood white cells or bone marrow cells</td>
</tr>
<tr>
<td></td>
<td>• Peripheral blood basophils at least 20%</td>
</tr>
<tr>
<td></td>
<td>• Persistent thrombocytopenia (&lt;100 X10^9/L) unrelated to therapy, or persistent thrombocytosis (&gt;1000 X10^9/L) unresponsive to therapy</td>
</tr>
<tr>
<td></td>
<td>• Increasing spleen size and increasing WBC count unresponsive to therapy</td>
</tr>
<tr>
<td></td>
<td>• Cytogenetic evidence of clonal evolution (i.e., the appearance of an additional genetic abnormality that was not present in the initial specimen at the time of diagnosis of chronic phase CML)</td>
</tr>
<tr>
<td></td>
<td>• Megakaryocytic proliferation in sizable sheets and clusters, associated with marked reticulosis or collagen fibrosis, and/or severe granulocytic dysplasia, should be considered as suggestive of CML-AP. These findings have not yet been analyzed in large clinical studies, however, so it is not clear if they are independent criteria for accelerated phase.</td>
</tr>
<tr>
<td>CML, blast phase (BP-CML)</td>
<td>Diagnose if one or more of following is present:</td>
</tr>
<tr>
<td></td>
<td>• Blasts 20% or more of peripheral blood white cells or bone marrow cells</td>
</tr>
<tr>
<td></td>
<td>• Extramedullary blast proliferation</td>
</tr>
<tr>
<td></td>
<td>• Large foci or clusters of blasts in bone marrow biopsy</td>
</tr>
</tbody>
</table>
Imatinib (Gleevec, Novartis) a small molecule tyrosine kinase inhibitor
Treatment Milestones for CML

**Definitions of Responses to Treatments**

**Hematologic Response**

<table>
<thead>
<tr>
<th>Complete Hematologic response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Normal PB counts (WBC &lt; 10 and plt &lt; 450)</td>
</tr>
<tr>
<td>2) Normal WBC differential</td>
</tr>
<tr>
<td>3) No Dz symptoms</td>
</tr>
<tr>
<td>4) Normalization of the size of the liver and spleen</td>
</tr>
</tbody>
</table>

**Cytogenetic Responses: Ph+ Metaphases**

| 1) complete: 0% |
| 2) partial: 1% - 35% |
| 3) minor: 36% - 65% |
| 4) minimal: 66% - 95% |
| 5) none: 96% - 100% |

**Molecular Responses: ratio of Bcr-Abl/Abl**

Major Molecular Response

3-log$_{10}$ reduction from initial diagnosis sample (i.e. 25 $\rightarrow$ 0.025)
# 2009 ELN Recommendations for Response Assessment for Treatment


<table>
<thead>
<tr>
<th>Evaluation Time, Months</th>
<th>Optimal</th>
<th>Suboptimal</th>
<th>Failure</th>
<th>Warnings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>High risk; CCA/Ph+*</td>
</tr>
<tr>
<td>3</td>
<td>CHR and at least minor CgR (Ph+ ≤ 65%)</td>
<td>No CgR (Ph+ &gt; 95%)</td>
<td>Less than CHR</td>
<td>NA</td>
</tr>
<tr>
<td>6</td>
<td>At least PCgR (Ph+ ≤ 35%)</td>
<td>Less than PCgR (Ph+ &gt; 35%)</td>
<td>No CgR (Ph+ &gt; 95%)</td>
<td>NA</td>
</tr>
<tr>
<td>12</td>
<td>CCGR</td>
<td>PCgR (Ph+ 1% to 35%)</td>
<td>Less than PCgR (Ph+ &gt; 35%)</td>
<td>Less than MMolR†</td>
</tr>
<tr>
<td>18</td>
<td>MMolR†</td>
<td>Less than MMolR†</td>
<td>Less than CCGR</td>
<td>NA</td>
</tr>
<tr>
<td>Any time during treatment</td>
<td>Stable or improving MMolR†</td>
<td>Loss of MMolR†; mutations‡</td>
<td>Loss of CHR; loss of CCGR; mutations§; CCA/Ph+</td>
<td>Increase in transcript levels¶; CCA/Ph–</td>
</tr>
</tbody>
</table>

