HAEMATOLOGY-5

CHRONIC MYELOGENOUS LEUKEMIA

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CML: Definition

- Chronic Myelogenous Leukemia (CML); also known as Chronic Myeloid Leukemia or Chronic Myelocytic Leukemia; is a haemopoietic stem cell neoplasm characterized by:
- Anemia
- Extreme blood granulocytosis and granulocytic immaturity
- Basophilia
- Thrombocytosis
- Splenomegaly
- Characteristic genetic change – Reciprocal translocation between chromosomes 9 and 22 (Philadelphia chromosome).

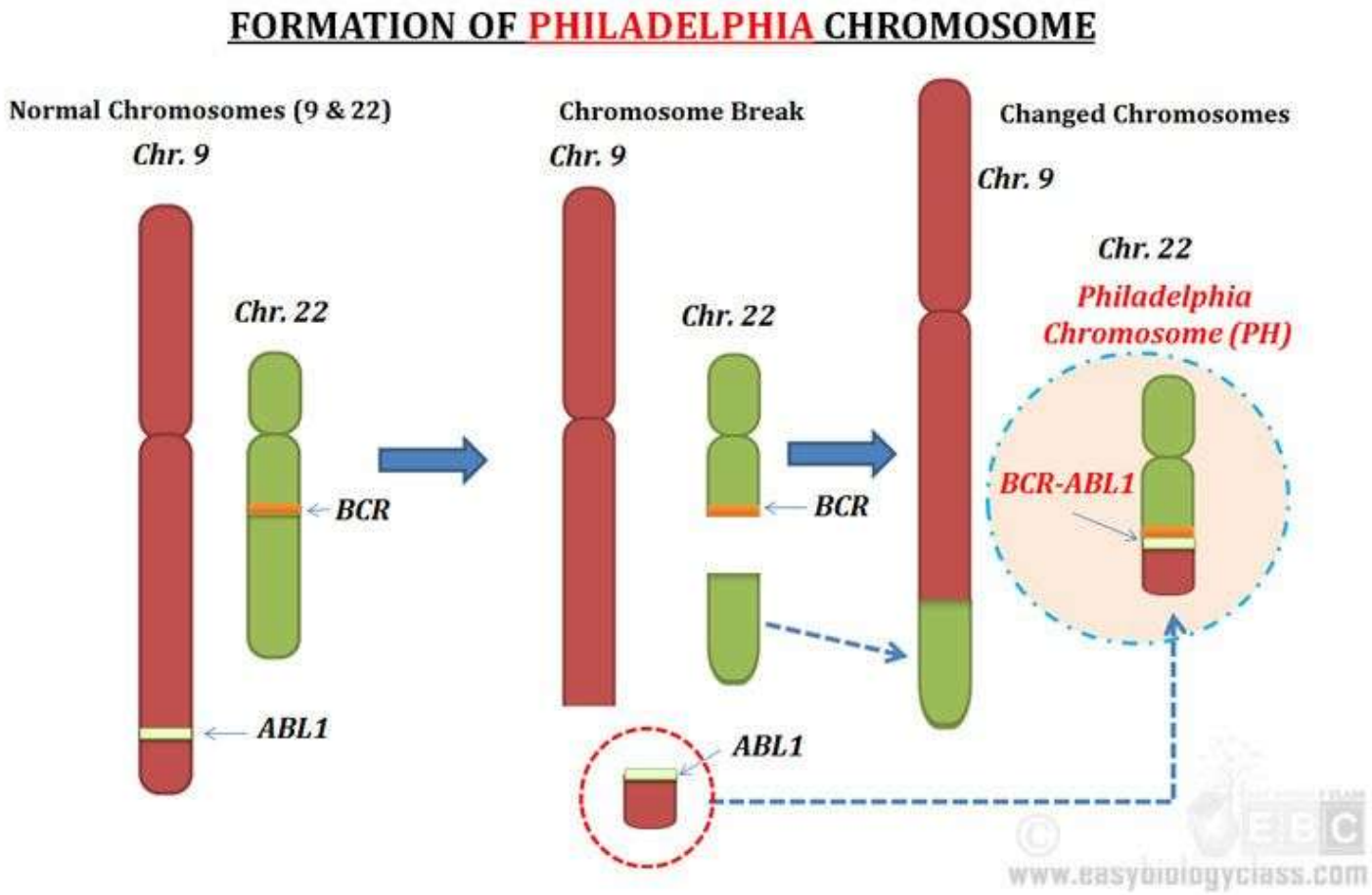
CML: EPIDEMIOLOGY

- CML accounts for 15 %of all cases of leukemia world-wide.
- In India it accounts for 50-70% of all leukemias.
- Male preponderance (M:F :: 1.4:1).
- Average age at presentation is 40 – 50 yrs.
- Note: Amongst the Myeloid Neoplasms CML belongs to the category of Myeloproliferative Neoplasms that are associated with the increased production of mature myeloid elements (granulocytes i.e. myelocytes, metamyelocytes and neutrophils in the case of CML) in the bone marrow with elevated peripheral blood counts. CML is discussed here as a leukemia. The other myeloproliferative neoplasms shall be discussed separately, later.

CML: ETIOPATHOGENESIS

- **Etiology:**
- Radiation
- Chemicals like benzene
- HLA-CW3 and CW4 associated with increased genetic susceptibility to CML.
- **Pathogenesis:**
- The disease arises from malignant transformation of a single stem cell. The driving mutation is t(9,22) resulting in formation of **Philadelphia chromosome** and a fusion **bcr-abl gene**. The Ph chromosome or its molecular equivalent is found not only in the granulocytic precursors but also in other myeloid lineages (erythroid and megakaryocytic) as well as the B-cells and possibly T-lymphoid cells, indicating that the target of transformation is a **Pluripotent Haemopoietic Stem Cell**.

