Alkaline Phosphatase Isoenzymes and Leukocyte Alkaline Phosphatase Score in Patients with Acute and Chronic Disease: A Brief Review

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Background
The alkaline phosphatase test (ALP), usually, is ordered of clinicians to help detect liver diseases or bone disorders.

Content
In the laboratories of medical analyses, the total ALP activity is typically measured colorimetrically using the p-nitrophenol method. ALP isoenzymes can be measured by an electrophoretically method with Titan III on a supporting media. A mouse monoclonal antibody specific to the bone alkaline phosphatase isoenzyme (BAP) has been adapted to an immunoassay reaction and an alkaline phosphatase score can be used in patients with acute and chronic diseases.

Summary
Isoenzyme testing is crucial in an accurate diagnosis and should be considered when signs and symptoms of diseases fail to provide a clear answer to clinical and laboratory features.

Keywords: alkaline phosphatase, bone alkaline phosphatase, neutrophil alkaline phosphatase.
Introduction

Alkaline phosphatase (ALP) is an enzyme found in all human tissues, but is particularly concentrated in liver, bone, kidney, intestine, and placenta. ALP exists in multiple forms, some of which are coded on specific genetic loci, while others differ only by post-translational modification (primarily glycosylation). The activity of ALP measured in human serum is mostly a composite of bone, liver, and kidney isoenzymes; with smaller amounts of intestinal ALP. Fractionation and measurement of ALP isoenzymes may be helpful in determining the organ or tissue from which ALP elevation in serum originates.¹

ALP is an enzyme that has very variable reference ranges during the lifetime of an individual. For example, different reference ranges exist for newborns (110-450 U/L), 1-month (120-720 U/L), 3-years (110-650 U/L), 10-years olds (130-700). Additional reference ranges must be used during puberty (49-587 U/L), depending on the Tanner developmental stage of the patient. Finally, adult males and females also have different ALP concentrations (adults males 90-190 U/L and females 85-165 U/L)².

After age 60, reference limits increase in women, although studies have not consistently evaluated for the presence of osteoporosis, which can increase alkaline phosphatase activity in serum. Assays for alkaline phosphatase activity should have total analytical error of ≤10-15% at the upper reference limit.

In the liver, ALP is concentrated in cells of the bile duct³. ALP in bone is produced by osteoblasts, therefore bone ALP (BAP) reflects the activity of these cells, ALP being a
sensitive indicator of bone metabolism. An alternate reason for elevated ALP activity is hyperreactivity of osteoblasts.

Elevated total ALP activity in serum is observed when osteoblastic activity is increased (e.g., hyperparathyroidism, osteomalacia, and primary and metastatic neoplasms), in hepatobiliary diseases characterized by some degree cholestasis, and in sepsis, chronic inflammatory bowel disease, and thyrotoxicosis.

Smaller increases of ALP activity are seen in liver cancer and cirrhosis, with use of drugs toxic to the liver and, in hepatitis. Any condition causing excessive bone formation, including bone disorders such as Paget's disease and others such as rheumatoid arthritis and healing fractures, can cause increased ALP activity. This test may also sometimes be used to monitor treatment of other bone conditions, such as vitamin D deficiency in children or to adolescents which typically have higher blood ALP levels because their bones are still growing.

**Body of review of ALP**

The bone and liver tissue contain the highest concentrations of ALP. Alkaline phosphatase activities are also extremely high in patients taking certain drugs, particularly drugs that treat psychiatric disorders. Other drugs may also affect ALP levels; for example, oral contraceptives may cause a decrease in ALP activity and antiepileptics may cause an increase.

Low or undetectable levels of ALP activity are relatively uncommon. Errors in detection of decrease of ALP activity can occur without bivalent cations, which are necessary as cofactors in reaction. In a review of nearly 70,000 ALP results for adult patients, Lum found low levels of activity in only 0.19%. In half the cases reviewed, no explainable cause was found for the low results. The most common explainable causes of low or non-detectable ALP activity were hypophosphatasia, malnutrition with low magnesium and cardiac surgery. All these causes can be associated with low levels of cations, such as zinc, or the presence of chelators, such as citrate, in transfusions that lower ALP activity.

