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

Aurelian Udristioiu¹, Radu G. Iliescu², Manole Cojoraru³
1Department of Hematology, Emergency County Hospital Targu Jiu, Clinical Laboratory, Gorj, Romania, aurelianu2007@yahoo.com
2Department of Researches, Polytechnic Institute of New York University, Brooklyn, New York, radugiliescu@yahoo.com
3Titu Maiorescu University, Medicine Faculty, Physiology, Bucharest, E-mail: mancojocaru@yahoo.com

Corresponding Author,
Aurelian Udristioiu, MD
aurelianu2007@yahoo.com
Background

The alkaline phosphatase test (ALP) is used to help detect liver disease or bone disorders. In conditions affecting the liver, damaged liver cells release increased amounts of ALP into the blood. High ALP usually means that either the liver has been damaged or a condition causing increased bone cell activity is present. If other liver tests such as bilirubin, aspartate aminotransferase (AST) or alanine aminotransfere (ALT) are also high, usually the ALP is coming from the liver. If it is not clear from signs and symptoms or from other routine tests whether the high ALP is due to liver or bone, then a test for ALP isoenzymes, produced by different types of tissue, may be necessary to distinguish between bone and liver ALP.

There are 4 genes in the ALP gene family: intestinal (found on chromosome 2); placental (2); germ cell (2) and non-tissue specific (1). The tissue non-specific isoenzyme includes the common serum forms of ALP from bone and liver.

Total ALP activity is typically measured colorimetrically using the p-nitrophenol method. ALP isoenzymes can be measured by a method described by the Japanese Society of Clinical Chemistry, in which the ALP isoenzymes are separated electrophoretically with Titan III supporting media. (Helena Laboratories, Corporation's Beaumont, TX location, 77704, USA.). A mouse monoclonal antibody specific to the bone alkaline phosphatase isoenzyme (BAP) is available and, has been adapted to an immunoassay.

Key words
ALP-alkaline phosphatase; BAP-bone alkaline phosphatase; NAP-neutrophil alkaline phosphatase.
Introduction

Alkaline Phosphatase (ALP) is a non-specific metalloenzyme which hydrolyzes many types of phosphate esters at an alkaline pH in the presence of zinc and magnesium ions. There are different isoenzymes (gene products) and isoforms (posttranslationally modified gene products). Alkaline phosphatase was 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) (1).

Different tissues contain different isoenzymes or isoforms and this may allow identification of the source of ALP in some cases. The tissues which contain highest amounts of ALP are liver, bone, placenta, and intestine. 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 (2).

ALP is present mainly on the cell membrane in various tissues and hydrolyzes a variety of monophosphate esters into inorganic phosphoric acid and alcohol. Human ALPs are classified into four types: tissue-nonspecific, intestinal, placental, and germ cell types. Based on studies of hypophosphatasia, which is a systemic skeletal disorder resulting from a tissue-nonspecific ALP (TNSALP) deficiency, TNSALP was suggested to be indispensable for bone mineralization (3).

These results suggest that variation in TNSALP may be an important determinant of age-related bone loss in humans and that the phosphate metabolism pathway may provide a novel target for the prevention and treatment of osteoporosis. Biochemically, Alkaline phosphatases (AP, EC 3.1.3.1) belong to a ubiquitous family of dimeric metalloenzymes which catalyse the
hydrolysis of phosphomonoesters under alkaline conditions with release of inorganic phosphate. One can distinguish between four isoenzymes in humans: placenta-specific AP, germ cell specific (placental) AP, intestinal AP and the tissue non-specific AP (TNAP). The production of TNAP is strongest in the liver, kidney and bones (4).

Alternatively, it has been suggested that TNAP could be a plasma membrane transporter for inorganic phosphate, an extracellular calcium ion binding protein that stimulates calcium phosphate precipitation and orients mineral deposition in osteoid. TNAP is known to be a marker of osteoblast differentiation. To our knowledge, however, there have been no previous reports of cell surface expression of TNAP by immature multipotential cells (5).

Serum ALP (total, isoenzymes, isoforms and LAP score) and evaluation methods

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

- **Neutrophil alkaline phosphatase (NAP)**

  Neutrophil alkaline phosphatase is detectable in differentiated neutrophils or monocytes and is the product of the liver/bone/kidney-type ALP gene. The enzyme activity is 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 (6).

- **Hepatic-bile alkaline phosphatase (HBAP)** presents 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 (7).

- **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 (8).

Numerous studies support the concept that the non-haemopoietic cells of the bone marrow (BM), which include fibroblasts, adipocytes, chondroblasts, smooth muscle cells, osteoblasts and other cellular elements of bone, are derived from a population of multipotential bone marrow mesenchymal precursor cells (MPC), residing somewhere in the bone marrow spaces and the surrounding connective tissue(9, 10).