PHILADELPHIA CHROMOSOME

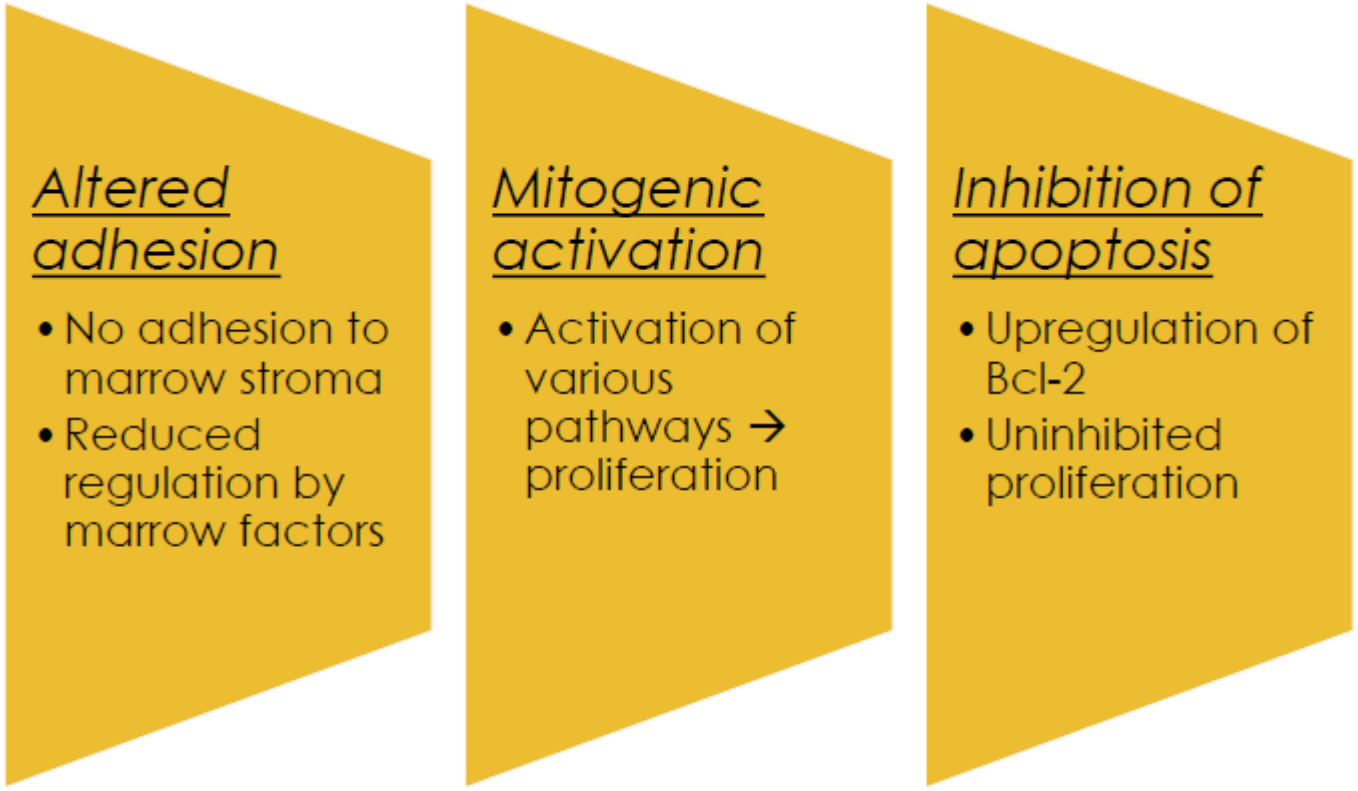


CML: PATHOGENESIS CONTD.

Molecular Pathology:

- CML is distinguished from other chronic myeloproliferative disorders by the presence of a distinctive molecular abnormality, a reciprocal translocation between the long arms of chromosomes 9 and 22 i.e. **t(9,22); (q34,q11).** A large portion of 22q is translocated to 9q and a smaller piece of 9q is moved to 22q resulting in overtly pre-shortened long arm of one of the chromosome pair number 22. **Thus, Philadelphia chromosome is a shortened chromosome 22 or 22q-.**
- As a result of this translocation the **Abelson** (**abl**) proto-oncogene located on Ch 9 is translocated adjacent to **breakpoint cluster region** (**bcr**) gene on Ch 22. Fusion gene bcr-abl directs the synthesis of a **210kD** protein with an **unregulated tyrosine kinase activity**. This protein causes malignant transformation of the pluripotent haemopoietic stem cell leading to uncontrolled proliferation and increased survival of the cells.

BCR-ABL TYROSINE KINASE



Etiopathogenesis

CML: PATHOGENESIS CONTD.

- A rearrangement of bcr is present in all subjects with CML, even the 10% without any overt Ph Ch abnormality. These cases which are Ph Ch negative at the level of light microscopic cytogenetics, demonstrate the bcr-abl gene at the molecular study level.
- The transformed stem cell undergoes unchecked proliferation but there is no differentiation block or maturation arrest. Although, multiple myeloid lineages, B lymphoid cells and possibly T lymphoid cells express the bcr-abl fusion protein, for unknown reasons, the constitutively active bcr-abl kinase is evident mainly in granulocytic progenitors and to a lesser degree in megakaryocytic progenitors. Hence, there is a marked increase in neoplastic granulocytes and their precursors in the bone marrow and the peripheral blood.
- Neoplastic extramedullary haematopoiesis within the splenic red pulp produces marked splenomegaly.

CML: CLINICAL PICTURE

- **A. Symptoms**
- At diagnosis – 70% symptomatic
- Easy fatiguability
- Decreased tolerance to exertion
- Anorexia
- Abdominal discomfort
- Weight loss
- Heat intolerance
- Excessive sweating

