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Original Research Paper

Pathology

CHRONIC MYELOID LEUKEMIA: REVIEW OF PATHOGENESIS AND DIAGNOSIS.

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ABSTRACT Chronic Myeloid Leukemia (CML) is one of the commonest leukemias in adults. Its pathogenesis involves translocation between chromosome 9 and 22 which results in abnormal tyrosine kinase activity. The disease has three phases with different criteria for diagnosis. Various modalities are used for making a diagnosis of CML as well as for predicting prognosis and for therapeutic planning. The following article, therefore, is written to review the current concept of pathogenesis and the different diagnostic modalities.

KEYWORDS : chronic myeloid leukemia, philadelphia chromosome, FISH, PCR.

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasms having an incidence of 1–2 cases per 100 000 adults. It accounts for approximately 15% of newly diagnosed cases of leukemia in adults(1). Being one of the commonest adult leukemia CML accounts for 30% to 60% of all adult leukemias(2).

PATHOGENESIS

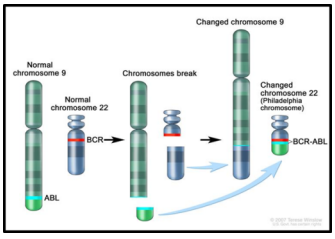

Fig 1 .The translocated abl gene inserts into the bcr gene. The two genes fuse.The altered abl gene functions improperly, resulting in CML*.

*Illustration taken from www.cancer.gov, site under National Institute of Health ,U.S.A.

Pathogenesis of CML , a clonal myeloproliferative disorder of the hematopoetic stem cell involves the fusion of the Abelson murine leukemia (ABL1) gene on chromosome 9 with the breakpoint cluster region (BCR) gene on chromosome 22. 90-95% of cases of CML have the characteristic t(9;22)(q34;q11.2) reciprocal translocation that results in Ph chromosome.

This results in expression of an oncoprotein termed BCR-ABL1(3). BCR-ABL1 is a constitutively active tyrosine kinase that promotes growth and replication through downstream signalling pathways such as RAS, RAF, JUN kinase, MYC, and STAT(4–10). A cytokine independent cell cycle with aberrant apoptotic signals is formed in response to cytokine withdrawal and that directly influences leukemogenesis.

The remaining cases either have variant translocation that involve a third or even a fourth chromosome in addition to chromosomes 9 and 22, or have a cryptic translocation of 9q34 and 22q11.2 that cannot be identified by routine cytogenetic abnormalities.The site of the breakpoint in the BCR gene may influence the phenotype of the disease.

In CML, the breakpoint in the BCR is almost always in the major breakpoint cluster region (M-BCR), spanning exons 12-16 and an abnormal fusion protein p210,is formed which has increased tyrosine kinase activity.

Rarely, the breakpoint in the BCR gene occurs in the µ-BCR region, spanning exons 17-20 and a larger fusion protein p230, is encoded. Patients with this fusion may demonstrate prominent neutrophilic maturation and/or conspicuous thrombocytosis.

m-BCR (minor) leads to a shorter fusion protein p190, is most frequently associated with Ph positive ALL, small amounts of the p190 transcript can also be detected in >90% of patients with classical p210 CML as well, due to alternative splicing of the BCR gene.However, this breakpoint may also be seen in rare cases of CML that are distinctive for having increased number of monocytes and thus can resemble chronic myelomonocytic leukemia.

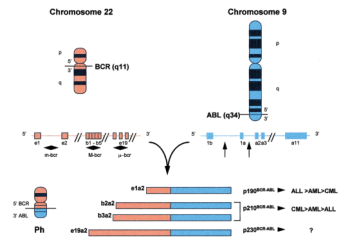

Fig 2. Shows molecular pathology involved in CML.**

**Quintás-Cardama, A., & Cortes, J. (2009). Molecular biology of bcr-abl1–positive chronic myeloid leukemia. Blood, 113(8), 1619-1630

A drastic change in the treatment landscape of CML came with the development of small molecule tyrosine kinase inhibitors (TKIs) which acts and interferes with interaction between adenosine triphosphate (ATP) and BCR/ABL1 oncoprotein thereby terminating the cellular proliferation. With this the 10 year survival rate has increased miraculously from 20% to 80%-90%(1,11,12).

