



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

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Authors' contributions

This work was carried out in collaboration between all authors. All authors designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript, managed the analyses in the study and managed the literature searches. All authors read and approved the final manuscript.

Review Article

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ABSTRACT

Aims: To conduct a review of the literature concerning existing methods to detect alkaline phosphatase (ALP) in human serum and to examine the dietary factors that modulate ALP-intestinal isoenzyme (IAP) activity, in light of new findings about its additional functions.

Background: Alkaline phosphatase (ALP) testing is used to detecting liver diseases and bone disorders. When the liver is impaired, damaged hepatocytes release increased amounts of ALP into the blood. If the results of other liver tests, such as for bilirubin, aspartate aminotransferase (AST), and/or alanine aminotransferase (ALT), are high, usually the ALP is usually coming from the liver. If it is not clear from the patient's signs and symptoms or from the results of other routine tests whether the high ALP originates

from is due to liver or bone, then a test for ALP isoenzymes, produced by different types of tissue, may be necessary to distinguish the sources of APL.

There are 4 gene ALP families: 1), intestinal (found on chromosome 2); placental (2); germ cell (3) and non-tissue-specific (4). The tissue nonspecific isoenzyme includes the common serum forms of ALP from bone and liver.

Discussion of Testing Methods: The total ALP activity is typically measured colorimetrically using the p-nitrophenol method. ALP isoenzyme levels can be measured via 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 (BAP) is available and, has been adapted to an immunoassay to detection this enzyme.

Conclusion: Isoenzyme testing is crucial before an accurate diagnosis can be made; this option should be considered when the signs and symptoms of certain diseases fail to provide a clear answer that explains clinical or laboratory features in acute or chronic diseases.

Keywords: Alkaline phosphatase; bone alkaline phosphatase; neutrophil alkaline phosphatase; tissue-nonspecific alkaline phosphatase; intestinal alkaline phosphatase.

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.

1. INTRODUCTION

Alkaline phosphatase (ALP) tests may be ordered by physicians and other health care professional as the part of a routine laboratory profile testing, often with a group of other tests called the liver panel. Biochemically ALP is an enzyme (AP, EC 3.1.3.1) that belongs to a ubiquitous family of dimeric metalloenzymes and is present mainly in the cell membrane in various tissues; its main action is to hydrolyze many types of phosphate esters at an alkaline pH, in the presence of zinc and magnesium ions.

ALP is found in all human tissues, and is particularly is concentrated in liver, bone, kidney, intestines, placenta, and in mature or immature leukocytes, as neutrophil ALP (NAP). This enzyme exists in multiple forms; some are coded on specific genetic loci, whereas others (such as isoforms enzymes) differ only by post-translational modification (primary glycosylation) [1].

Measurement of ALP isoenzymes may be helpful in determining which organ and tissues contain elevated ALP [2]. Based on the results of studies of hypophosphatemia a systemic skeletal disorder resulting from a tissue-nonspecific ALP (TNAP) deficiency, TNAP has been

suggested to be indispensable for bone mineralization [3]. 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.

Four isoenzymes can be distinguished in the human body: the placental-specific ALP (PLAP), germ cell-specific ALP, ALP-intestinal isoenzyme (IAP) and TNAP. The production of TNAP is strongest in the liver, kidney, and bones [4].

Alternately, it has been suggested that TNAP could be a plasma membrane transporter for inorganic phosphate; also, TNAP is known to be a marker of osteoblast differentiation. However, there have been no previous reports, to our knowledge, of cell-surface expression of TNAP by immature cells [5].

2. TYPES OF ALP ISOENZYMES

2.1 NAP

NAP 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 due to cell damaged or death, the neutrophils in infections may influence the release of NAP into the bloodstream [6].

Hepatic ALP (HAP) presents activities that are routinely measured during screening for liver disease. In some forms of liver diseases, such as hepatitis, the level of HAP is usually much less elevated than that of AST and ALT. Some individual who are at risk of liver disease include those who have been exposed to hepatitis viruses;, heavy drinkers;, those 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;, and those with an inherited disorder that affect the liver, such as Wilson disease [7].

2.2 Bone-Type ALP, (BAP)

An increased level of BAP should not trigger a misdiagnosis for at pathologic conditions, such as thyroid disease (eg. 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 type of misdiagnosis should also be avoided in adult female patients with osteoporosis who are 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].

Two studies [9,10] support the concept that the nonhaemopoietic 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 multipotent BM mesenchymal precursor cells (MPCs). These cells, reside somewhere in the BM spaces and the surrounding connective tissue.

