Overview of Molecular Quantification of the BCR-ABL Oncogene in CML Patients

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Chronic myeloid leukemia (CML) is considered a common blood cancers and accounts for approximately 15–20% of the total cases of leukemia. Recent studies indicated that above 95% of patients suffering of CML have been found with a distinctive Philadelphia chromosome that originates from a mutual translocation between both arms of chromosomes 9 and 22. During this mutation the translocation of the ABL gene located on chromosome 9 get transferred to the breakpoint cluster region (BCR) of chromosome 22 as an effect of a joined BCR-ABL gene. Furthermore, BCR-ABL oncogene is characteristically found in CML, causing cells to divide uncontrollably and inducing severe consequences among CML patients. In line with this, applying quantification technique of the BCR-ABL gene using molecular approaches is crucial for patient controlling, initiation of the proper treatment, measurement of response to therapy, and prediction of relapse. Of greater significance, molecular assay and monitoring of the BCR-ABL gene in CML using quantitative RT-PCR provides physicians with essential diagnostic and prognostic information.

Keywords: BCR-ABL; CML; leukemia; Genetics.

Leukemia

Leukemia is a type of cancer that takes place when the bone marrow start to release an enormous number of atypical white blood cells that accumulate in the blood and in different organs. Two methods of leukemia classification are used: the first is by its speed of progression (acute or chronic), and the second is by the type of white blood cells involved (lymphoid or myeloid). Myeloid leukemias start in neutrophils and monocytes, which are bacterial and fungal fighters, while the progression of lymphocytic leukemias occurs in natural killer cells, B lymphocytes, and T lymphocytes, which are important in the production of antibodies that fight infections.

The main types of leukemia are chronic myeloid leukemia (CML), acute lymphocytic leukemia, chronic lymphocytic leukemia and acute myeloid leukemia. Common leukemia symptoms include fever, weakness, frequent infections, and enlarged liver, spleen, and lymph nodes. Leukemia is usually diagnosed by physical examination followed by blood count and bone marrow biopsies in the early stages. Recently, Molecular analysis is
playing a significant role where RT-PCR competent to identify leukemia-specific gene or mutation1.

There are no clear causes for the development of different types of leukemia; however, most cases are caused by genetic alterations in the DNA that trigger oncogenes or disable the function of tumor suppressor genes4. In addition, some genetic disorders and chromosomal abnormalities, such as Down Syndrome, can be considered risk factors. Other environmental factors include radiation, chemicals, and viruses5.

CML is styled by abnormal growth and increase of myeloid cells in the blood. It is a class of myeloproliferative neoplasm linked to chromosomal translocation identified as the “Philadelphia chromosome”6, which involves chromosomes 9 and 22 switching places, and leads to fusion between part of the breakpoint cluster region (BCR) of chromosome 22 and the ABL gene on chromosome 97. This increases the speed of cell division and activates the proteins responsible for the regulation of the cell cycle8. In addition, BCR-ABL fusion affects DNA repair and decreases genome stability, which results in other genomic abnormalities that speed up cell division9.

**Current WHO criteria for the diagnosis and classification of CML**

World health organization classified the CML into three main significantly prognostic clinical phases before tyrosine kinase inhibitor (TKI) treatment. The main and essential pathological characteristics of Philadelphia chromosome (+) CML in the WHO 2016 classification are defined as the following three phases10.

**Chronic phase CML**

Chronic phase CML defined as a myeloproliferative neoplasm which is characterized by chromosomal translocation (t9;22) (q34.1;q11.2), that results in the formation of BCR-ABL fusion gene11. This gene fusion causes the formation of the abnormal chromosome (Philadelphia chromosome), that results in an increase in peripheral blood granulocyte cells and bone marrow myeloid precursors12.

**Accelerated CML phase AP**

In this phase, CML cells number is fast growing and symptomatic, including fever, fatigue, splenomegaly, and weight loss13. The typical bone marrow histopathology for the accelerated CML phase was explained before the TKI-treatment era. After the TKI treatment era, the criteria were adjusted considering the therapy14. CML cases that show respond to the treatment by TKI-era are characterized by normalization finding of the bone marrow cellular composition after treatment15.

This phase expresses the presence of BCR-ABL or t(9;22) (q34.1;q11.2) by molecular or chromosomal analysis (karyotyping), together with TKI resistance or genomic-cytogenetic evolution16. Increasing abnormal white blood cell (WBC) counts occur despite TKI treatment (WBC count more than 10×10^9/L), thrombocytosis (platelet count more than 1000×10^9/L), or thrombocytopenia (platelet count less than 100×10^9/L) unrelated to therapy (between 10 to 19 % blast cells and around 20 % or more basophils)17.

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**Fig. 1.** Peripheral blood smear shows myeloid precursors and a few blasts seen in chronic myelogenous leukemia. Source, MLS Collection, University of Alberta, https://doi.org/10.7939/R3NZ8155H

**Fig. 2.** Bone marrow aspiration shows myeloid hyperplasia and a few blasts seen in chronic myelogenous leukemia. Source, University of Alberta, https://doi.org/10.7939/R38P5VR48
Fig. 3. Gene mutations in CML case include the fused gene called BCR-ABL and the abnormal Philadelphia chromosome. Source, https://www.cancersupportcommunity.org/chronic-myeloid-leukemia

**Blastic phase CML (BP-CML)**

The CML blastic phase involves the presence of significantly increased blast infiltration, accordance with the typical CML blastic phase clinical presentation\(^1\). Further, the blastic phase shows the presence of BCR-ABL gene or \(t(9;22)\) (q34.1;q11.2) by molecular or chromosomal analysis (karyotyping), with genomic-cytogenetic progress or resistance of TKI treatment\(^1\). This involves at least 20% blast cells presence in the peripheral blood or bone marrow with or without the presence of extra medullary-blastic infiltration in organ or tissue\(^2\).