**Clinical significance of ALP**

ALP may be ordered as part of a routine lab testing profile, often with a group of other tests called a liver panel. Signs and symptoms of liver involvement may include: weakness, fatigue loss of appetite nausea, vomiting, abdominal swelling and/or pain, jaundice, dark urine, light colored stool itching. Some examples of the signs and
Symptoms suggesting a bone disorder include: bone and/or joint pain, increased frequency of bone fractures.

**ALP Isoenzymes**

The following types of ALP isoenzymes can be found in the human body:

- Neutrophil alkaline phosphatase (NAP)
  Neutrophil alkaline phosphatase is detectable in differentiated neutrophils and monocytes; it is the product of the liver/bone/kidney-type ALP gene. Neutrophil alkaline phosphatase messenger RNA and enzyme activity are induced by treatment of neutrophils with granulocyte colony-stimulating factor (G-CSF). Leakage of ALP from damaged or dead neutrophils in infections may influence the release of neutrophil alkaline phosphatase into the bloodstream.

- Hepatic-bile alkaline phosphatase (HBAP) with activities which are routinely measured during screening for liver disease. In some forms of liver disease, such as hepatitis, HBAP is usually much less elevated than AST and ALT. Some of those who are at risk of liver disease include the following: people who have been exposed to hepatitis viruses, heavy drinker, people who take medication that can be toxic to the liver or who are exposed to other liver toxins, those who are obese and have metabolic syndrome or insulin resistance, people with an inherited disorder affecting the liver such as Wilson disease.

- Bone-type alkaline phosphatase (BAP)
  An increased level of bone-type ALP should not be misdiagnosed as representing a pathologic condition such as thyroid disease (hyperthyroidism), in which osteomalacia is present; hyperparathyroidism (primary or secondary); chronic renal failure with renal osteodystrophy; diabetes mellitus with osteomyelitis; or metastatic cancer such as prostate cancer, in which osteoblastic activity is observed. This misdiagnosis should also be avoided in adult female patients with osteoporosis being treated with biophosphonates. Low levels of BAP may be observed temporarily after blood transfusions or heart bypass surgery. Zinc deficiency may also cause decreased levels of BAP.

- Placental alkaline phosphatase (PLAP)
  PLAP is a normally ALP isoenzyme which occur during pregnancy (Quarter 3 of pregnancy), but the form of isoenzyme named Regan isoenzyme is that form of the isoenzyme that is associated with malignancy.
Physiologically, API activity is associated with individuals with blood group O or B but its activity is increased in cirrhosis, intra-hepatic cholestasis, enteritis and chronic hemodialysis.\textsuperscript{11,12}

Organizing ALP analytic methods of measurement of total serum ALP activity, isoenzymes and LAP score

There are at least four major methods to measure ALP isoenzymes: electrophoresis based, immunoassay based techniques, heat inactivation and substrate specificity based technique. Enzyme and isoenzymes ALP activities in serum and plasma are measured in the different laboratories using various commercially available routine measurement systems at 37\degree C.

Only results obtained with the International Federation of Clinical Chemistry (IFCC) in compatible measuring systems are recommended for estimation of the enzyme reference intervals in laboratories of medical analyses. An internationally accepted standard method for serum enzyme activity assessment ALP is the IFCC/AACC reference method which recommends that, the accuracy of the measuring each device must be verified with a gallium cell\textsuperscript{13}.

- Japanese Society of Clinical Chemistry\textsuperscript{14} proposes the measurement serum ALP activity in the electrophoretic separation and this method is performed using Titan III support media (Helena Laboratories, Beaumont, Texas).

- Immune enzymatic method: a mouse monoclonal antibody specific to BAP is used in an immune enzymatic assay and the antibody-antigen (Ab-Ag) complex is measured on a luminescence analyzer. Normal values of BAP per this measurement are lower than 90 U/L (eg, 30\% of total ALP)\textsuperscript{15}. Separation of tissue nonspecific ALP forms (bone, liver, and kidney) is difficult owing to structural similarity; high resolution electrophoresis and isoelectric focusing are the most useful techniques. Bone-specific ALP can be measured by heat inactivation (a poor method), immunological and by electrophoretical methods.
Immunoassays of bone ALP are now available from several sources and can be used to monitor patients with bone disease\textsuperscript{16}.