Due to the lack of well-defined markers, little is known of the regulated changes in the phenomaturation of human MPCs into lineage-committed progeny [11]. Another study [12] has shown that human MPCs have been quickly differentiated in the presence of serum and have begun the markers expression of associated with commitment to osteogenesis and other cell lineages.

2.3 PLAP

PLAP is a normally ALP isoenzyme that occurs during the third trimester of pregnancy (quarter 3 of pregnancy). However the form of isoenzyme named Regan isoenzyme is that form of the isoenzyme that is associated with malignancy [13].

2.4 IAP

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 controls bacterial endotoxin-induced inflammation via 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. Thus, IAP has a pivotal role in intestinal homeostasis, and its activity could be increased through the diet. This is especially true in pathological situations, (eg.) inflammatory bowel diseases) in which the involvement of commensal bacteria is suspected and when the level of IAP is too low to detoxify a sufficient amount of bacterial lipopolysaccharide [14].

Physiologically, IAP activity is associated with individuals with blood group O or B; however, but its activity is increased in cirrhosis, intrahepatic cholestasis, enteritis, and chronic hemodialysis [15,16]. The diverse nature of intestinal alkaline phosphatase (IAP) functions has remained elusive;, and was finding only recently were 4 additional major functions of IAP discovered. The present review analyzes, in earlier literature, the dietary factors modulating IAP activity, as reported earlier in the literature, in light of these new findings.

3. METHODS OF ALP ISOENZYMES MEASUREMENT

At least 4 major methods are available to measure ALP isoenzymes: electrophoretic separation, immunoassay-based techniques, heat inactivation, and the substrate specificity-based technique. Enzyme and isoenzymes ALP activities in serum and in plasma can be measured in the different laboratories using various commercially available routine measurement systems.

An international accepted standard method for serum enzyme activity assessment ALP is the International Federation of Clinical Chemistry/American Association for Clinical Chemistry (AACC) reference method. This method recommended that, the accuracy of the measurement obtained using each device must be verified using gallium cell testing [17].

Other accepted standard methods are as follows:

- The Japanese Society of Clinical Chemistry proposes the measurement of serum ALP activity using the electrophoretic separation; and this method is performed using Titan III support media (Helena Laboratories, Beaumont, Texas) [18].
- Immun enzymatic method: a mouse monoclonal antibody specific to BAP is used in an immunoenzymatic assay; and the antibody-antigen (Ab-Ag) complex is measured using on a luminescence analyzer [19].
- The method used to test total ALP levels in the widest cases used is the p-nitrophenylphosphate method created by Bowers et al. Complexing agents, such as citrate, oxalate, and ethylenediaminetetraacetic acid (EDTA) bind cations such as zinc and magnesium, which are necessary cofactors for ALP activity measurement; this causes falsely decreased values. Transfusion of blood (containing citrate) causes a transient decrease in ALP through a similar mechanism. Also, in the colorimetric method, p-Nitrophenol reagent absorbs wavelengths of light in the 400-nm region; however 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].

3.1 Semi-Quantitative Method for Determining the Presence of NAP

NAP is an old method that was used to the differentially diagnosis uncertain hematologic diseases, to decide whether the cause of the elevated leukocyte count is a reactive process or a malignancy neoplasm, has been useful in present times for small laboratories, because it is a very inexpensive method. Using the protocol of the work NAP in vitro test, (Code SP 910 from Gailand Chemical Company), on the blood smear from peripheral-blood smear, the lysosomes from granulocytes appear as dark blue or black grains in cell cytoplasm. The interpretation of the chemical reaction is be determined according to the score generated by analysis of 100 segmented granulocytes, in which the intensity of the color of the grains is be recorded on a scale from 1 through- 4. The LAP score is 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 score and the calculated score according to intensity of color of granulocytic granules (based on the observations reported by Kaplow [(1921)]), are listed in the (Table 1).

Table 1. NAP score in Granulocytic Cells, calculated in function of color 366 intensity, based on the observations reported by Kaplow, [19].

Cells, No.	Cytoplasm Volume Displaying Color, %	Intensity of Color from Granulocytic Cells, Normal Score	Intensity of Color Indicating Granulocytic Cells, ALP score
0	NA	Absent = 60	Absent = 0
≥1	50	Small = 20	Mild = 20
≥2	50 - 80	Small = 14	Mild to powerful = 28
≥3	80 - 100	Mild = 5	Powerful = 15
≥4	100	Strong = 1	Very powerful = 4
Total	NA	100	67

Abbreviation: ALP, alkaline phosphatase; NA, not applicable.