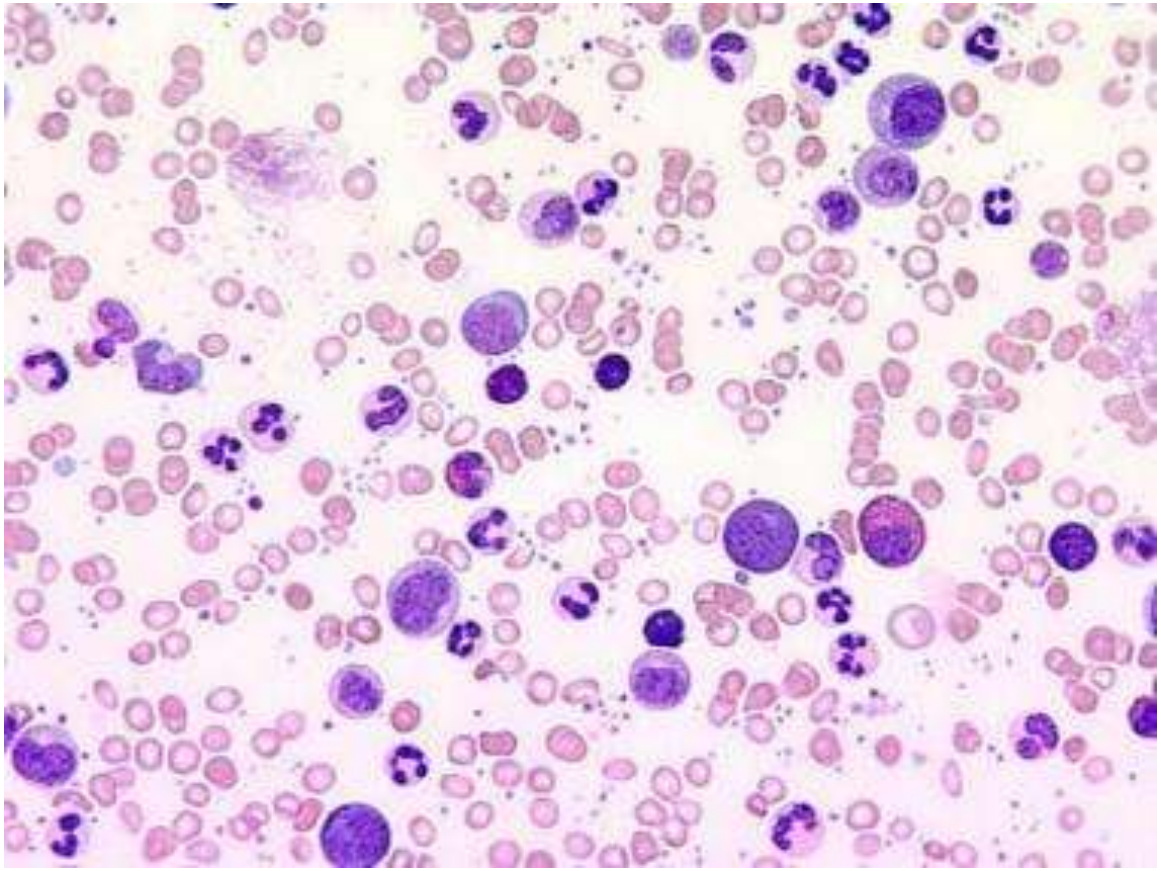
- **B. Signs**
- Pallor
- **Splenomegaly**
- Sternal tenderness
- Rarely hepatomegaly, lymphadenopathy – Poor prognostic indicators
- Sweet Syndrome is an acute febrile dermatosis seen in CML.
- Infection, bleeding. Thrombosis due to neutrophil and platelet dysfunction.