PD-INEL
Mechanisms of Imatinib Resistance

### Primary resistance
- failure to achieve preset hematologic and/or cytogenetic milestones
  - IRIS data indicates a rate of ~ 15% by failing to achieve a PCyR at 12 months and 24% by failing to achieve a CCyr by 18 months of therapy.
- rates higher in accelerated and blast phase disease

### Secondary resistance
- loss of a previously achieved hematologic or cytogenetic milestone
  - rates may be 10-15% on Imatinib, but become rarer as time on therapy progresses
  - rates higher in accelerated and blast phase disease

### Resistance Mechanisms

1) **Bcr-Abl Kinase mutations**
   - > 50 known mutations within Abl sequence which inhibits Imatinib from binding
   - mutations identified in 30-80% of individuals with resistant disease

2) **Bcr-Abl duplication**
   - duplication of the *Bcr-Abl* sequence has been identified in cell lines with Im resistance
   - rates higher in accelerated and blast phase disease

3) **Pgp over-expression**
   - export pump of many chemotherapeutics leading to lower intracellular Im concentration

4) **hOct-1 under-expression**
   - import pump for Im which may lead to lower intracellular levels of IM

5) **Src-Family kinase (SFK) expression**
   - activation may circumnavigate the Bcr-Abl ‘addiction’ of the transformed cell
Bcr-Abl

Mut. Bcr-Abl

imatinib

imatinib

dasatinib

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Bosutinib</th>
<th>Imatinib</th>
<th>Dasatinib</th>
<th>Nilotinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parental</td>
<td>38.31</td>
<td>10.78</td>
<td>&gt; 50</td>
<td>38.43</td>
</tr>
<tr>
<td>WT</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P-LOOP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L248V</td>
<td>2.97</td>
<td>3.54</td>
<td>5.11</td>
<td>2.80</td>
</tr>
<tr>
<td>G250E</td>
<td>4.31</td>
<td>6.86</td>
<td>4.45</td>
<td>4.56</td>
</tr>
<tr>
<td>Q252H</td>
<td>0.81</td>
<td>1.39</td>
<td>3.05</td>
<td>2.64</td>
</tr>
<tr>
<td>Y253F</td>
<td>0.96</td>
<td>3.58</td>
<td>1.58</td>
<td>3.23</td>
</tr>
<tr>
<td>E255K</td>
<td>9.47</td>
<td>6.02</td>
<td>5.61</td>
<td>6.69</td>
</tr>
<tr>
<td>E255V</td>
<td>5.53</td>
<td>16.99</td>
<td>3.44</td>
<td>10.31</td>
</tr>
<tr>
<td>C-Helix</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D276G</td>
<td>0.60</td>
<td>2.18</td>
<td>1.44</td>
<td>2.00</td>
</tr>
<tr>
<td>E279K</td>
<td>0.95</td>
<td>3.55</td>
<td>1.64</td>
<td>2.05</td>
</tr>
<tr>
<td>ATP binding region (drug contact sites)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V299L</td>
<td>26.10</td>
<td>1.54</td>
<td>8.65</td>
<td>1.34</td>
</tr>
<tr>
<td>T315I</td>
<td>45.42</td>
<td>17.50</td>
<td>75.03</td>
<td>39.41</td>
</tr>
<tr>
<td>F317L</td>
<td>2.42</td>
<td>2.60</td>
<td>4.46</td>
<td>2.22</td>
</tr>
<tr>
<td>SH2-contact</td>
<td>M351T</td>
<td>0.70</td>
<td>1.76</td>
<td>0.86</td>
</tr>
<tr>
<td>Substrate binding region (drug contact sites)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F359V</td>
<td>0.93</td>
<td>2.86</td>
<td>1.49</td>
<td>5.16</td>
</tr>
<tr>
<td>A-LOOP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L384M</td>
<td>0.47</td>
<td>1.28</td>
<td>2.21</td>
<td>2.33</td>
</tr>
<tr>
<td>H399P</td>
<td>0.43</td>
<td>2.43</td>
<td>1.07</td>
<td>2.41</td>
</tr>
<tr>
<td>H399R</td>
<td>0.81</td>
<td>3.91</td>
<td>1.63</td>
<td>3.10</td>
</tr>
<tr>
<td>G398R</td>
<td>1.16</td>
<td>0.35</td>
<td>0.69</td>
<td>0.49</td>
</tr>
<tr>
<td>C terminal lobe</td>
<td>F486S</td>
<td>2.31</td>
<td>8.10</td>
<td>3.04</td>
</tr>
</tbody>
</table>