The NAP score was calculated and, for the microscopic internal control, compared with the scores of specimens analyzed via a 2-slide blood-smear series. 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 and, unsegmented neutrophils, as well as eosinophils; any basophils that do not test NAP-negative must not be counted toward the ALP score. The NAP testing allows for discrimination between the normal and pathologic chemical activity of neutrophils as displayed in the microscopic field, as well as morphologic characteristics in the functioning of benign or malignant disease (Fig. 1).

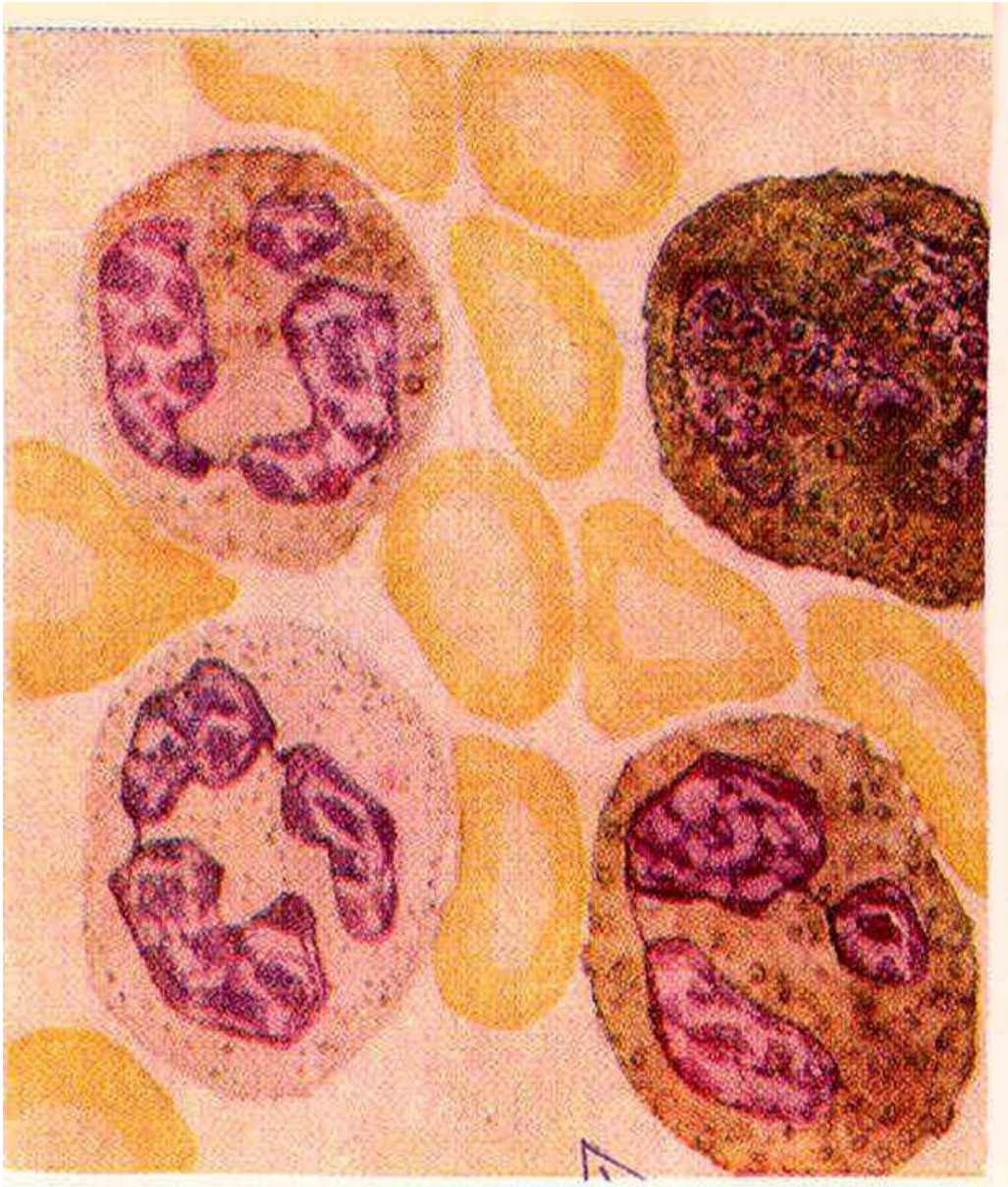


Fig. 1. Leukocytes with positive reaction via neutrophil alkaline phosphatase testing NAP, (Leonard-Israels-Wilkinson stain, original magnification. Alkaline phosphatase with positive reaction, + and ++ are, showing as black granules. In the lower left area of the image, down, a neutrophil granulocyte without alkaline phosphatase activity can be observed (arrow)

4. DISCUSSION

4.1 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 pain, jaundice, dark urine, light-colored stool, and itching. Some examples of the signs and symptoms that suggesting a bone disorder include: pain in the bones and/or joints pain, and increased frequency of bone fractures [21].

