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The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

Editorial

Chronic myelogenous (or myeloid) leukemia (**CML**), also known as **chronic granulocytic leukemia (CGL)**, is a cancer of the white blood cells. It is a form of leukemia characterized by the increased and unregulated growth of predominantly myeloid cells in the bone marrow and the accumulation of these cells in the blood. CML is a clonal bone marrow stem cell disorder in which proliferation of mature granulocytes (neutrophils, eosinophils, and basophils) and their precursors is the main finding. It is a type of myeloproliferative disease associated with a characteristic chromosomal translocation called the Philadelphia chromosome. CML is now largely treated with targeted drugs called tyrosine kinase inhibitors (TKIs), such as Gleevec/Glivec (imatinib), Sprycel (dasatinib), Tasigna (nilotinib), or Bosulif (bosutinib) which have led to dramatically improved long term survival rates (95.2%) since the introduction of Gleevec in 2001. These drugs have revolutionized treatment of this disease and allow most patients to have a good quality of life when compared to the former chemotherapy drugs. The **DISEASE DIAGNOSIS** segment of this issue delves deep into the clinico - diagnostic issues related to CML.

At the request of our friends from a Central Asian Republic, this whole issue is dedicated to the diagnosis of CML.

Thus, **TROUBLESHOOTING** section outlines “Leucocyte Alkaline Phosphatase” for you and at the same time it briefly presents the special staining techniques as applied to various haematologic malignancies. The process is explained in layman’s language.

The **INTERPRETATION** portion completes the CML diary by discussing Philadelphia Chromosome in ample detail.

Hereafter, never should we fumble while diagnosing CML, it should be as easy as reporting iron deficiency anaemia.

The Crux is now almost a decade old. For the tenth time hence, we wish you A VERY HAPPY PROSPEROUS, PEACEFUL AND PLEASURABLE NEW YEAR.

Tulip Group

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DISEASE DIAGNOSIS

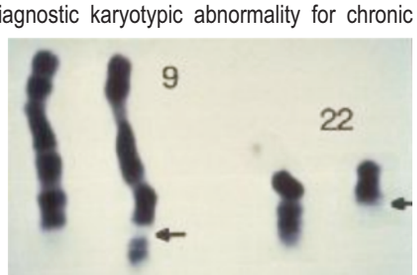
CHRONIC MYELOID LEUKEMIA

Background

Chronic myelogenous leukemia (CML), also known as chronic myeloid leukemia, is a myeloproliferative disorder characterized by increased proliferation of the granulocytic cell line without the loss of their capacity to differentiate. Consequently, the peripheral blood cell profile shows an increased number of granulocytes and their immature precursors, including occasional blast cells. CML is one of the few cancers known to be caused by a single, specific genetic mutation. More than 90% of cases result from a cytogenetic aberration known as the Philadelphia chromosome. CML progresses through 3 phases: chronic, accelerated, and blast. In the chronic phase of disease, mature cells proliferate; in the accelerated phase, additional cytogenetic abnormalities occur; in the blast phase, immature cells rapidly proliferate. Approximately 85% of patients are diagnosed in the chronic phase and then progress to the accelerated and blast phases after 3-5 years. The diagnosis of CML is based on the histopathologic findings in the peripheral blood and the Philadelphia chromosome in bone marrow cells. CML accounts for 20% of all leukemias affecting adults. It typically affects middle-aged individuals. Uncommonly, the disease occurs in younger individuals. Younger patients may present with a more aggressive form of CML, such as in accelerated phase or blast crisis. Uncommonly, CML may appear as a disease of new onset in elderly individuals. The goals of treatment are to achieve hematologic, cytogenetic, and molecular remission. Although a variety of medications have been used in CML, including myelosuppressive agents and interferon alfa, the tyrosine kinase inhibitor imatinib mesylate is currently the agent of choice, and other drugs in this category are playing increasingly important roles. However, allogeneic bone marrow transplantation is currently the only proven cure for CML.

Pathophysiology

CML is an acquired abnormality that involves the hematopoietic stem cell. It is characterized by a cytogenetic aberration consisting of a reciprocal translocation between the long arms of chromosomes 22 and 9 [t(9;22)]. The translocation results in a shortened chromosome 22, an observation first described by Nowell and Hungerford and subsequently termed the Philadelphia (Ph1) chromosome after the city of discovery. The Philadelphia chromosome, which is a diagnostic karyotypic abnormality for chronic myelogenous leukemia, is shown in this picture of the banded chromosomes 9 and 22. Shown is the result of the reciprocal translocation of 22q to the lower arm of 9 and 9q (c-abl to a specific breakpoint cluster region [bcr] of chromosome 22 indicated by the arrows). This translocation relocates an oncogene called *ABL* from the long arm of chromosome 9 to a specific breakpoint cluster region (*BCR*) in the long arm of chromosome 22. The *ABL* oncogene encodes a tyrosine protein kinase. The resulting *BCR/ABL* fusion gene encodes a chimeric protein with strong tyrosine kinase activity. The expression of this protein leads to the development of the CML phenotype, through processes that are not yet fully understood. The presence of *BCR/ABL* rearrangement is the hallmark of CML, although this rearrangement has also been described in other diseases. It is considered diagnostic when present in a patient with clinical manifestations of CML. The initiating factor of CML is still unknown, but exposure to ionizing radiation has been implicated, as observed in the increased prevalence among survivors of the atomic bombing of Hiroshima and Nagasaki. Other agents, such as benzene, are possible causes.