**Sensitive** ≤ 2  
**Moderately resistant** 2.01–4  
**Resistant** 4.01–10  
**Highly resistant** > 10
What do you see?
It is more obvious,
Diagnostic Criteria for Primary PV

**Polycythemia Vera Study Group (PVSG) Criteria for PV**

**Major Criteria**
- Elevated RBC mass
  - >36 cc/kg in men
  - >32 cc/kg in women
- Oxygen saturation >92%
- Splenomegaly

**Minor Criteria**
- Platelet count > 400,000
- WBC > 12,000
- Elevated LAP score (>100)
- Serum vitamin B12 >900 pg/mL or serum unbound B12 binding capacity >2,200 pg/mL

→ All 3 major criteria OR the first 2 major and any 2 minor criteria ←

**2008 WHO Diagnostic Criteria for Primary Polycythemia Vera**

**Major Criteria**
1) Hgb > 18.5 g/dl (♂) or 16.5 g/dl (♀)

- or
- Hgb or Hct > 99%

- or
- Hgb > 17 g/dl (♂) or 15 g/dl (♀) and a documented increase of 2 g/dl

- or
- RBC mass > 25% of mean normal

2) Presence of a JAK2 V617F or similar mutation

→ two major or first major and two minor criteria ←

Tefferi et al. Leukemia (2008) 22, 14–22
JAK2 Mutations and MPNs

- Receptor Tyrosine Kinase - maps to chromosome 9p
- **Valine to phenylalanine** substitution at amino acid 617 (V617F) in pseudokinase domain of JAK2 allows for the constitutive activation of the receptor
- Somatic acquired mutation
- High incidence in PCV (~95%)
  - Not present in every patient with PCV
- Lower incidence in ET (~50%) and PMF (~50%)
What do you see and which dxs are possible?

myelocyte

nucleated rbc
Tear Drop Cells (or Tear Drop Poikilocytes)
## Conditions Associated With Myelofibrosis and/or Leukoerythroblastosis

<table>
<thead>
<tr>
<th>Nonmalignant</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis</td>
<td>Myeloproliferative</td>
</tr>
<tr>
<td>Gaucher's disease</td>
<td>Acute/chronic leukemias</td>
</tr>
<tr>
<td>Radiation</td>
<td>Hodgkin's disease</td>
</tr>
<tr>
<td>Renal osteodystrophy</td>
<td>Non-Hodgkin's lymphomas</td>
</tr>
<tr>
<td>Paget's disease of bone</td>
<td>Plasma cell myeloma</td>
</tr>
<tr>
<td>Benzene</td>
<td>Metastatic carcinomas</td>
</tr>
<tr>
<td>Congenital syphilis</td>
<td></td>
</tr>
</tbody>
</table>

normal marrow trephine

- cells
- fat
- bone
myelofibrosis

fibrosis

new bone (arrows)
What do you see and which dxs are possible?
Idiopathic Myelofibrosis, Myelofibrosis with myeloid metaplasia (MMM)

Extramedullary hematopoiesis with myelofibrosis (unknown etiology ? Stem cell disorder)

- **Peripheral blood**
  - Anemia (50%), leukocytosis (40%), leukopenia (20%)
  - Thrombocytosis (20%) thrombocytopenia
  - Leukoerythroblastic picture (100%)
  - Poikilocytosis and tear-drop RBC (100%)
Diagnostic algorithm for suspected primary myelofibrosis.