-The method for total ALP in the widest cases used is the p-nitrophenylphosphate method of Bowers, McComb and Kelly. Complexing agents such as citrate, oxalate, or EDTA bind cations such as zinc and magnesium, necessary cofactors for ALP activity measurement, causing falsely decreased values, as low as zero. Blood transfusion (containing citrate) causes transient decrease in ALP through a similar mechanism. Also, in colorimetric method, p-Nitrophenol reagent absorbs wavelengths of light in the 400-nm region but some metabolic components and drugs (bilirubin, methotrexate, nitrofurantoin, etc) that significantly absorb light in the 400-nm region can cause a special type of interference\textsuperscript{17}.

Semi-quantitative method for determining the presence of NAP (LAP score)

LAP score is an old test that was used to the differential diagnosis of certain hematologic diseases, to decide if the cause of the elevated leukocyte count is a reactive process or a malignancy and in present is useful for in small laboratories, because of is very cheap method.

In the neutrophils of healthy individuals, ALP is localized predominantly to the secretory vesicles. On blood smear from peripheral blood, using the protocol of work NAP in vitro test\textsuperscript{18}, Code SP 910, from Gailand Chemical Co, Isoenzyme from granulocytes appear as dark blue or black grains in cell cytoplasm. Interpretation of the chemical reaction will be determined according to the score generated by analysis of 100 segmented granulocytes, in which the intensity of the color of the grains will be recorded on a scale from 1 to 4. The leukocyte-alkaline-phosphatase score will be given as the product of the number of cells counted and the percentage values\textsuperscript{20}. Normal scores are defined as being between 60 and 100. Characteristics of the NAP and the calculated score according to intensity of color of granulocytic granules (based on the observations reported by Kaplow\textsuperscript{19}) are listed in table.

The NAP enzyme must be examined only in segmented, un-segmented neutrophils and eosinophils; some basophiles test NAP-negative must not be counted toward the ALP
score. The NAP test allows the discrimination between normal and pathologic chemical activity of neutrophils which display in microscopic field, morphologic characteristics in function of benign or malign disease [Figure 1].

**Clinical interpretation of results from semi-quantitative method NAP**

Neutrophil alkaline phosphatase activity does not appear to be involved in serum ALP activity in healthy individuals. However, ALP enzyme activity increases in cases of bacterial infection. A leukemoid reaction is an excessive but reactive outpouring of leukocytes that involves the appearance of immature forms (blast cells, myelocytes, and metamyelocytes); however, this reaction is distinct from leukemia. The leukemoid reaction appears in response to infection, as well as toxic, inflammatory, and neoplastic disorders. It may also appear in acute or chronic form with numerous granulocytes; it rarely appears with numerous lymphocytes.

The major pathologic manifestations associated with leukemoid reaction are acute or chronic infection, especially in children, severe hemolysis and various solid tumors (especially of the breast, kidney, and lung, as well as metastatic cancers), and other illnesses bone on arrival at the point-of-care department. The total leukocyte count is increased, typically 50 000 to 100 000/mm³. The granulocytes observed with May-Grünwald Giemsa staining display predominant toxic granulation (eg, Döhle bodies); ALP activity is observed to be extremely high. Lack of the Philadelphia chromosome, or extremely-low-score NAP, in chronic myeloid leukemia (CML), is usually sufficient that this malignant disease to be distinguished from a leukemoid reaction with very high NAP activity.

Increased NAP activity is funded and in some myeloproliferative diseases, such Hodgkin’s disease and polycythemia vera (PV). Granulocytes from healthy individuals and patients with PV or CML preferentially express bone-type ALP transcriptions. Neutrophil alkaline phosphatase activity is substantially decreased in hematopoietic stem cell disorders such as CML, acute myelocytic leukemia (AML) and paroxysmal nocturnal hemoglobinuria (PNH).
In myelopoiesis, NAP production in neutrophils is induced by GCS-F, and NAP is released into the bloodstream, perhaps through leakage of ALP from damaged or dead neutrophils. Fosså and colleagues reported leukocytosis and increased serum ALP in response to GCS-F treatment; they suggested that increased serum ALP activity was related to release of the enzyme resulting from the increased leukocyte count. In experiments in which GCS-F was administered to rats, reported by Tsuruta et al increased serum ALP activity was traced to neutrophils.

The PNA score [Table 1] was calculated in a microscopic internal control, compared with specimens analyzed via a 2-slide series of blood smear. This technique juxtaposes results of a sure positive control (eg, blood from a patient with sepsis) and a sure negative sample (eg, blood from a healthy individual).