Clinical History

The clinical manifestations of chronic myelogenous leukemia (CML) are insidious. The disease is often discovered incidentally in the chronic phase, when an elevated white blood cell (WBC) count is revealed by a routine blood count or when an enlarged spleen is found on a general physical examination. Nonspecific symptoms of fatigue and weight loss may occur long after the onset of the disease. Loss of energy and decreased exercise tolerance may occur during the chronic phase after several months. Patients often have symptoms related to enlargement of the spleen, liver, or both. The large spleen may encroach on the stomach and cause early satiety and decreased food intake. Left upper quadrant abdominal pain described as "gripping" may occur from spleen infarction. The enlarged spleen may also be associated with a hypermetabolic state, fever, weight loss, and chronic fatigue. The enlarged liver may contribute to the patient's weight loss. Some patients with CML have low-grade fever and excessive sweating related to hypermetabolism. In some patients who present in the accelerated, or acute, leukemia phase of the disease (skipping the chronic phase), bleeding, petechiae, and ecchymoses may be the prominent symptoms. In these situations, fever is usually associated with infections. Bone pain and fever, as well as an increase in bone marrow fibrosis, are harbingers of the blast phase.

Physical Examination

Splenomegaly is the most common physical finding in patients with chronic myelogenous leukemia (CML). In more than 50% of the patients with CML, the spleen extends more than 5 cm below the left costal margin at time of discovery. The size of the spleen correlates with the peripheral blood granulocyte counts, with the largest spleens being observed in patients with high WBC counts. A very large spleen is usually a harbinger of the transformation into an acute blast crisis form of the disease. Hepatomegaly also occurs, although less commonly than splenomegaly. Hepatomegaly is usually part of the extramedullary hematopoiesis occurring in the spleen. Physical findings of leukostasis and hyperviscosity can occur in some patients, with extraordinary elevation of their WBC counts, exceeding 300,000-600,000 cells/µL. Upon funduscopy, the retina may show papilledema, venous obstruction, and hemorrhages. The blast crisis is marked by an increase in the bone marrow or peripheral blood blast count or by the development of soft-tissue or skin leukemic infiltrates. Typical symptoms are due to increasing anemia, thrombocytopenia, basophilia, a rapidly enlarging spleen, and failure of the usual medications to control leukocytosis and splenomegaly.

Diagnostic Considerations

Problems to be considered include the following: Acute myeloid leukemia, Chronic myelomonocytic leukemia, Chronic neutrophilic leukemia, Thrombocythemia, Leukemoid reactions from infections (chronic granulomatous [eg, tuberculosis]), Tumor necrosis.

Differential Diagnoses

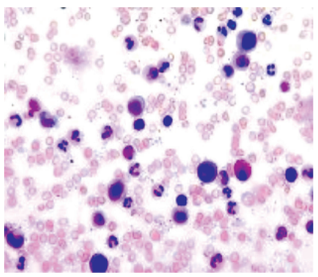
Agnogenic Myeloid Metaplasia with Myelofibrosis, Essential Thrombocytosis, Myelodysplastic Syndrome, Myeloproliferative Disease, Polycythemia Vera.

Approach Considerations

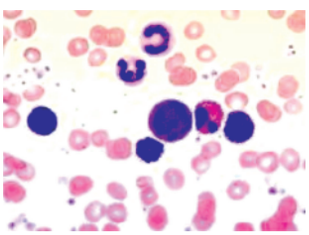
The workup for chronic myelogenous leukemia (CML) consists of a complete blood count with differential, peripheral blood smear, and bone marrow analysis. Although typical hepatomegaly and splenomegaly may be imaged by using a liver/spleen scan, these abnormalities are often so obvious clinically that radiologic imaging is not necessary. The diagnosis of CML is based on the histopathologic findings in the peripheral blood and the Philadelphia (Ph1) chromosome in bone marrow cells. Other laboratory abnormalities include hyperuricemia, which is a reflection of high bone marrow cellular turnover, and markedly elevated serum vitamin B-12–binding protein (TC-I). The latter is synthesized by the granulocytes and reflects the degree of leukocytosis.

Blood Count and Peripheral Smear

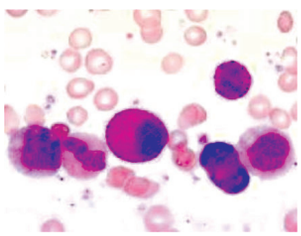
In CML, the increase in mature granulocytes and normal lymphocyte counts (low percentage due to dilution in the differential count) results in a total WBC count of 20,000-60,000 cells/µL. A mild increase in basophils and eosinophils is present and becomes more prominent during the transition to acute leukemia. These mature neutrophils, or granulocytes, have decreased apoptosis (programmed cell death), resulting in accumulation of long-lived cells with low or absent enzymes, such as alkaline phosphatase (ALP). Consequently, the leukocyte alkaline phosphatase stains very low to absent in most cells, resulting in a low score. The peripheral blood smear in patients with CML shows a typical leukoerythroblastic blood picture, with circulating immature cells from the bone marrow. Blood film at 400X magnification demonstrates leukocytosis with the presence of precursor cells of the myeloid lineage.



In addition, basophilia, eosinophilia, and thrombocytosis can be seen. The transitional or accelerated phase of CML is characterized by poor control of blood counts with myelosuppressive medication, the appearance of peripheral blast cells (≥ 15%), promyelocytes (≥ 30%), basophils (≥ 20%), and reduction in platelet counts to less than 100,000 cells/µL unrelated to therapy. Promyelocytes and basophils are shown in the images below.