**CML diagnosis**

Many patients with CML are asymptomatic when they diagnosed first time. The most common finding of CML is leukocytosis (abnormal high WBC count). Often discovered during routine blood tests (complete blood count (CBC)) or checkups for an unrelated health problem\(^3\). Usually, to discover and diagnose new CML cases, physicians request a variety of routine laboratory tests to examine blood cells and bone marrow\(^4\). A hematopathologist (who specializes in identifying hematologic disorders) reviewing white blood cells morphology and count using a microscope to evaluate the bone marrow and peripheral blood smear\(^5\).

**Laboratory tests used to diagnose CML**

**Complete Blood Count (CBC) with differential**

The CBC is a routine hematology test used to measure the number of red blood cells, total white blood cell count, and platelets. Also, CBC measures the amount of hemoglobin in the red blood cells (MCH) and the percentage of red blood cells to the whole blood (HCT)\(^6\). The CBC test includes a differential white cell count to measure the different types and count of white blood cells in peripheral blood sample. Figure 1, demonstrate the presence of blast cells in CML peripheral blood smear. CML patients often have a leukocytosis (greatly increased WBC count), decreased red blood cell count (anemia), and an increase or decrease in platelet count (thrombocytopenia) based on the severity of the disease\(^7\).

**Bone marrow aspiration and biopsy**

Bone marrow examinations is a hematopathologic test used to identify abnormalities in bone marrow cells. The bone marrow aspiration and biopsy are taken by the hematologist from the hip bone. Figure 2, shows bone marrow aspiration and the presence of blast cells in CML patient. Both sample aspiration and biopsy are examined by hematopathologist under a microscope to look for abnormal cells (such as blast cells) and indicate chromosomal abnormality and other blood cell changes\(^8\).

**Cytogenetic analysis**

The cytogenetic analysis test is an effective laboratory test that involves the study of chromosomal abnormalities in patient using bone marrow aspiration sample\(^9\). Figure 1, illustrate the gene mutation in CML (Philadelphia chromosome). A bone marrow aspiration sample is examined by cytogenetic using a microscope for Philadelphia chromosome detection and any chromosomal changes or abnormalities\(^10\).

**Fluorescence in situ hybridization (FISH)**

FISH is a cytogenetic lab test that is used to examine chromosomes and genes in patient cells\(^11\). FISH test is more sensitive and accurate method for detecting CML by identifying the presence of the BCR-ABL gene than standard cytogenetic tests that identify the Philadelphia chromosome\(^12\).

**Quantitative polymerase chain reaction (qPCR)**

qPCR is considered as an effective technique and actual sensitive method for
distinguishing and measuring the quantity of BCR-ABL gene\textsuperscript{30}. Using bone marrow or blood sample, More further, qPCR technique is able to detect a low amounts of the BCR-ABL gene particularly when Philadelphia chromosome cannot be identified in blood or bone marrow cells in cyrogenetic testing\textsuperscript{31}. The BCR-ABLE qPCR assay is a fundamental tool for aiding in CML diagnosis from the genetic side\textsuperscript{32}. Likewise, physicians practice BCR-ABLE to assess the ongoing patient situation and subsequently prescribe appropriate medication and the ideal dose\textsuperscript{33}. Consistently, pre-analysis and analysis for such genetic testing are very sensitive, and not complying with the correct techniques could affect the final outcomes\textsuperscript{34}.

Processing the sample for qPCR BCR-ABL testing requires extraordinary parameters, such as using an EDTA tube for blood collection in which the anticoagulant substance provides the ideal preservation of cellular components and the blood cells morphology\textsuperscript{35}. In addition, it is crucial to perform the processing step as soon as the specimens are received in order to avoid any changes to the WBCs that subsequently affect the final yield of extracted DNA\textsuperscript{36}. Approaching the highest quality standards in terms of processing samples will reflect the eventual result, which will be elaborated on in the patient’s treatment plan\textsuperscript{37}. Furthermore, the BCR-ABLE assay needs to be carried out by skilled personnel as a vital part of quality\textsuperscript{38}. Programming the optimal time for blood collection is a vital part of the quality standard, which assists in testing BCR-ABL with no postponement and avoiding subsequent freezing of the patient’s specimens\textsuperscript{39}. Considering these parameters will eventually support accomplishing the perfect end result, which will lead to prescribing precise medication\textsuperscript{40}.

CONCLUSION

Molecular inspection for BCR-ABLE gene is being a crucial method to evaluate patients with CML illness in early phase to avoid inadequate diagnosis. More in depth, The BCR-ABL molecular results provide valuable guidance for starting medication. In addition, BCR-ABL results should be engaged into consideration before proceeding any type of clinical decisions. Likewise, quantitative BCR-ABL testing affords the advantage of detecting small quantity of BCR-ABL oncogene that act to reflect tendencies in tumor burden.

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Competing interests

The authors have no conflicts of interest. We declare no conflict of interest, including any financial, personal, or other relationships with other people or organizations, within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, our work.

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