In leukocytosis, NAP activity allow for the distinction between CML, in which NAP activity is absent, and PV, in which NAP activity are beyond the normal range. Also, secondary polycythemia, in which NAP activity is decreased, can be distinguished from essential malignant PV, in which NAP activity is highly increased. In addition, NAP activity allows to distinguish between the following types of acute leukemia (in the absence of cortisol medications): acute myeloblastic leukemia (AML), in which NAP has low activity or is absent in mature neutrophils, acute lymphoblastic leukemia (ALL), in which NAP activity is decreased in mature neutrophils, hairy cell leukemia (HCL) with severe neutropenia, in which NAP activity is very high and non-Hodgkin lymphoma (NHL), in which NAP activity is decreased. At children with the diagnosis of trisomy 21 (Down syndrome), NAP activity is increased due to surplus chromosomal.

**Conclusion**

The alkaline phosphatase is assessed by different methods for to help the clinicians to make the correct decisions concerning treatment of hospitalized patients with benign or malignant diseases. Interpretation of alkaline phosphatase results using appropriate biological references is particularly important in children because of the reference limits differ little in adult males and females.
Reference


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<th>Cells, No.</th>
<th>Cytoplasm Volume Displaying Color, %</th>
<th>Intensity of Color from Granulocytic Cells, Normal Score</th>
<th>Intensity of Color Indicating Granulocytic Cells, ALP score $^b$</th>
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**Table 1.** NAP score in Granulocytic Cells, calculated in function of color intensity, based on the observations reported by Kaplow$^{19}$.  
Abbreviation: ALP, alkaline phosphatase; NA, not applicable.
Fig 1. Leukocytes with positive reaction alkaline phosphatase, + and ++, in cytoplasm showing as black granules. In the left down, is present a neutrophil granulocyte without alkaline phosphatase activity.
Table 1. Characteristics of the neutrophil alkaline phosphatase (NAP) and the calculated score according to intensity of color from neutrophils granules (Observation of Kaplow)

Fig 1. Leukocytes with positive reaction alkaline phosphatase, + and ++, in cytoplasm showing as black granules. In the left down, is present a neutrophil granulocyte without alkaline phosphatase activity.
Continuing Education

After completing this article, readers should understand the importance of alkaline phosphatase isoenzymes, which are the various types of ALP isoenzymes and how their activity can be distinguished between bone and liver diseases, as well as between the different types of acute leukemia.

1. Pathological conditions can increase the activity of the following isoenzyme(s):
   A. NAP and HAP
   B. HAP only
   C. BAP only
   D. NAP, HAP, and BAP

2. Which of the following is not an ALP isoenzyme?
   A. NAP
   B. PLAP
   C. LDH
   D. ALPI

3. ALP activity in a mature female are elevated and further testing shows that BAP levels are also elevated. These results indicate that:
   A. The patient is healthy and is going through a growth spurt
   B. The patient could be suffering from a bone disease or a cancer spread to the bone
   C. The bile ducts are blocked, causing the elevated BAP levels
D. The patient is in the third trimester of pregnancy and the placental ALP is increased.

4. NAP values are useful in distinguishing between (choose all that apply):
   
   A. Polycythemia Vera and Secondary Polycythemia
   
   B. Different types of bone cancer
   
   C. Acute and Chronic Lymphocytic Leukemia
   
   D. Liver diseases and Obstructive biliary disease

5. Low ALP activity is seen in the following case(s) (choose all that apply):
   
   A. Malnutrition and magnesium deficiency
   
   B. Bone or liver disease
   
   C. Acute leukemia
   
   D. Leukemoid reaction

Abbreviations

ALP, alkaline phosphatase; BAP, bone alkaline phosphatase; NAP, neutrophil alkaline phosphatase; HBAP, hepatic-bile alkaline phosphatase; TBIL, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; G-CSF, granulocyte colony-stimulating factor; PLAP, placental ALP; ALPPL2, alkaline phosphatase placental-like 2; ALPI, alkaline phosphatase–intestinal; Ab-Ag, antibody-antigen; CML, chronic myelocytic leukemia; PV, polycythemia vera; AML, acute myeloblastic leukemia; ALL, acute lymphoblastic leukemia; HCL, hairy cell leukemia; NHL, non-Hodgkin lymphoma.