Blood film at 1000X magnification shows a promyelocyte, an eosinophil and 3 basophils.

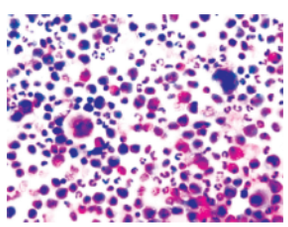


Blood film at 1000X magnification demonstrates the whole granulocytic lineage, including an eosinophil and a basophil.

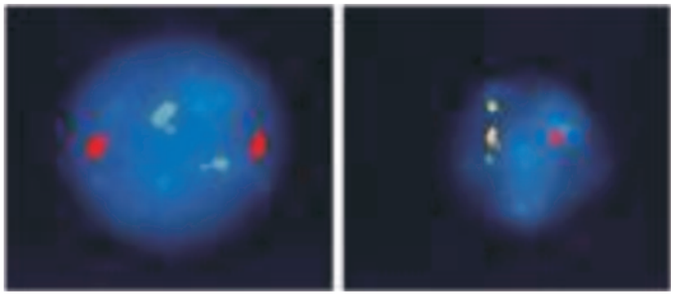
Signs of transformation or accelerated phase in patients with CML are poor control of blood counts with myelosuppression or interferon, increasing blast cells in peripheral blood with basophilia and thrombocytopenia not related to therapy, new cytogenetic abnormalities, and increasing splenomegaly and myelofibrosis. In approximately two thirds of cases, the blasts are myeloid. However, in the remaining one third of patients, the blasts exhibit a lymphoid phenotype, further evidence of the stem cell nature of the original disease. Additional chromosomal abnormalities are usually found at the time of blast crisis, including additional Ph1 chromosomes or other translocations. Early myeloid cells such as myeloblasts, myelocytes, metamyelocytes, and nucleated red blood cells are commonly present in the blood smear, mimicking the findings in the bone marrow. The presence of the different midstage progenitor cells differentiates CML from the acute myelogenous leukemias, in which a leukemic gap (maturation arrest) or hiatus exists that shows absence of these cells. A mild to moderate anemia is very common at diagnosis and is usually normochromic and normocytic. The platelet counts at diagnosis can be low, normal, or even increased in some patients (>1 million in some).

Bone Marrow Analysis

The bone marrow is characteristically hypercellular, with expansion of the myeloid cell line (eg, neutrophils, eosinophils, basophils) and its progenitor cells. Megakaryocytes (see the image below) are prominent and may be



increased. Mild fibrosis is often seen in the reticulin stain. Bone marrow film at 400X magnification demonstrates clear dominance of granulopoiesis. The number of eosinophils and megakaryocytes is increased. Cytogenetic studies of the bone marrow cells, and even peripheral blood, should reveal the typical Ph1 chromosome, which is a reciprocal translocation of chromosomal material between chromosomes 9 and 22. This is the hallmark of CML (though it can also be found in certain other conditions too), found in almost all patients with the disease and present throughout the entire clinical course of CML. In addition, the chimeric *BCR/ABL* messenger RNA (mRNA) that characterizes CML can be detected by polymerase chain reaction (PCR). This is a sensitive test that requires just a few cells and is useful in monitoring minimal residual disease (MRD) to determine the effectiveness of therapy. *BCR-ABL* mRNA transcripts can also be measured in peripheral blood. Karyotypic analysis of bone marrow cells requires the presence of a dividing cell without loss of viability because the material requires that the cells go into mitosis to obtain individual chromosomes for identification after banding. This is a slow, labor-intensive process. The new technique of fluorescence in situ hybridization (FISH) uses labeled probes that are hybridized to either metaphase chromosomes or interphase nuclei, and the hybridized probe is detected with fluorochromes. This technique is a rapid and sensitive means of detecting recurring numerical and structural abnormalities.



Fluorescence in situ hybridization using unique-sequence, double-fusion DNA probes for bcr (22q11.2) in red and c-abl (9q34) gene regions in green. The abnormal bcr/abl fusion present in Philadelphia chromosome–positive cells is in yellow (right panel) compared with a control (left panel).

Two forms of the *BCR/ABL* mutation have been identified. These vary according to the location of their joining regions on *bcr* 3' domain. Approximately 70% of patients who have the 5' DNA breakpoint have a b2a2 RNA message, and 30% of patients have a 3' DNA breakpoint and a b3a2 RNA message. The latter is associated with a shorter chronic phase, shorter survival, and thrombocytosis. CML should be differentiated from Ph1-negative diseases with negative PCR results for *BCR/ABL* mRNA. These diseases include other myeloproliferative disorders and chronic myelomonocytic leukemia, which is now classified with the myelodysplastic syndromes. Additional chromosomal abnormalities, such as an additional or double Ph1-positive chromosome or trisomy 8, 9, 19, or 21; isochromosome 17; or deletion of the Y chromosome, have been described as the patient enters a transitional form or accelerated phase of the blast crisis as the Ph chromosome persists. Patients with conditions other than CML, such as newly diagnosed acute lymphocytic leukemia (ALL) or nonlymphocytic leukemia, may also be positive for the Ph1 chromosome. Some consider this the blastic phase of CML without a chronic phase. The chromosome is rarely found in patients with other myeloproliferative disorders, such as polycythemia vera or essential thrombocythemia, but these are probably misdiagnosed chronic myelogenous leukemia (CML). It is rarely observed in myelodysplastic syndrome.