ALP is an enzyme with highly variable reference ranges during the human lifetime of an individual. For example, different reference ranges exist for newborns (110-450 U/L), infants in the first month of life (120-720 U/L), children at 3 -years of age (110-650 U/L), and children aged past 10 -years or older (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, men and women also have different ALP concentrations: between 90 and -190 U/L values for men and between 85 and -165 U/L for women [22].

After 60 years, reference limits increase in women; however, although studies have not consistently evaluated for the presence of osteoporosis, which can increase ALP activity in serum. Assays to determine ALP activity should have a total analytical error of less than or equal to 10 -15% at the upper reference limit [23].

In the liver, ALP is concentrated in the cells of the bile duct, [24]. ALP in bone is produced by osteoblasts, therefore, BAP reflects the activity of these cells, because ALP is a sensitive indicator of bone metabolism. An alternate reason for elevated ALP activity is osteoblast hyperreactivity of osteoblasts. Elevated total ALP activity in serum is observed when osteoblastic activity is increased (eg, hyperparathyroidism, osteomalacia, or metastatic neoplasms) and in hepatobiliary diseases characterized by some degree of cholestasis.

Smaller increases of ALP activity are seen in liver cancer and cirrhosis, with use of drugs toxic to the liver, as well as and, in hepatitis. Any condition causing excessive bone formation, including bone disorders such as Paget's disease and other conditions as rheumatoid arthritis and healing fractures, can cause increased ALP activity. ALP testing may also sometimes be used to monitor treatment of other bone conditions in children and adolescents, such as vitamin D deficiency to children or to adolescents, which typically involve higher blood ALP levels because their bones of these patients are still growing, (25).

ALP activities are also extremely high in patients taking certain drugs, particularly drugs that treat psychiatric disorders. Also other drugs may also affect ALP levels; for example, oral contraceptives may cause a decrease in ALP activity and antiepileptic drugs may cause an increase in ALP activity. In a review of nearly 70 000 ALP results for adult patients, low levels of activity were found in only 0.19% of patients.

The most common explainable causes of low or non detectable ALP activity levels were hypophosphatasia, malnutrition with low magnesium levels, 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 [26].

4.2 Clinical Relevance/Interpretation of the Results Derived from the Semi-quantitative NAP Method

NAP is not easily detected in the human serum of healthy persons; however 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 (eg, blast cells, myelocytes, and metamyelocytes); however, this reaction is distinct from leukemia. The leukemoid reaction appears in response to infection, as well as to 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 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 borne on arrival at the point-of-care department. In leukemoid reaction the total leukocyte count is increased, typically in values of 50 000 to 100 000 per/ mm³, and the granulocytes, observed via optic microscopy using May-Grünwald/Giemsa staining, display predominant toxic granulation (eg, Döhle bodies). Absence of the Philadelphia chromosome, or an extremely low score NAP, in chronic myeloid leukemia (CML), is usually sufficient to distinguish that this malignant disease to be distinguished from a leukemoid reaction, which involves with very high NAP activity.

Increased NAP activity is in some myeloproliferative diseases, such as Hodgkin's lymphoma and polycythemia vera (PV). NAP is substantially decreased in hematopoietic stem cell disorders such as CML, acute myelocytic leukemia (AML), and paroxysmal nocturnal hemoglobinuria (PNH) [27].

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 et al. [28] 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. [29] increased serum ALP activity was traced to neutrophils.

Also NAP activity allows the health care professional to distinguish between among 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. In children diagnosed with the diagnosis of trisomy 21 (eg. Down syndrome), NAP activity is increased due to surplus chromosomes. NAP values are useful in distinguishing between PV (increased NAP activity) and secondary polycythemia (decreased NAP activity).

5. CONCLUSION

Laboratory results that quantify ALP activity, which are obtainable via different methods, help clinician doctors, to make the correct decisions concerning treatment of hospitalized patients with benign or malignant diseases.

Interpretation of ALP results, using appropriate references of populations, is particularly important in children. Isoenzymes testis is crucially before an accurate diagnosis can be made; and this should be considered when the signs and symptoms of certain diseases fail to provide a clear answer that explains clinical and laboratory features in acute or chronic diseases.

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ETHICAL APPROVAL

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