CML: LAB DIAGNOSIS

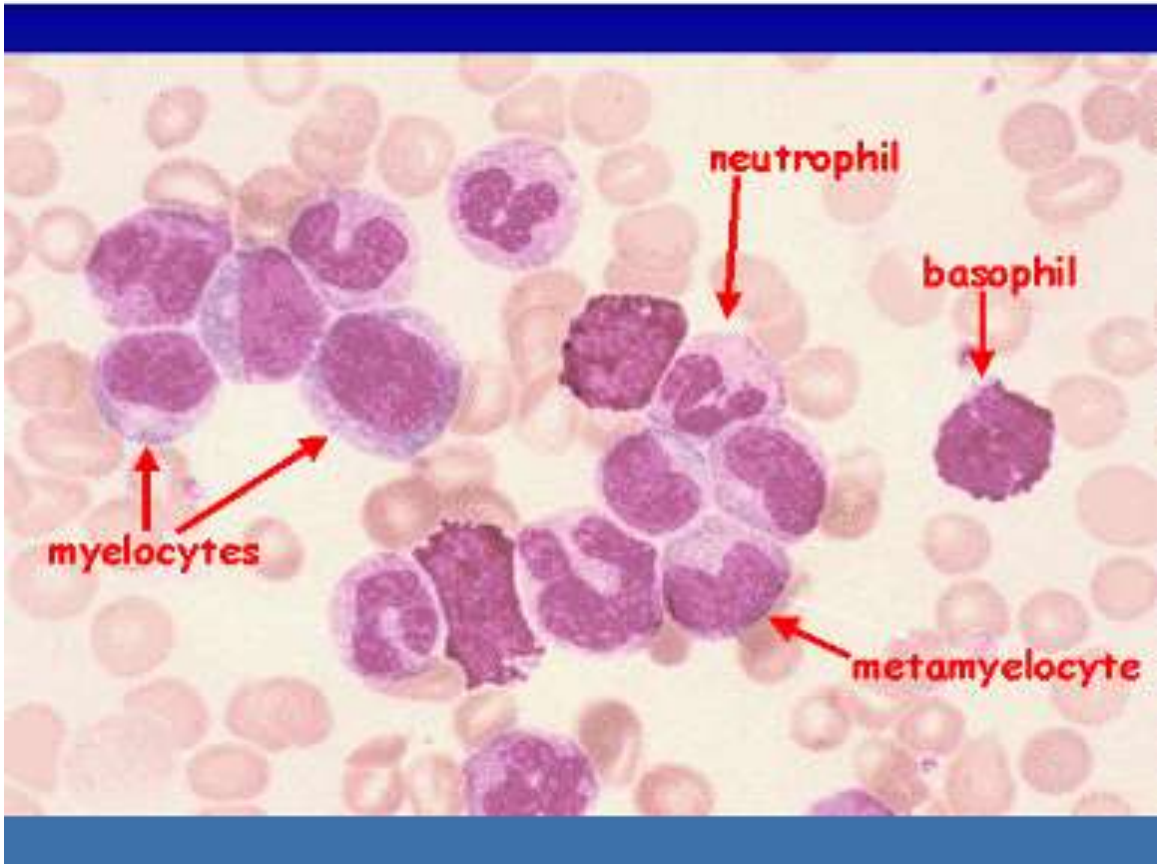
- Lab Diagnosis of CML is based on the following:
- Moderate to marked splenomegaly
- TLC ≥ 25,000/µl with marked granulocytosis and basophilia on peripheral blood film.
- Low leukocyte/neutrophil alkaline phosphatase (LAP/NAP) score.
- Demonstration of Philadelphia chromosome on cytogenetic studies or bcr-abl rearrangement on molecular studies.

CML: LAB DIAGNOSIS
Peripheral Blood

- Hb and haematocrit reduced. Mild normocytic normochromic anaemia. Nucleated RBCs may be present.
- **TLC nearly always > 25,000/µl**. Half the patients have TLC> 100,000/µl.
- DLC shows **marked granulocytosis with basophilia and eosinophilia**. Granulocytes in all stages of maturation are seen. Neutrophils and myelocytes are predominant. **Blasts** average around 3% (range is from 0.5% to **<10**% in the chronic phase). Basophils range from 2-10%.
- Platelets – mild to moderate **thrombocytosis**.
- Leucocyte/ neutrophil alkaline phosphatase score **(LAP/NAP score) is reduced** to almost zero in CML (normal score is 40-100).



PBF in CML showing granulocytosis with full range of maturation and thrombocytosis.



PBF in CML. A higher magnification showing different cell types.

CML: LAB DIAGNOSIS
Bone Marrow

- Markedly **hypercellular.**
- Granulopoiesis is dominant. **M:E ratio markedly increased to 10:1 to 30:1.**
- **Myeloblasts** are usually <5% in the chronic phase, **always <10%.**
- Eosinophils, basophils and their precursors are increased.
- Erythropoiesis is usually reduced.
- Megakaryocytes are increased and may show dysplastic features.
- Mitotic figures are increased.
- Macrophages mimicking Gaucher cells are seen. Macrophages engorged with lipids (ceroid pigment - imparting a and bluish cast to the cytoplasm) called *sea-blue histiocytes,* are also present in the marrow.
- **Marrow reticulin fibrosis** (type III collagen is increased).