MANIFESTATION AND STAGING

CML can be classified into three stages Chronic phase, Accelerated phase and Blast Phase

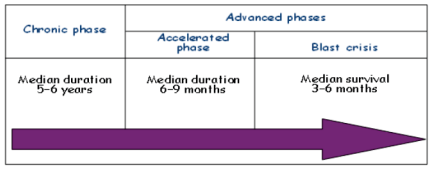

Fig 3. Clinical course : Phases of Untreated CML***

*** Faderl S et al. Ann Intern Med 1999;131:207-219

Typical Laboratory Parameters by Phase of CML

Parameter	Phase of CML: Chronic	Accelerated	Blast Crisis
WBC count	≥20 × 10⁹/L	—	—
Blasts	1%–15%	≥15%	≥30%
Basophils	↑	≥20%	—
Platelets	↑ or normal	↓ or ↑	↓
Bone marrow	Myeloid hyperplasia		
Cytogenetics	Ph+		
Bcr-Abl	+	+	+

WBC = white blood cell; Ph+ = Ph chromosome–positive

Patients usually appear in chronic phase with several signs and symptoms and around 50 % appear asymptomatic and often diagnosed in routine examination. Common signs and symptoms of CML-CP include Fatigue, weight loss, malaise, easy satiety and left upper quadrant fullness or pain. splenomegaly is present in 46–76%[13,14]. Rare manifestation include bleeding (associated with a low platelet count and/or platelet dysfunction), thrombosis (associated with thrombocytosis and/or marked leukocytosis), gouty arthritis (from elevated uric acid levels), priapism (usually with marked leukocytosis or thrombocytosis), retinal hemorrhages, and upper gastrointestinal ulceration and bleeding (from elevated histamine levels due to basophilia)[14]. Hepatomegaly is less common (less than 10%). Lymphadenopathy and infiltration of skin or other tissues are rare. When present, they favor Ph-negative CML or AP or BP of CML. Headaches, bone pain, arthralgias, pain from splenic infarction, and fever are more frequent with CML transformation. CML-AP might be insidious or present with worsening anemia, splenomegaly and organ infiltration; CML-BP presents as an acute leukemia (myeloid in 60%, lymphoid in 30%, megakaryocytic or undifferentiated in 10%) with worsening constitutional symptoms, bleeding, fever and infections.

Criteria for CML, accelerated phase

Any 1 or more of the following hematologic/cytogenetic criteria or response-to-TKI criteria:	
• Persistent or increasing WBC (>10 × 10⁹/L), unresponsive to therapy	"Provisional" response-to-TKI criteria
• Persistent or increasing splenomegaly, unresponsive to therapy	• Hematologic resistance to the first TKI (or failure to achieve a complete hematologic response* to the first TKI) or
• Persistent thrombocytosis (>1000 × 10⁹/L), unresponsive to therapy	• Any hematological, cytogenetic, or molecular indications of resistance to 2 sequential TKIs or
• Persistent thrombocytopenia (<100 × 10⁹/L) unrelated to therapy	• Occurrence of 2 or more mutations in BCR-ABL1 during TKI therapy
• 20% or more basophils in the PB	
• 10%-19% blasts† in the PB and/or BM	
• Additional clonal chromosomal abnormalities in Ph⁺ cells at diagnosis that include "major route" abnormalities (second Ph, trisomy 8, isochromosome 17q, trisomy 19), complex karyotype, or abnormalities of 3q26.2	
• Any new clonal chromosomal abnormality in Ph⁺ cells that occurs during therapy	

DISEASE TRANSFORMATION

The molecular basis of disease transformation is still largely unknown.

Progression is usually associated with clonal evolution and at the time of transformation to AP or BP, 80% of patients demonstrate cytogenetic changes in addition to the Ph chromosome, such as an extra Ph,+8,+19,or i(17q).

Genes shown to be altered in the transformed stages include TP53, RB1, MYC, p16(INK4A), RAS, AML1 and EVI1 , but their role in the transformation, if any, is unknown

BONE MARROW : A NECESSITY??

Bone marrow aspiration is mandatory for all patients in whom CML is suspected, as it will confirm the diagnosis (eg, cytogenetic analysis), and provide information needed for staging in terms of the blast and basophil percentages.

DIAGNOSIS

Peripheral Blood Smear: Arises suspicion!

CHRONIC PHASE	ACCELARATED PHASE	BLAST PHASE
• Blast usually accounts for fewer than 5% in blood • Absolute basophilia is invariably present. • Eosinophilia is a common finding. • Platelet count usually normal to increased. • Monocytosis (p190 isoform) can be found	• Persistent or increasing WBC (>10x109/l) and/or persistent or increasing splenomegaly unresponsive to therapy. • Persistent thrombocytosis(>1000x109/L) unresponsive to therapy. • Persistent thrombocytopenia(<100x109/L) unrelated to therapy. • Clonal cytogenetic evolution occurring after the initial diagnostic karyotype. • 20% or more basophils in the peripheral blood smears. • 10-19% myeloblasts in the blood	• Blast equal or greater than 20% of peripheral blood WBC or of the nucleated cells of BM. • Extra medullary blast proliferation most commonly present in the skin, lymph node and spleen • In approx. 70% of cases, the blast lineage is myeloid and may include neutrophilic, eosinophilic, basophilic monocytic, megakryocytic or erythroid blasts or any combination. In approx. 20-30% of cases the blast are lymphoblasts