BM biopsy, reticulin stain, cytogenetic studies & mutation screening for JAK2V617F

- Ph chromosome (+) → CML
- V617F (+) or del(13q) → PMF likely but use histology to exclude other myeloid neoplasm
- Other cytogenetic abnormalities → Could be PMF but also MDS or other myeloid neoplasm
- Normal cytogenetics and V617F (-) → If megakaryocytes dwarf consider FISH for BCR-ABL otherwise use histology for specific diagnosis
CIMF

- **Prefibrotic phase**
  - Mild hepatosplenomegaly
  - Mild to moderate ↑WBC, ↑plts.
  - Neutrophil, meg proliferation
  - Minimal fibrosis

- **Fibrotic phase**
  - Moderate hepatosplenomegaly
  - Variable plts, WBC.
  - Leukoerythroblastosis
  - Marrow fibrosis
  - Meg proliferation
Diagnostic Criteria for PMF

2008 WHO Diagnostic Criteria for Primary Myelofibrosis

**Major:**
1. Megakaryocytic proliferation and atypia with either reticulin or collagen fibrosis
   or
   If no fibrosis, mekakaryocytic expansion must be assn. w/ increased BM cellularity
2. Does not meet WHO criteria for CML, PV, MDS, or other myeloid neoplasm
3. Demonstration of the JAK2 V617F mutation or other clonal marker
   or
   no other evidence of a reactive marrow fibrosis

**Minor:**
1. Leukoerythroblastosis (immature RBCs and WBCs in the PB)
2. Increased LDH
3. Anemia
4. splenomegaly

→ Diagnosis of primary myelofibrosis (PMF) requires meeting all three major criteria and two minor criteria ←

## DDx of Myelofibrosis

### Myeloid Neoplasms
- PMF
- CML
- ET
- PV
- MDS
  - Acute myelofibrosis (potentially assn. w/ FAB M7 AML)
- AML
- Mast Cell Disease

### Lymphoid Neoplasms
- lymphoma
- Hairy Cell Leukemia
- Multiple Myeloma

### Non-Hematologic Disorders
- Metastatic cancer
- Connective tissue diseases
  - Rickets
  - Infections
  - Renal Osteodystrophy
What is your diagnosis?

note giant platelets
Diagnostic Criteria for Essential Thrombocythemia

2008 WHO Diagnostic Criteria for Essential Thrombocythemia

1. Platelet count > 450,000
2. Megakaryocytic proliferation with large, mature morphology and with little granulocytic or erythroid expansion
3. Not meeting WHO criteria for CML, PV, PMF, MDS or other myeloid neoplasm
4. Demonstration of the JAK2V617F or other clonal marker or lack of evidence of a secondary (reactive thrombocytosis)

→ Diagnosis of essential thrombocythemia requires meeting all four major criteria ←

Teferri et al. Leukemia (2008) 22, 14–22
Peripheral blood mutation screening for JAK2V617F

- **V617F (+)**
  - ET, PV or PMF highly likely
  - BM biopsy & cytogenetics
  - Use 2008 WHO criteria for specific diagnosis
  - Consider FISH for BCR-ABL in the absence of the Ph chromosome but presence of dwarf megakaryocytes

- **V617F (-)**
  - ET and PMF still possible & CML should be considered as well
Causes of Thrombocytosis