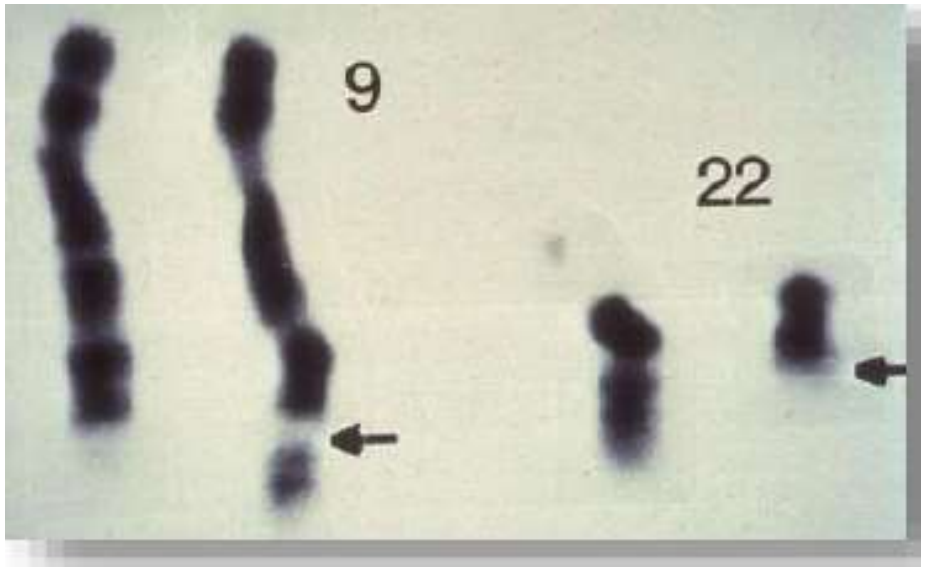
CML: LAB DIAGNOSIS
Biochemical tests

- Serum B12 and transcobalamin increased (>10 ULN).
- **Serum uric acid increased**.
- Serum Lactate dehydrogenase increased.
- Mean histamine levels increased.
- Hypercalcemia and hypokalemia may occur during chronic phase but more common when disease transforms to acute leukemia.
- Serum cholesterol decreases.

CML: LAB DIAGNOSIS
Cytogenetic and molecular studies

- **Cytogenetics** – study of the number and structure of chromosomes. Cytogenetics cannot identify complex translocations.
  - **Karyotyping** to detect Philadelphia chromosome i.e. shortened Ch 22.
- **Molecular Probes** – detect the bcr-abl fusion gene on Ch 22 or the mRNA transcription of mutant fusion protein.
  - **FISH**
  - **Southern Blot**
  - **Polymerase chain reaction**

Karyotype:Philadelphia chromosome



CML: COURSE AND PROGNOSIS

The natural course of CML is divided into three phase:

1. **Chronic Phase**
2. **Accelerated Phase**
3. **Blast Crisis**

- The average span of all the 3 phases has increased significantly with advent of tyrosine kinase inhibitor therapy.
- CML may terminate in a spent phase characterized by **marrow fibrosis** and peripheral blood cytopenias.

CML: COURSE AND PROGNOSIS

- **1. Chronic Phase:**
- Without treatment CML follows a stable course for a variable period, averaging 3 years. This is called the **chronic phase.**
- The clinical and the laboratory features discussed so far pertain to the chronic phase of CML.
- With the advent of therapy with Tyrosine Kinase inhibitors (TKI) the duration of the chronic phase has now been prolonged significantly.
- Characterized by marked peripheral blood granulocytosis, basophilia and thrombocytosis.
- Splenomegaly.
- **PB and BM blasts <10%.**

CML: COURSE AND PROGNOSIS

- **2. Accelerated Phase:** 50% of the patients in chronic phase enter a phase of transformation called the accelerated phase after a variable period.
- **Criteria for CML, accelerated phase (WHO 2016)**
- Persistent or increasing TLC (>$10x10^9$/L) unresponsive to therapy.
- Persistent or increasing splenomegaly unresponsive to therapy.
- Persistent thrombocytosis (>$1000x10^9$/L) unresponsive to therapy.
- Persistent thrombocytopenia (<$100x10^9$/L) unrelated to therapy.
- 20% or more basophils in the peripheral blood (PB).
- 10%-19% blasts in the PB or BM.
- Additional clonal chromosomal abnormalities in Ph^+ cells at diagnosis or new abnormalities detected during therapy (additional Ph+, trisomy8 or 19 and isochromosome 17q).
- Resistance to treatment with TKI.