METHODS OF DETECTING BCR-ABL FUSION

CHRONIC PHASE	ACCELARATED PHASE	BLAST PHASE
• The BM cellularity is increased due to granulocytic proliferation • Pseudo Gaucher cells are commonly found in chronic phase. These histiocytes are secondary to increased cell turnover, are derived from the neoplastic clone. • The megakaryocytes of CML are smaller than normal and have hypolobated nuclei.(Dwarf megakaryocytes), although they may be normal or slightly decreased in number.	• BM is hypercellular and myelodysplasia is seen. • The increase in myeloid lineage blasts may be readily appreciated with stain for CD34 performed on biopsy. • Large clusters or sheets of small, abnormal megakaryocytes associated with marked reticulin and collagen fibrosis are commonly observed.	• Large aggregates or clusters of blasts in the bone marrow • During transformation lymphoblasts are also seen

1. **Conventional cytogenetics (Karyotyping)**
2. **FISH**
3. **PCR**

1.CONVENTIONAL CYTOGENETICS (KARYOTYPING)

CONVENTIONAL CYTOGENETICS

Figure 4. - Cytogenetic analysis by G-banding identified 46,XX,t(9;22)(q34;q11.2)**.**

****Ai, D., Liu, W., Lu, G., Patel, K.P., & Chen, Z. (2015). Extramedullary blast crisis as initial presentation in chronic myeloid leukemia with the e1a2 BCR-ABL1 transcript: A case report. Molecular and Clinical Oncology, 3, 1319-1322.

ADVANTAGES

- All chromosomes evaluated simultaneously, so in addition to Philadelphia chromosome, other abnormalities can also be detected.
- Can be performed on peripheral blood or bone marrow

DISADVANTAGES

- Lower sensitivity

- Requires dividing or metaphase cells
- Takes 2-3 weeks
- Inherent sensitivity is low which further decreases by 5% when peripheral blood is used.
- 5% patients are ph- because of cryptic BCR-ABL rearrangement. These require FISH/RT-PCR.

FLUORESCENE IN SITU HYBRIDIZATION

A FISH analysis relies on the colocalization of large genomic probes specific to the BCR and ABL genes. Comparison of simultaneous marrow and blood samples by FISH analysis shows high concordance. FISH studies may have a false positive range of 1%-5% depending on the probes used.

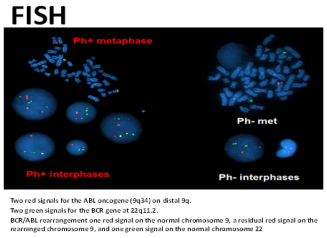

Fig 5. BCR-ABL1 fusion in bone marrow cells detected by fluorescence in situ hybridization. Ph, Philadelphia chrom osome***.**

*****Ai, D., Liu, W., Lu, G., Patel, K.P., & Chen, Z. (2015). Extramedullary blast crisis as initial presentation in chronic myeloid leukemia with the e1a2 BCR-ABL1 transcript: A case report. Molecular and Clinical Oncology, 3, 1319-1322

ADVANTAGES

- Can be done interphase or metaphase cells
- Fast results (within 24hrs)
- Higher sensitivity compared to conventional cytogenetics.
- Evaluates more number of cells (200-500 cells at a time)
- Cryptic translocations can be detected

DISADVANTAGES

- Detects only one abnormality at one time
- Lower sensitivity than PCR
- Cannot detect Minimal residual disease

POLYMERASE CHAIN REACTION(PCR)

Reverse transcriptase-polymerase chain reaction (RT-PCR) amplifies the region around the splice junction between BCR and ABL1. It is highly sensitive in detecting minimal residual disease. PCR testing can either be qualitative (QPCR), providing information about the presence of the BCR-ABL1 transcript, or quantitative, assessing the amount of BCR-ABL1 transcripts. Qualitative PCR is useful for diagnosing CML; quantitative PCR is ideal for monitoring residual disease.

Simultaneous peripheral blood and marrow QPCR studies show a high level of concordance. False-positive and false-negative results can happen with PCR. False-negative results may be from poor-quality RNA or failure of the reaction; false-positive results can be due to contamination(15,16).

Baseline cytogenetic analysis allows the detection of clonal evolution, particularly i(17)(q10)-7/del7q, and 3q26.2 rearrangements, associated with a relatively poor prognosis[16]

Baseline reverse transcriptase-polymerase chain reaction is imperative to identify the specific type of rearrangement that can be appropriately followed when assessing for response to TKI therapy.

About 2%-5% of patients have e13a3 or e14a3 (not e13a2 or e14a2) variants of p210 BCR-ABL1 or p230 transcripts that may yield a false negative PCR by routine probes and (if not tested at diagnosis) would give the false impression that a patient is in "complete molecular response" on TKI.

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