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) is used to help detecting liver diseases or bone disorders. In conditions in which the liver is affected, damaged liver cells release increased amounts of ALP into the blood. If other liver tests such as bilirubin, aspartate aminotransferase (AST) or alanine aminotransfere (ALT) are high, usually the ALP is coming from the liver. If it is not clear from the 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 the surses of APL. There are 4 gene ALP family: intestinal (found on chromosome 2); placental (2); germ cell (2) and non-tissue specific. The tissue non-specific isoenzyme includes the common serum forms of ALP from bone and liver(1).

The 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. A mouse monoclonal antibody specific to the bone alkaline phosphatase enzyme (BAP) is available and, has been adapted to an immunoassay in detection of this enzyme.

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

Alcaline phosphatase tests may be ordered of clinician doctors as the part of a routine lab profile testing, often with a group of other tests called the liver panel. Biochemically, the alkaline phosphatase (ALP), is an enzyme (AP, EC 3.1.3.1) which belongs to an ubiquitous family of dimeric metalloenzymes and is presented mainly on the cell membrane in various tissues, with the action to hydrolyse many types of phosphate esters at an alkaline pH, in the presence of zinc and magnesium ions.

ALP was found in all human tissues, but particularly is concentrated in liver, bone, kidney, intestine, placenta and in mature or imature leukocytes, as neutrophil alacaline phophatase(NAP). This enzyme exists in multiple forms, some are coded on specific genetic loci, while others (isoforms enzymes) differ only by post-translational modification (primary glycosylation), (1).

Measurement of ALP isoenzymes may be helpful in determining the organ or tissues with elevated ALP, (2). Based on studies of hypophosphatasia, which is a systemic skeletal disorder resulting from a tissue-nonspecific ALP (TNAP) deficiency, TNAP was suggested to be indispensable for bone mineralization (3).

These results suggest that variation in TNAP 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. which catalyse the hydrolysis of phosphomonoesters under alkaline conditions with release of inorganic phosphate. Can be distinguished four isoenzymes in human body: placental-specific ALP, germ cell specific ALP, intestinal ALP and the 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 and also TNAP was known to be a marker of osteoblast differentiation. However, there have been no previous reports of cell surface expression of TNAP by immature multipotential
Types of ALP isoenzymes

- Neutrophil alkaline phosphatase (NAP)

Neutrophil alkaline phosphatase is detectable in differentiated neutrophils and monocytes and is the product of the liver/bone/kidney-type gene ALP. The enzyme activity is induced by treatment of neutrophils with granulocyte colony-stimulating factor (G-CSF). Leakage of ALP from damaged or death, the 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 diseases, 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 a misdiagnosis for the pathologic conditions 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 an osteoblastic activity is observed. This misdiagnosis should also be avoided in adult female patients with osteoporosis being treated with biophosphonats. 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 regulated changes in phenmaturation of human MPC into lineage-committed progeny(11). Other studies have shown that human MPC have been quickly differentiated in the presence of serum and begun the markers expression of 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 was finding only recently four additional major functions of IAP. The present review analyzes, in earlier literature, 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 IAP is too
low to detoxify a sufficient amount of bacterial lipopolysaccharide (15).

- Placental alkaline phosphatase (PLAP)

PLAP is a normally ALP isoenzyme which occurs 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).

**Methods of ALP isoenzymes measurement**

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

An international accepted standard method for serum enzyme activity assessment ALP is the IFCC/AACC reference method which recommended 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, (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. 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 uncertain hematologic diseases, to decide if the cause of the elevated leukocyte count is a reactive process or a malignancy, in present been useful for small laboratories, because of is a very cheap method.

Using the protocol of work NAP in vitro test, (Code SP 910 from Gailand Chemical Co), on the blood smear from peripheral blood, 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 PNA score was calculated and for 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).

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 displays in microscopic field, morphologic characteristics in function of benign or malign disease [Figure 1].

**Clinical relevance/interpretation of serum ALP**

The 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 and past 10-years olds (130-700 U/L). 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 ≤10-15% 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. And other drugs may also affect ALP levels, for example, oral contraceptives may cause a decrease in ALP activity or antiepileptic drugs may cause an increase. In a review of nearly 70 000 ALP results for adult patients was found low levels of activity in only 0.19%..

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, (27).

**Clinical relevance/interpretation of the results from semi-quantitative method NAP**

Neutrophil alkaline phosphatase activity (NAP) does not appear as detected in human serum at healthy persons but its activity increases in cases of bacterial infection. A leukemoid reaction is an excessive 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. In leukemoid reaction the total leukocyte count is increased, typically in value of 50 000 to 100 000/mm³ and the granulocytes, observed in optic microscopy with May-Grünwald Giemsa staining, display predominant toxic granulation (e.g., Dohle bodies). 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 lymphoma and polycythemia vera (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.

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. NAP values are useful in distinguishing between Polycythemia Vera (increased NAP activity) and Secondary
Polycythemia (decreased NAP activity).

**Conclusion**

The laboratory results of ALP activity, obtained by different methods, 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 references of populations, is particularly important especially at children. Isoenzymes testing is crucial for an accurate diagnosis and this should be considered when the signs and symptoms of diseases fail to provide a clear answer to clinical and laboratory features in acute or chronic diseases.
Reference


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<th>Cells, No.</th>
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<th>Intensity of Color Indicating Granulocytic Cells, ALP scores</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. 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. 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 leukemias.

1. Pathological conditions which 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 spur
   **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 Limphocytic 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.