Clonal Thrombocytosis
- Essential thrombocythemia
- Polycythemia vera
- Primary myelofibrosis
- Chronic myeloid leukemia
- Refractory anemia with ringed sideroblasts and thrombocytosis
- 5q-minus syndrome

Reactive (secondary) thrombocytosis

Transient thrombocytosis
- Acute blood loss
- Recovery from thrombocytopenia (rebound thrombocytosis)
- Acute infection or inflammation
- Response to exercise
- Response to drugs (vincristine, epinephrine, all-trans-retinoic acid)

Sustained thrombocytosis
- Iron deficiency
- Splenectomy or congenital absence of spleen
- Malignancy
- Chronic infection or inflammation
- Hemolytic anemia

Familial thrombocytosis

Spurious thrombocytosis
- Cryoglobulinemia
- Cytoplasmic fragmentation in acute leukemia
- Red cell fragmentation
- Bacteremia
Platelet count >450 x 10^9/l

Review blood film
Acute phase reactants (e.g. CRP, ESR)
Iron status

Acute phase response

Reactive thrombocytosis

Normal

Iron deficiency
Treat
Repeat blood count

Repeat blood count

Persistent unexplained thrombocytosis

Further investigation

Molecular genetics
(JAK2 V617F; MPL)
Bone marrow examination (aspirate and trephine biopsy)
Cytogenetics

Diagnosis
(see text for diagnostic features)
What is your diagnosis?
Diagnostic Criteria for Systemic Mastocytosis

**Major Criteria**
- Multifocal, dense infiltrates of mast cells (≥15 mast cells in an aggregate) detected in sections of marrow and/or other extracutaneous organ(s).

**Minor Criteria**
- a. In biopsy sections of marrow or other extracutaneous organs, >25% of the mast cells in the infiltrate are spindle shaped or have atypical morphology, or, of all mast cells in marrow aspirate smears, >25% are immature or atypical mast cells.
- b. Detection of a point mutation in *KIT* at codon 816 in marrow, blood, or other extracutaneous organ
- c. Mast cells in marrow, blood, or other extracutaneous organs that coexpress CD117 with CD2 and/or CD25
- d. Serum total tryptase persistently >20 ng/mL (if there is an associated myeloid disorder, this criterion is not valid)

The diagnosis of systemic mastocytosis can be made if one major and one minor criterion are present or if three minor criteria are met.
## Summary of MPDs

<table>
<thead>
<tr>
<th></th>
<th>CML</th>
<th>PV</th>
<th>Prefibrotic PF</th>
<th>Fibrotic PF</th>
<th>ET</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood</strong></td>
<td>↑ myeloids, plts</td>
<td>↑ myeloids, plts, rbc's</td>
<td>↑ myeloids, plts</td>
<td>↑ or ↓ myeloids, plts</td>
<td>↑ plts</td>
</tr>
<tr>
<td><strong>Marrow</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>cellularity</strong></td>
<td>Increased</td>
<td>Increased</td>
<td>Increased</td>
<td>Can be decreased</td>
<td>Increased</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Proliferating</strong></td>
<td>Grans, megs</td>
<td>Erythroids, grans, megs</td>
<td>Grans, megs</td>
<td>Grans, megs</td>
<td>Megs</td>
</tr>
<tr>
<td><strong>lineages</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Meg</strong></td>
<td>Small, hypolobated</td>
<td>Large, hyperlobated</td>
<td>Large, hyperlobated; can be variable in size.</td>
<td>Variable in size, bizarre morphology</td>
<td>Giant, hyperlobated</td>
</tr>
<tr>
<td><strong>morphology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fibrosis</strong></td>
<td>Minimal</td>
<td>Minimal</td>
<td>Minimal</td>
<td>Present</td>
<td>Minimal</td>
</tr>
</tbody>
</table>

Adapted from Practical Diagnosis of Hematologic Disorders (2006), Kjeldsberg, p. 537.