Laboratory investigation of chronic myeloid leukemia

Introduction

Chronic Myeloid Leukemia (CML) is a disorder of hematopoietic stem cells characterized by chromosomal translocation between chromosomes 9 and 22, leading to the production of an oncoprotein which causes the hallmark features of leukemia. It is characterized by the infiltration of large numbers of immature myeloid progenitor cells in the blood stream leading to symptoms such as anemia, fatigue, malaise, splenomegaly, night sweats, low appetite and breathlessness (Simoneau, 2012). It is best identified by a complete blood count, peripheral blood smear, karyotyping analysis, fluorescence in situ hybridization (FISH), and polymerase chain reaction (PCR)-based methods. This paper aims to provide an overview of the pathophysiologic progression of CML and the widely adopted diagnostic measures.

Pathogenesis of Chronic Myeloid Leukemia (CML)

i. Genetic basis

CML is a genetic disorder, considered to be associated with the Philadelphia chromosome. This chromosome is the result of an exchange between the BCR (Breakpoint Cluster Region) and the ABL (Abelson) genes located on chromosomes 9 and 22 respectively. The fusion gene product of these two genes gives rise to an oncoprotein, $p210^{BCR/ABL}$. This induces malignancy due to an increased tyrosine kinase activity and increased affinity for the actin cytoskeleton. The presence of this oncoprotein leads to leukemic cell growth in hematopoietic stem cells due to achievement of growth factor independence. Hence, this disease features as presence of malignant myeloid cells in the circulation, activation of antiapoptotic and mutagenic pathways, and abnormal functioning of cytoskeleton (Deininger et al., 2000).

Like other oncoproteins, the oncoprotein p210 causes the initiation of numerous downstream signaling pathways responsible for the promotion of abnormal cell growth, suppression of cell death pathways, alteration of cell adhesion pathways and instability in the genetic makeup. Large numbers of myeloid cells in different stages of maturation are seen in bone marrow, spleen and peripheral circulation (Thielen et al., 2011).

ii. Cellular basis

Cellularly, CML is a disorder of hematopoietic stem cells and it is clonal and myeloproliferative in nature. It begins with the accumulation of myeloid progenitors in the blood and extramedullary tissues and this manifests as the chronic phase. After an extended period of about 3 to 4 years, there occurs a sudden halt in the maturation of the myeloid or the lymphoid lineage (Shet et al., 2002). Hematopoietic stem cells are distinguished by two important properties – capacity for self-renewal and capacity to give rise to all hematopoietic cell lineages. Hence, this is how the underlying genetic abnormality is passed on to an entire lineage of cells resulting in an accumulation of abnormal myeloid cells in the circulation (Thielen et al., 2011).

CML is a heterogeneous disorder and encompasses the myeloid, erythroid, megakaryocytic, B-lymphoid and T-lymphoid elements. The presence of the genetic abnormality in CML cells leads to a decreased interaction between the affected hematopoietic cell and the stromal matrix. This leads to a delay in cell differentiation capturing the cells in an immature stage of their life cycle (Kantarjian et al., 1993).

The uncontrolled myeloid cell production leads to a steady increase in white blood cell count, and occasionally, involvement of platelets. When the bone marrow is completely filled

with these abnormal leukemic cells, they overflow into the bloodstream and are found in the peripheral blood circulation (Marley and Gordon, 2005).

Diagnostic tests for CML

i.Full blood count and blood film

A complete blood count (CBC) and a blood film are the most basic and initial tests for the presumptive diagnosis of CML and elimination of differential diagnoses. In a patient with CML, the white blood cell (WBC) count can range anywhere between 50,000/l to 8 lacs/µl (Davis et al., 2014). A peripheral blood smear shows a range of WBCs such as promyelocytes, myelocytes, mature neutrophils and blast cells (contributing less than 10% of all WBCs). An important feature of a blood film of a CML patient is basophilia which is typically less than 20%. An absence of basophilia often rules out CML (Talwar and Saha, 2010). Other features include thrombocytosis - up to 10 lacs/µl, normocytic normochromic anemia, low levels of leukocyte alkaline phosphatase, increased vitamin B12 binding capacity of serum, and an elevated count of uric acid and lactate dehydrogenase (Patel et al., 2013).

This technique is not very reliable for the confirmatory diagnosis of CML as the findings can change with the phase of the disease. In the early stages, leukocytosis may not be very prominent and may be overlooked. However, basophilia is invariably present even in the very early stages. A large number of segmented neutrophils may be seen in some cases pointing to chronic neutrophilic leukemia (CNL). If an insufficient leukocytosis is accompanied by prominent thrombocytosis, it may indicate thrombocythemia. Prominent monocytosis may indicate chronic myelomonocytic leukemia (Vardiman, 2009).

Treatment response monitoring of CML patients is usually done after 3 months of commencing treatment. For an effective treatment response, the WBC count should be lower than 10,000 cells/µl and there should be no immature WBCs seen in the peripheral blood smear. The platelet count should be less than 4.5 lac cells/µl and there should be no signs of leukostasis (Erter and Garzon, 2009).

ii. Cytogenetic analysis

The cytogenetic analysis of a suspected CML specimen is done by three methods – identification of the Philadelphia chromosome and fluorescence in situ hybridization (FISH) (Buyukasik et al., 2010). The underlying genetic abnormality is best identified by G-banding karyotyping, where 25 to 30 metaphase (or dividing) cells from the bone marrow are analyzed (Wei, 2007).