Therapeutic Approach Considerations

The goals of treatment of chronic myelogenous leukemia (CML) are threefold and have changed markedly in the past 10 years. They are as follows: Hematologic remission (normal complete blood cell count [CBC]

and physical examination [ie, no organomegaly]). Cytogenetic remission (normal chromosome returns with 0% Ph-positive cells). Molecular remission (negative polymerase chain reaction [PCR] result for the mutational *BCR/ABL* mRNA), which represents an attempt for cure and prolongation of patient survival. Typically, CML has 3 clinical phases: an initial chronic phase, during which the disease process is easily controlled; then a transitional and unstable course (accelerated phase); and, finally, a more aggressive course (blast crisis), which is usually fatal. In all 3 phases, supportive therapy with transfusions of red blood cells or platelets may be used to relieve symptoms and improve quality of life. In Western countries, 90% of patients with CML are diagnosed in the chronic phase. These patients' white blood cell (WBC) count is usually controlled with medication (hematologic remission). The major goal of treatment during this phase is to control symptoms and complications resulting from anemia, thrombocytopenia, leukocytosis, and splenomegaly. The standard treatment of choice is now imatinib mesylate, which is a specific small-molecule inhibitor of *BCR/ABL* in all phases of CML. The chronic phase varies in duration, depending on the maintenance therapy used: it usually lasts 2-3 years with hydroxyurea (Hydrea) or busulfan therapy, but it may last for longer than 9.5 years in patients who respond well to interferon-alfa therapy. Furthermore, the advent of imatinib mesylate has dramatically improved the duration of hematologic and, indeed, cytogenetic remissions. Some patients with CML progress to a transitional or accelerated phase, which may last for several months. The survival of patients diagnosed in this phase is 1-1.5 years. This phase is characterized by poor control of the blood counts with myelosuppressive medication and the appearance of peripheral blast cells (≥15%), promyelocytes (≥30%), basophils (≥20%), and platelet counts less than 100,000 cells/µL unrelated to therapy. Many of the treatment decisions in CML, including possible bone marrow or stem cell transplantation and investigative options for younger patients, are extremely complex and in constant flux. Individualized decisions should be made in conjunction with consultation with physicians familiar with the recent literature. New agents that are currently under study may prolong the survival of patients with CML and offer the possibility of eventual cure. Physicians should refer their patients to tertiary care centers for clinical trials involving these therapies.

Myelosuppressive Therapy: Myelosuppressive therapy was formerly the mainstay of treatment to convert a patient with CML from an uncontrolled initial presentation to one with hematologic remission and normalization of the physical examination and laboratory findings. However, it may soon fall out of favor as the new agents prove to be more effective, with fewer adverse events and longer survival.

Hydroxyurea: Hydroxyurea (Hydrea), an inhibitor of deoxynucleotide synthesis, is the most common myelosuppressive agent used to achieve hematologic remission. The initial blood cell count is monitored every 2-4 weeks, and the dose is adjusted depending on the WBC and platelet counts. Most patients achieve hematologic remission within 1-2 months. This medication causes only a short duration of myelosuppression; thus, even if the counts go lower than intended, stopping treatment or decreasing the dose usually controls the blood counts. Maintenance with hydroxyurea rarely results in cytogenetic or molecular remissions.

Busulfan: Busulfan (Myleran) is an alkylating agent that has traditionally been used to keep the WBC counts below 15,000 cells/µL. However, the myelosuppressive effects may occur much later and persist longer, which makes maintaining the numbers within normal limits more difficult. Long-term use can cause pulmonary fibrosis, hyperpigmentation, and prolonged marrow suppression lasting for months.

Leukapheresis: Leukapheresis using a cell separator can lower WBC counts rapidly and safely in patients with WBC counts greater than 300,000 cells/µL, and it can alleviate acute symptoms of leukostasis, hyperviscosity, and tissue infiltration. Leukapheresis usually reduces the WBC count only temporarily. Thus, it is often combined with cytoreductive chemotherapy for more lasting effects.

Interferon alfa: In the past, interferon alfa was the treatment of choice for most patients with CML who were too old for bone marrow transplantation (BMT) or who did not have a matched bone marrow donor. With the advent of tyrosine kinase inhibitors, interferon alfa is no longer considered first-line therapy for CML. It may be used in combination with newer drugs for treatment of refractory cases.