CML: COURSE AND PROGNOSIS

- **3. Blast Crisis:** Within 6-12 months (without treatment), accelerated phase terminates in a picture indistinguishable from **acute leukemia,** called the **blast crisis.** In 50% patients blast crisis may occur without an intermediate accelerated phase. 2/3 of the cases develop myeloblastic –M1,2,4,5,6,7 (CD 34+. TdT+, CD 13+, 33+, 117+) type of blast phase, and 1/3 develop lymphoblastic crisis (CD10+, TdT+,CD19+). Lymphoid blast crisis responds better to chemotherapy. Patients in blast crisis present with rapidly progressing anaemia, splenomegaly and weight loss. Prognosis is poor and survival is 6-12 months.
- **WHO criteria for CML blast phase:**
- Blasts ≥20% in PB or BM.
- Extramedullary blast proliferation in skin (chloromas), lymph nodes, spleen, bone or CNS.
- Large foci of the blasts in the marrow biopsy.

CML: DIFFERENTIAL DIAGNOSIS

- CML needs to be distinguished from :
- **1. Other chronic myeloproliferative disorders (myeloproliferative neoplasms):**

➢ Polycythemia vera

➢ Essential thrombocythemia

➢ Primary myelofibrosis

- Presence of marked peripheral blood granulocytosis with geanulocytic immaturity, a low NAP score (NAP values often increase once CML transforms from chronic phase to accelerated phase or blast crisis) and Ph Chromosome or bcr-abl rearrangement distinguish CML from the above conditions.
- **2. Myeloid Leukemoid reaction**

CML: DIFFERENTIAL DIAGNOSIS
LEUKEMOID REACTION

- **A leukemoid reaction is an increase in WBC count which can mimic leukemia**. The reaction is actually due to an infection or another disease and is not a neoplastic condition. Blood counts often return to normal once the underlying condition is treated. Leukemoid reaction may be **myeloid type** (neutrophilia with presence of a few immature granulocytes in PB) or **lymphoid type** (presence of lymphocytes and immature lymphoid cells in PB). Causes of leukemoid reaction:

- Bacterial infections- septicaemia, pyogenic meningitis, burns etc.
- Infectious mononucleosis
- Cytomegalovirus infection
- Pertussis
- Tuberculosis
- Malignancy

LEUKEMOID REACTION VS CML

	Leukemoid Reaction	CML
Clinical picture	Features of underlying cause	Fatigue, weight loss, abdominal distension
Splenomegaly	Absent	Present
Anaemia	Mild or absent	Present and progressive
TLC	Gen < 50,000/µl	May be >100,000/µl
DLC	Neutrophilia, myelocytes <15%, basophilia and eosinophilia not seen. Blasts <5%.	Myelocytes, meta-myelocytes and bands 20-40%. Basophilia+, eosinophilia+, Blasts may be >5%
Toxic changes in neutrophils	Present (Dohle bodies, toxic granulation, vacuoles)	Absent
LAP score	Normal or increased	Low
Bone marrow	Mild myeloid hyperplasia, Blast count is low, marrow reticulin fibres not increased	Marked myeloid hyperplasia, blasts increased, marrow reticulin fibrosis.
Ph+ chr/bcr-abl	Absent	Present

CML: TREATMENT

- Tyrosine Kinase Inhibitor (TKI) Therapy has revolutionized the management of CML. Imatinib is the prototype drug that inhibits bcr-abl induced kinase activity.
- Interferon-α therapy
- Chemotherapy
- Allogeneic Stem Cell Transplantation is the only modality that offers complete cure.

CML CONCLUDED