Due to the lack of well defined markers, little is known of the precise developmentally regulated changes in phenotype and patterns of gene expression, which occur during the differentiation and maturation of human MPC into lineage-committed progeny(11).

Further studies have shown that ex vivo expanded human MPC quickly differentiate in the presence of serum, and begin expressing many of the markers associated with commitment to the osteogenic and other cell lineages(12).

-Alkaline phosphatase–intestinal isoenzyme (IAP)

Physiologically, AIP 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(13, 14).

The diverse nature of intestinal alkaline phosphatase (IAP) functions has remained elusive, and it is only recently that four additional major functions of IAP have been revealed. The present review analyzes the earlier literature on the dietary factors modulating IAP activity in
light of these new findings. IAP regulates lipid absorption across the apical membrane of enterocytes, participates in the regulation of bicarbonate secretion and of duodenal surface pH, limits bacterial transepithelial passage, and finally controls bacterial endotoxin-induced inflammation by dephosphorylation, thus detoxifying intestinal lipopolysaccharide. Many dietary components, including fat, protein, and carbohydrate, modulate IAP expression or activity and may be combined to sustain a high level of IAP activity. In conclusion, IAP has a pivotal role in intestinal homeostasis and its activity could be increased through the diet. This is especially true in pathological situations (e.g., inflammatory bowel diseases) in which the involvement of commensal bacteria is suspected and when intestinal AP is too low to detoxify a sufficient amount of bacterial lipopolysaccharide(15).

- **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 (16).

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°C.

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(17).

- Japanese Society of Clinical Chemistry proposes the measurement serum ALP activity in the electrophoretic separation and this method is performed using Titan III support media (Helena Laboratories, Beaumont, Texas), (18).

- 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), (19).

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 (19).

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 (20).

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.

On blood smear from peripheral blood, using the protocol of work NAP in vitro test, (Code SP 910 from Gailand Chemical Co), the lysosomes from granulocytes appear as dark blue or black grains in cell cytoplasm. The 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 1-4. The leukocyte-alkaline-phosphatase score will be given as the product of the number of cells counted and the percentage values. 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 (21), are listed in the [Table 1].

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 relevance/interpretation of serum ALP evaluation (total, isoenzymes and isoforms; no methods in this section).**

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, aundice, 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 (22).

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), in first month (120-720 U/L), at 3-years (110-650 U/L) an dat 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 between 90-190 U/L values for men and 85-165 U/L for women, (23).

After 60 years, 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 }
15% at the upper reference limit (24).

In the liver, ALP is concentrated in cells of the bile duct (25). 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 (eg, hyperparathyroidism, osteomalacia, and primary and metastatic neoplasms), in hepatobiliary diseases characterized by some degree cholestasis.

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 to children or to adolescents which typically have higher blood ALP levels because their bones are still growing (26).

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 was found low levels of activity in only 0.19% (27).

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 relevance/interpretation of the 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(6).

The major pathologic manifestations associated with leukemoid reaction are acute or chronic infections, 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- Grunwald Giemsa staining, display predominant toxic granulation (eg, Dohle 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 Hodgkins's disease and polycythemia vera (PV). Granulocytes from healthy individuals and patients with PV. Neutrophil alkaline phosphatase activity is substantially decreased in hematopoietic stem cell disorders such as CML, acute myelocytic leukemia (AML) and paroxysmal nocturnal hemoglobinuria(PNH). (28).

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.
Fossa and colleagues (29) 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 (30) 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 juxtaposed 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 trisomy21(Down syndrome), NAP activity is increased due to surplus chromosomal.

Conclusion

Interpretation of alkaline phosphatase results using appropriate reference populations is particularly important in children. Reference limits differ little in adult males and females between the ages of 25 and 60. Separate reference ranges are required for children and pregnant women. Isoenzymes testing is crucial for an accurate diagnosis and should be considered when signs and symptoms of diseases fail to provide a clear answer to clinical and
laboratory features.

The assessments of laboratory results of ALP activity, by different methods, help clinicians to make the correct decisions concerning treatment of hospitalized patients with benign or malignant diseases.

Reference


<table>
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<tr>
<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</th>
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<td>Total</td>
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**Table 1.** NAP score in Granulocytic Cells, calculated in function of color intensity, based on the observations reported by Kaplow. Abbreviation: ALP, alkaline phosphatase; NA, not applicable.
Fig 1. Leukocytes with positive reaction NAP, Leonard-Israels-Wilkinson stain. Alkaline phosphatase with positive reaction, + and ++, showing as black granules. In the left down, a neutrophil granulocyte without alkaline phosphatase activity.
Figure Captions List:

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.
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
   The patient is in the third trimester of pregnancy and the 453 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.