Transplantation: Allogeneic bone marrow transplantation (BMT) or stem cell transplantation is currently the only proven cure for CML. Ideally, it should be performed in the chronic phase of the disease rather than in the transformation phase or in blast crisis. Candidate patients should be offered the procedure if they have a matched or single–antigen-mismatched related donor available. In general, younger patients fare better than older patients. BMT should be considered early in young patients (< 55 y) who have a matched sibling donor. All siblings should be typed for human leukocyte antigen (HLA)-A, HLA-B, and HLA-DR. If no match is available, the HLA type can be entered into a bone marrow registry for a completely matched unrelated donor. Allogeneic BMT with matched unrelated donors has yielded very encouraging results in this disease. The procedure has a higher rate of early and late graft failures (16%), grade III-IV acute graft versus host disease (50%), and extensive chronic graft versus host disease (55%). The overall survival rate ranges from 31% to 43% for patients younger than 30 years and from 14% to 27% for older patients. Benefits and risks should be assessed carefully with each patient. The mortality rate associated with BMT is 10-20% or less with a matched sibling and 30-40% with an unrelated donor. The bone marrow registry approximates the cure rate for patients with CML at 50%. Transplantation has been relegated to patients who do not achieve molecular remissions or show resistance to imatinib and failure of second-generation bcr-abl kinase inhibitors such as dasatinib. Previous exposure to imatinib before transplantation does not adversely affect posttransplant outcomes such as overall survival and progression-free survival. A retrospective analysis that included 70 patients with CML (44% in accelerated phase or blast crisis) who had received imatinib before stem cell transplantation showed 90% engraftment and estimated transplant-related mortality of 44% and estimated relapse mortality of 24% at 24 months. Graft versus host disease rates were 42% for acute and 17% for chronic. Most data are from allogeneic transplantations from HLA-matched sibling donors and a few syngeneic transplantations from an identical twin. Data show that allogeneic transplantations have better results than syngeneic transplantations because of some graft versus leukemia effects. Autologous BMT is investigational, but, relatively recently, chemotherapy combinations or interferon have been found to induce a cytogenetic remission and allow harvesting of Ph-negative CD34 hematopoietic stem cells from the patient's peripheral blood. The advent of imatinib therapy has overshadowed allogeneic hematopoietic stem cell transplantation in newly diagnosed CML. However, it has been suggested that patients with a poor-risk Sokal score (see Prognosis) but good risk for allogeneic hematopoietic stem cell transplantation be transplanted early or upfront. No current consensus exists on these issues. However, a widely accepted consensus is that patients who progress beyond chronic phase on imatinib should be offered hematopoietic stem cell transplantation if this is an option. With patients in blast crisis who are imatinib naive, the drug is used in combination with induction regimens similar to those used in acute myelogenous or lymphoblastic leukemia. However, because a high percentage of imatinib-resistant mutations exist in these patients, relapses occur more frequently and at an earlier time from induction. Thus, all efforts are made to perform an allogeneic hematopoietic stem cell transplantation as soon as possible. Most patients with minimal residual disease (MRD) after transplantation require interferon maintenance therapy. Alternatively, they may require a reinfusion of T cells collected from the donor.

TROUBLESHOOTING

LEUCOCYTE ALKALINE PHOSPHATASE

How the Test is Performed

Blood is typically drawn from a vein, usually from the inside of the elbow or the back of the hand. The site is cleaned with germ-killing medicine (antiseptic). The health care provider wraps an elastic band around the upper arm to apply pressure to the area and make the vein swell with blood. Next, the health care provider gently inserts a needle into the vein. The blood collects into an airtight vial or tube attached to the needle. The elastic band is removed from your arm. Once the blood has been collected, the needle is removed, and the puncture site is covered to stop any bleeding. In infants or young children, a sharp tool called a lancet may be used to puncture the skin and make it bleed. The blood collects into a small glass tube called a pipette, or onto a slide or test strip. A bandage may be placed over the area if there is any bleeding. A laboratory specialist separates the white blood cells from the rest of the blood sample and watches to see if any substances stick to specific colored dyes. Substances that contain phosphate, such as ALP, attach to certain colored dyes.

How to Prepare for the Test

One should not eat or drink for 6 hours before the test. Certain medicines may affect the test results. Your health care provider may tell you to stop taking such medications. These medications include: Allopurinol, Androgens, Anti-inflammatory medicines, Birth control pills, Certain antibiotics, Certain arthritis drugs, Certain diabetes drugs (taken by mouth), Chlorpromazine, Cortisone, Methyldopa, Narcotics, Propranolol, Tranquilizers, Tricyclic antidepressants. No medicine should be stopped without medical advice.

Why the Test is Performed

ALP is found in different forms throughout the body. This test is done to confirm a number of different medical conditions, including certain types of anemia and leukemia. It may also be done if a person has an increase in platelet levels in the blood.

Normal Results

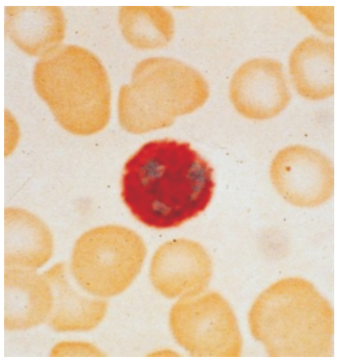
A staining score of 20 - 100 (out of a maximum of 400) is considered normal. Note: Normal value ranges may vary slightly among different laboratories. Talk to your doctor about the meaning of your specific test results. The examples above show the common measurements for results for these tests. Some laboratories use different measurements or may test different specimens.

What Abnormal Results Mean

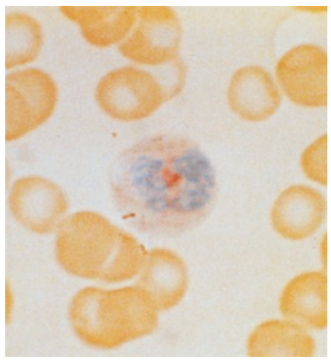
Higher-than-normal results may be due to: Essential thrombocytosis, Leukemoid reaction, Myelofibrosis, Polycythemia vera.

Lower-than-normal results may be due to: Aplastic anemia, Chronic myeloid leukemia, Pernicious anemia.