FISH is used to locate the positions of the BCR and ABL genes in the chromosomes of about 200 interphase cells. A BCR-binding fluorescent probe and an ABL-binding fluorescent probe are designed with two distinct colors and allowed to bind to the respective genes in denatured chromosomes in the sample. A triple probe FISH may also be used where a third fluorescent probe is designed to bind to the breakpoint region of BCR or ABL making the technique more accurate. Similarly, four probes may also be used in double FISH to span the breakpoints of both the genes (Cortes et al., 2011).

The main limitation of karyotyping is the limited number of cells used for analysis. Hence, the sensitivity of detection of leukemic cells by this method is just 3 to 4%. However, this is still the preferred method of diagnosis especially in treatment response monitoring due to its ability to quantify the number of leukemic cells in a given sample. The problem with FISH is its

high rate of false positives of up to 3 to 10% making it unreliable for cases identifying less than 10% leukemic cells in a sample (Wei, 2007).

iii. Molecular analysis

This is mainly done by the amplification of the BCR-ABL gene by real-time quantitative polymerase chain reaction (q-PCR). This aims to detect the presence of the BCR-ABL mRNA in peripheral blood samples (Cortes et al., 2011).

Treatment response monitoring is done molecularly by tracking the decline in the number of mRNA transcripts over a specific period of time, usually 3 months. It is a highly preferred tool for monitoring as it is currently the most sensitive method for determination of the level of CML in a sample (Baccarani et al., 2012).

This technique is more sensitive than cytogenetic techniques as it can identify even low levels of leukemic cells in a sample (Cortes et al., 2011). However, it is subject to both false positive and false negative results. False negative results might occur due to poor quality of the mRNA sample or errors in performing the reaction. False positives might occur due to contamination of the sample (Jabbour and Kantarjian, 2014).

Conclusion

In conclusion, the genetic nature of CML makes it quite difficult to achieve a complete cure in a short period of time and continuous monitoring at regular intervals of time is essential for proper management outcomes. The most reliable technique, by far, is molecular analysis due to its high sensitivity and accuracy. Cytogenetic analysis methods have very low sensitivity and cannot be used as confirmatory tests. A blood workup may lead to a number of differential diagnoses and may require further tests to provide a confirmatory diagnosis of CML.

References

Baccarani, M., Pileri, S., Steegmann, J. L., Muller, M., Soverini, S., and Dreyling, M., 2012. Chronic Myeloid Leukemia: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*, 23 (Supplement 7), pp. vii72-vii77.

Buyukasik, Y., Haznedaroglu, Y., and Ilhan, O., 2010. Chronic Myeloid Leukemia: practical issues in diagnosis, treatment and follow-up. *International Journal of Hematology and Oncology*, 20 (2).

Cortes, J., Quintas-Cardama, A., and Kantarjian, H. M., 2011. Monitoring molecular response in chronic myeloid leukemia. *Cancer*, 117, pp. 1113-1122.

Davis, A. S., Viera, A. J., and Mead, M. D., 2014. Leukemia: an overview for primary care. *American Family Physician*, 89 (9), pp. 731-738.

Deininger, M. W. N., Goldman, J. M., and Melo, J. V., 2000. The molecular biology of chronic myeloid leukemia. Blood, 96 (10), pp. 3343-3356.

Erter, J. W., and Garzon, R., 2009. Current approach to treating chronic myeloid leukemia. *Hematology Board Review Manual*, 4 (1).

Jabbour, E., and Kantarjian, H., 2014. Chronic Myeloid Leukemia: 2014 update on diagnosis, monitoring, and management. *American Journal of Hematology*, 89, pp. 547-556.

Kantarjian, H. M., Deisseroth, A., Kurzrock, R., Estrov, Z., and Talpaz, M., 1993. Chronic Myelogenous Leukemia: A concise update. *Blood*, 82 (3), pp. 691-703.

Marley, S. B., and Gordon, M. Y., 2005. Chronic Myeloid Leukemia: stem cell derived but progenitor cell driven. *Clinical Science*, 109, pp. 13-25.

Patel, A. A., Patel, K. M., and Jain, A., 2013. Chronic Myeloid Leukemia in childhood. *GCSMC Journal of Medical Science*, 3 (2).

Shet, A. S., Jahagirdar, B. N., and Verfaillie, C. M., 2002. Chronic Myelogeneous Leukemia: mechanisms underlying disease progression. *Leukemia*, 16, pp. 1402-1411.

Simoneau, C., 2012. Treating Chronic Myeloid Leukemia: improving management through understanding of the patient experience. *Clinical Journal of Oncology Nursing*, 17 (1), pp. E13-E20.

Talwar, V., and Saha, R., 2010. Chronic Myeloid Leukemia: Overview and recent advances. *Medicine Update*, 20, pp. 609-612.

Thielen, N., Ossenkoppele, G. J., Schuurhuis, G. J., Janssen, J. J. W. M., 2011. New insights into the pathogenesis of chronic myeloid leukemia: towards a path to cure. *The Netherlands Journal of Medicine*, 69 (10), pp. 430-440.

Vardiman, J. W., 2009. Chronic Myelogenous Leukemia, BCR-ABL1+. *American Journal of Clinical Pathology*, 132, pp. 250-260.

Wei, Y., 2007. *Molecular monitoring of chronic myeloid leukemia treated with Imatinib Mesylate*. PhD Thesis, Goteborg University.