Leukocyte alkaline phosphatase



STRONG LAP POSITIVE NEUTROPHIL



LAP NEGATIVE NEUTROPHIL

What if one suspects CML but doesn't have access to a cytogenetic or molecular lab (to look for the Philadelphia chromosome or the bcr-abl translocation)? Well, first you'd look for all the morphologic clues you could. CML usually presents with a marked leukocytosis (the WBC is often over 100,000), with a left shift all the way back to myeloblasts (though there are relatively few myeloblasts around). A benign left shift usually presents with a mild to moderate leukocytosis (the neutrophil count is often just above normal; it's generally nowhere near the magnitude often seen in CML), and the neutrophils are shifted back to the metamyelocyte or myelocyte stages (you'll very rarely see promyelocytes, and you'll virtually never see myeloblasts). Also, CML tends to have a "bulge" at the metamyelocyte stage, whereas a benign left shift does not (the cells are more or less present in decreasing amounts by stage of maturation, i.e., there are more segmented than band neutrophils, more bands than metamyelocytes, more metamyelocytes than myelocytes, more myelocytes than promyelocytes and blasts are basically nonexistent). Finally, CML has a basophilia, whereas a benign left shift does not. But if you wanted more proof that your case was CML, you could do a leukocyte (or neutrophil) alkaline phosphatase (LAP). This test is not done as much as it used to be, because now everyone goes right to cytogenetics or molecular testing in order to find the Philadelphia chromosome or the bcr-abl translocation. But it's still a good test, and it would be a good thing to do if you couldn't look for the Philadelphia chromosome. Here's the principle behind the test. LAP is an enzyme present in normal neutrophils, but absent (or present at very low concentrations) in malignant neutrophils (i.e., the ones in CML). So if you have a whole bunch of neutrophils around, and the LAP is strongly positive in those cells, as in the top image, you can be quite sure that it is a benign bunch of neutrophils. However, if the LAP is negative, or only weakly positive, as in the bottom image, that probably means that those neutrophils are malignant and that you're dealing with a case of CML. You'd still want to send off a blood or bone marrow specimen to a cytogenetics and/or molecular diagnostics lab, but in the meantime, the LAP can help you quickly assess and triage your patient.

Classification of acute leukemias by morphologic and cytochemical criteria (modified from Löffler)

Stem cell leukemia		
peroxidase	0	undifferentiated type
periodic acid Schiff	0	
alpha-napthylacetate esterase	0	
Myeloblastic leukemia (AML)		
peroxidase	(-)	Peroxidase type 1 and 2
periodic acid Schiff	0-(+)	
alpha-napthylacetate esterase	(+)	
Promyelocytic leukemia		
peroxidase	+	Peroxidase type 3
periodic acid Schiff	(+)	
alpha-napthylacetate esterase	(+)	
Myelomonocytic leukemia		
peroxidase	+	Peroxidase esterase type
periodic acid Schiff	(+)	
alpha-napthylacetate esterase	+	
Monocytic leukemia		
peroxidase	(+)	Esterase type
periodic acid Schiff	0-(+)	
alpha-napthylacetate esterase	+	
Lymphoblastic leukemia (ALL)		
preoxidase	0	periodic acid Schiff type
periodic acid Schiff	+	
alpha-naphthylacetate esterase	0	
0 = negative; (+) = weakly positive < 25%; + = strongly positive > 50		

Acute eosinophilic leukemia is diagnosed by examination of the bone marrow, since eosinophils usually are not increased in the peripheral blood. The predominant marrow cells are abnormal eosinophils (immature, pleomorphic forms, some with coarse, dark-blue granules, cytoplasmic vacuoles, distinct nucleoli). Diagnosis relies on the cytochemical detection of naphthol-AS-D-chloracetate esterase in the granules. Auer rods are unusual. Acute basophilic leukemia is evidenced by an extreme increase in the basophilic granulated cells of granulocytopoiesis. The granules are very atypical (large, coarse, hyperchromic), and Auer rods may be present The diagnosis is confirmed by the metachromatic reaction to toluidine blue in the cells. Four forms are recognized: (1) basophilic blast crises in CML, (2) promyelocytic basophilic type, (3) histiobasophilic type, (4) basophilic-eosinophilic type. It is very difficult to establish a diagnosis of acute megakaryoblastic leukemia, designated M7 in the FAB classification of acute leukemias. The blast cells in the peripheral blood and bone marrow present a variety of morphologies. They may appear as small cells with a narrow cytoplasmic border and dense chromatin, resembling lymphoblasts (L1), or they may resemble L2 cells with or without granules. The nuclei are round, finely reticular, and have one to three prominent nucleoli. The cells vary greatly in size and may be two to three times larger than normal lymphocytes. Sometimes one finds cytoplasmic vesicles or differentiated megakaryocytes with adjacent platelets or bare nuclei nested in clusters of platelets. Occasional megakaryocytic nuclei are found in the peripheral blood. It is often difficult to obtain a bone marrow specimen by aspiration, and marrow biopsy is usually indicated. Cytochemical methods may contribute to the diagnosis. The Sudan black and peroxidase reaction are negative. The monocytes can be a source of confusion. Often they are positive for alpha-napthylesterase and naphthyl-ASD-acetate esterase, in which case these enzymes canbe inhibited by fluoride. While the monocytes usually show a diffuse positivity for these esterase enzymes, the reaction in megakaryoblasts tends to be localized. PAS and acid phosphatase positivity are also localized. A platelet peroxidase occuring on a nuclear membrane and in the endoplasmic reticulum of megakaryoblasts can distinquish these cells from myeloblasts on electron microscope examination. This can also be accomplished by the use of monoclonal or polyclonal platelet-specific antibodies.

BOUQUET

In Lighter Vein

--NO SPEAKAH DE ENGLISH
A bus stops and 2 Italian men get on. They sat down and engage in an animated conversation. The lady sitting next to them ignores them at first, but her attention is galvanized when she hears one of them say the following:
Emma come first.
Den I come.
Den two asses come together.
I come once-a-more!
Two asses, they come together again.
I come again and pee twice.
Then I come one lasta time.'
The lady can't take this anymore, "You foul- mouthed sex obsessed pig!" She retorted indignantly. 'In this country, we don't speak aloud in public places about our sex lives!"
'Hey, coola down lady,' said the man, 'Whooza talkin' about sex, I'm a justa tellin' my frienda how to spell ' Mississippi '...

--I asked my new girlfriend what sort of books she's interested in. She said: Cheque books.

-- What's the difference between a good lawyer and a great lawyer? A good lawyer knows the law. A great lawyer knows the judge.

-- Nurse: A beautiful woman who holds your hand for one full minute and then expects your pulse to be normal.

-- A Blonde enters kitchen, opens sugar container, looks inside and closes it. She does this again and again.
Why? Because his Doctor told her to check the sugar level regularly.

Wisdom Whispers

Always Look On The Bright Side
A pessimist sees the difficulty in every opportunity; an optimist sees opportunity in every difficulty.

Serenity
The real sign of serenity is not seen so much in the face, as found in the depth and stillness of the eyes.

Notice the Difference
Replace the words "I have to" with "I choose to" and notice the difference in how you feel.

Courage
Courage is taking a step forward into an area of difficulty without a solution in mind, trusting that whatever help you need will become available.

Self-Respect
Experience the bliss of Self-Respect and give respect to others at all times. When I am prejudiced against another, my narrow vision and small heart lower my self-dignity and self-worth.

Balance
Maintain the balance of responding to situations with a cool head and to people with a warm heart.

Pure and Gentle
Success is merged in every step of those who have a pure and gentle nature.

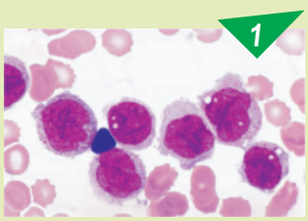
The Happiness You Give
The happiness you give makes you more happy than the happiness you receive.

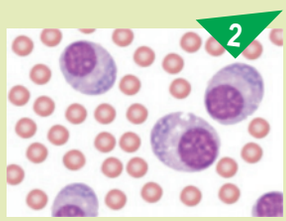
Lotus Life
The lotus is a symbol of purity.
Its roots are in the mud, but the flower remains above dirty water.
Live a lotus life. Be in the world, but unaffected by impurities.

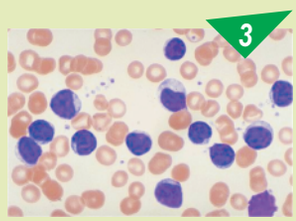
Determination
Determination brings the strength to continue, the steadiness to succeed, and the wisdom to slip past difficulties undisturbed.

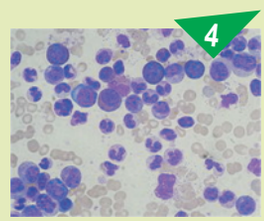
Brain Teasers

Diagnose the following haematologic malignancies from their peripheral smear images









Answers: 1 – Acute Myeloblastic Leukemia 2. – Plasma Cell Leukemia 3. – Chronic Lymphocytic Leukemia 4. – Chronic Myelocytic Leukemia.

INTERPRETATION

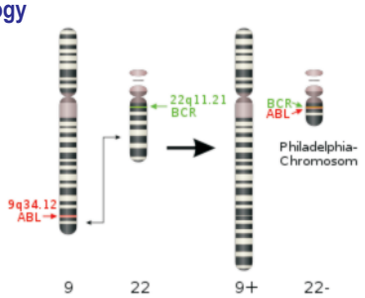
Ph CHROMOSOME (Philadelphia chromosome)

Philadelphia chromosome or **Philadelphia translocation** is a specific chromosomal abnormality that is associated with chronic myelogenous leukemia (CML). It is the result of a reciprocal translocation between chromosome 9 and 22, and is specifically designated t(9;22)(q34;q11). The presence of this translocation is a highly sensitive test for CML, since 95% of people with CML have this abnormality (the remainder have either a cryptic translocation that is invisible on G-banded chromosome preparations, or a variant translocation involving another chromosome or chromosomes as well as the long arm of chromosomes 9 and 22). However, the presence of the Philadelphia (Ph) chromosome is not sufficiently specific to diagnose CML, since it is also found in acute lymphoblastic leukemia (ALL, 25–30% in adult and 2–10% in pediatric cases) and occasionally in acute myelogenous leukemia (AML).

History

The Philadelphia chromosome was first discovered and described in 1960 by Peter Nowell from University of Pennsylvania School of Medicine and David Hungerford from the Fox Chase Cancer Center's Institute for Cancer Research and was therefore named after the city in which both facilities are located. In 1973, Janet D. Rowley at the University of Chicago identified the mechanism by which the Philadelphia chromosome arises as a translocation.

Molecular biology



Schematic representation of formation of the Philadelphia Chromosome

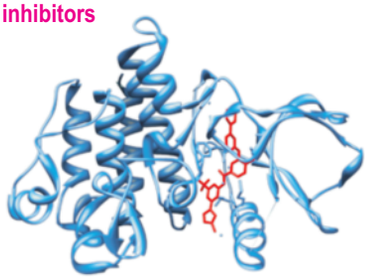
The exact chromosomal defect in Philadelphia chromosome is a translocation, in which parts of two chromosomes, 9 and 22, swap places. The result is that a fusion gene is created by juxtapositioning the *Abl1* gene on chromosome 9 (region q34) to a part of the *BCR* ("breakpoint cluster region") gene on chromosome 22 (region q11). This is a reciprocal translocation, creating an elongated chromosome 9 (*der 9*), and a truncated chromosome 22 (*the Philadelphia chromosome).* In agreement with the International System for Human Cytogenetic Nomenclature (ISCN), this chromosomal translocation is designated as t(9;22)(q34;q11). *Abl* stands for "Abelson", the name of a leukemia virus which carries a similar protein. The result of the translocation is the oncogenic BCR-ABL gene fusion, located on the shorter derivative 22 chromosome. This gene encodes the Bcr-abl fusion protein. Depending on the precise location of the fusion the molecular weight of the protein can range from 185 to 210 kDa. For this reason bcr-abl is sometimes called p210 or p185. Three clinically important variants are the p190, p210 and p230 isoforms. p190 is generally associated with acute lymphoblastic leukemia (ALL), while p210 is generally associated with chronic myeloid leukemia but can also be associated with ALL. p230 is usually associated with chronic neutrophilic leukemia. Additionally, the p190 isoform can also be expressed as a splice variant of p210. Because the Abl gene expresses a membrane-associated protein, a tyrosine kinase, the BCR-Abl transcript is also translated into a tyrosine kinase, adding a phosphate group to tyrosine. Although the BCR region also expresses serine/threonine kinases, the tyrosine kinase function is very relevant for drug therapy. Tyrosine kinase inhibitors (such as imatinib and sunitinib) are important drugs against a variety of cancers including CML, renal cell carcinoma (RCC) and gastrointestinal stromal tumors (GISTs). The fused *BCR-Abl* protein interacts with the interleukin-3 receptor beta(c) subunit. The ABL tyrosine kinase activity of *BCR-Abl* is elevated relative to wild-type ABL. Since ABL activates a number of cell cycle-controlling proteins and enzymes, the result of the *BCR-Abl* fusion is to speed up cell division. Moreover, it inhibits DNA repair, causing genomic instability and potentially causing the feared blast crisis in CML.

Nomenclature

Philadelphia chromosome is designated **Ph (or Ph') chromosome** and the translocation is termed **t(9;22) (q34.1;q11.2)**.

Therapy

Tyrosine kinase inhibitors



Crystal structure of Abl kinase domain (blue) in complex with 2nd generation tyrosine kinase inhibitor (TKI) nilotinib (red)
Main article: Bcr-Abl tyrosine-kinase inhibitor

In the late 1990s, STI-571 (imatinib, Gleevec/Glivec) was identified by the pharmaceutical company Novartis (then known as Ciba Geigy) in high-throughput screens for tyrosine kinase inhibitors. Subsequent clinical trials led by Dr. Brian J. Druker at Oregon Health & Science University in collaboration with Dr. Charles Sawyers and Dr. Moshe Talpaz demonstrated that STI-571 inhibits proliferation of BCR-ABL-expressing hematopoietic cells. Although it did not eradicate CML cells, it did greatly limit the growth of the tumor clone and decreased the risk of the feared "blast crisis". In 2000 John Kuriyan determined the mechanism by which STI-571 inhibits the Abl kinase domain. It was marketed in 2001 by Novartis as imatinib mesylate. Other pharmacological inhibitors are being developed, which are more potent and/or are active against the emerging Gleevec/Glivec resistant BCR-abl clones in treated patients. The majority of these resistant clones are point-mutations in the kinase of BCR-abl. New inhibitors include dasatinib and nilotinib, which are significantly more potent than imatinib and may overcome resistance. Treatment of pediatric Ph+ ALL with a combination of standard chemotherapy and RTK inhibitors may result in remission, but the curative potential is unknown.

Blood or marrow transplants

COG study AALL 0031, which examines the use of Gleevec with standard chemotherapeutic regimens and bone marrow transplant from HLA-matched related donors for high risk ALL (including Ph+ ALL), has concluded, and findings will be published in the near future. A potentially curative, but risky, option for pediatric Ph+ ALL or Ph+ CML is bone marrow transplant or cord blood transplant, but chemotherapy is favored by some for achieving first remission (CR1). For some, bone marrow transplant from a matched sibling donor or a matched, unrelated donor may be favored when remission is obtained. Cord blood transplant is favored by some when a 10/10 bone marrow match is not available, and cord blood transplant may have some advantages, including a reduced incidence of graft-vs-host disease (GVHD), which is a common and significant complication of transplant. However, transplant with cord blood sometimes requires longer periods of time for engraftment, which may increase the potential for complications due to infection. Regardless of the type of transplant, transplant-related mortality and relapse are possible, and the rates may change as treatment protocols improve. For second remission (CR2), if achieved, both chemotherapy and transplant options are possible, and many physicians prefer transplant.

Ensuring ABO Compatibility – Key to Safe Transfusion

Facts :

- **Globally ABO-incompatible transfusions are still a frequent cause of serious adverse transfusion reactions.**
- **Most errors result from human actions and thus may be preventable.**

In order to prevent life threatening Hemolytic Transfusion Reactions because of ABO-incompatible blood transfusion bedside ABO and Rh grouping of patient and donor's blood unit should be incorporated in pre transfusion testing.

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Ensuring Safe Transfusion...

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