Review Article

Investigative algorithms for disorders affecting human plasma alkaline phosphatase: a narrative review

Michael Irving[1], Alexa R. Shipman[2], Kate E. Shipman[3,4]

[1]Biochemistry and Immunology Department, University Hospital Coventry and Warwickshire NHS Trust, Coventry, UK; [2]Portsmouth Hospitals University NHS Trust, Portsmouth, UK; [3]University Hospitals Sussex NHS Foundation Trust, Worthing, UK; [4]Brighton and Sussex Medical School, Brighton, UK

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Correspondence to: Michael Irving, PhD. Biochemistry and Immunology Department, University Hospital Coventry and Warwickshire NHS Trust, Coventry CV2 2DX, UK. Email: Michael.irving@uhcw.nhs.uk.

Background and Objective: The following article is part of a special series to aid the physician in diagnosing the cause of various plasma abnormalities. Patients presenting with low or high alkaline phosphatase (ALP) activity can present a diagnostic challenge, particularly in the absence of symptoms. The objective is to provide information and algorithms to support the physician to order and interpret appropriate investigations when faced with this situation.

Methods: A narrative, focused literature review was performed of English language resources using PubMed, OMIM, ScienceDirect, and Google. References published from database inception to June 2023 were searched for from February 2023 to June 2023. Further articles were identified from reference lists.

Key Content and Findings: Bone and liver are the primary sources of ALP activity. When activity is low correlates with potassium, magnesium, and calcium concentrations which can help rule out specimen contamination and identify bone disease and malnutrition. When activity is high correlates with liver function tests, followed by a bone profile if normal. Analytical limitations include the range of isoforms present such as macro-ALP which can affect the results obtained.

Conclusions: Diagnostic algorithms are presented that should support healthcare professionals to efficiently and systematically approach people with these abnormalities.

Keywords: Alkaline phosphatase (ALP); low; high; diagnosis; algorithm

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Introduction

The alkaline phosphatases (ALPs) are a group of isoenzymes that catalyse the hydrolysis of organic phosphate esters. Located primarily in the cytoplasm they are anchored to the plasma membrane by a glycosylphosphatidylinositol anchor in almost all tissues (1,2), with a half-life of 7 days (3). Optimal enzyme conditions include an alkaline pH, between 8–11 (4). The ALP metalloenzyme family is encoded by multiple genes in humans, is expressed in multiple tissues including liver and bone, and each enzyme requires three metal ions, two Zn^{2+} and one Mg^{2+}, in its active site (5).

ALP is widely included in panels designed to assess liver and bone function with alanine aminotransaminase (ALT) and/or aspartate aminotransferase (AST), total protein, albumin, and bilirubin, or calcium, albumin with or without phosphate respectively (6). The International Federation of Clinical Chemistry (IFCC) stated adult range is 45–135 U/L (7). Diagnostic algorithms will be presented to provide an approach to investigate abnormalities of ALP activity. This article is not meant to replace current

Table 1 The search strategy summary

Items	Specification
Date of search	February 2023–June 2023
Databases and other sources searched	PubMed, OMIM, ScienceDirect, Google
Search terms used	"ALP", "Alkaline Phosphatase", "Hypophosphatasia", "isoenzyme", "pregnancy"
Timeframe	From database inception to June 2023
Inclusion criteria	All papers and reviews were included, restricted to English
Selection process	M.I. and K.E.S. conducted initial search, with refinement by all other authors to obtain consensus and agreement
Any additional considerations, if applicable	Seminal texts were also searched and the references of important articles and texts were obtained and checked for relevance

guidelines and reviews, nor replace thorough clinical assessment. Instead, these algorithms are aimed to enhance the understanding of the role of diagnostics in the clinical pathway. Quality and efficiency of patient care are promoted by the appropriate use of diagnostics. We present this article in accordance with the Narrative Review reporting checklist (available at https://jlpm.amegroups.org/article/view/10.21037/jlpm-23-63/rc).

Methods

The narrative literature review was undertaken with review of PubMed, OMIM, ScienceDirect, and Google and seminal texts (*Table 1*). The search was performed from February 2023 to June 2023 from database inception. Language was restricted to English. Diagnostic algorithms were created from synthesis of the information obtained from literature review.

Metabolic role of ALP

ALP is a zinc metalloenzyme; it is activated by magnesium, or other cations. Encoded by four distinct genes that encode the isoforms found in humans with a great many functions (*Table 2*) (14). The most abundant, *ALPL*, accounts for the tissue non-specific ALP (TNSALP) that is found in liver, bone, and kidney, encoded on chromosome 1. Post translational modification further results in distinct carbohydrate compositions between those produced in the liver (hepatocellular and biliary canalicular subtypes) and in bone osteoblasts (BALP) (5). *ALPP*, *ALPP2* and *ALPI* encode tissue specific ALP found in the placenta (syncitiotrophoblasts), germ cells, and intestines (enterocyte luminal surface) respectively (5). Tumours have been associated with excretion of variations of the placental isoform e.g., Regan isoenzyme (15). Both liver and intestinal types are found in the brush border of the renal proximal tubule (*Table 2*). The three major substrates for ALP are inorganic pyrophosphate (PP_i), pyridoxal-5-phosphate (PLP), and phosphoethanolamine (PEA) (16).

Although ubiquitous there is a considerable difference in relative enzyme activity between tissues (*Table 3*) (8). Although activity may be higher in the kidney, bone is heavier therefore the highest ALP tissue activity in total is found in the placenta followed by the intestine, bone, kidney, and liver in descending order (2). In health, human serum contains predominantly bone and liver isoforms, with approximately equal activity from both. Elevations of ALP activity are seen in health, e.g., during pregnancy and placenta formation (placental isoform), growing (bone isoform, and see later section on special states) or can represent a wide range of disorders of tissues (particularly those with high ALP activity) including endocrine and medication causes (2).

Liver based ALP is synthesised in many tissues including hepatocytes and osteoblasts (*Figures 1,2*) (3,14). At least 90% (8) of the ALP is attached to exterior surfaces, 3% is found in the cytosol and the rest in the extracellular fluid and vessels. ALP is eliminated by being taken up by hepatocytes and catabolised in lysosomes (14) (*Figure 1*). In adults, there is a continuous process of bone remodelling, involving the resorption of bone by osteoclasts, followed by the synthesis and maintenance of bone by osteoblasts (*Figure 2*). This is a process that is regulated by complex interactions between large numbers of factors and hormones and is highly co-ordinated (18). ALP, no matter the source, is not cleared by the kidneys

Table 2 Table of human alkaline phosphatase isozymes (8-13)

Human genes	Names	Tissue distribution	Function
ALPL	Tissue nonspecific alkaline phosphatase	Developing nervous system	Hydrolyses pyrophosphate supplying inorganic phosphate for mineralization, reduces extracellular pyrophosphate and phosphorylcholine concentration, dephosphorylation and detoxification of lipopolysaccharides, sphingosine 1-phosphate receptor signalling, antiendotoxin mediator and anti-inflammatory, regulation of adenosine concentrations
	Liver-bone-kidney type alkaline phosphatase	Skeletal tissue, liver, kidney	Hydrolyses a variable spectrum of phosphate-containing compounds, contributes to DNA synthesis, attenuates inflammation, influences mitochondrial respiration, extracellular matrix mineralization
ALPP	Placental alkaline phosphatase	Syncytio-trophoblast, tumours	Indicative of tissue having stem cell functions, tumour marker, detoxification of bacterial endotoxin
ALPP2	Germ cell alkaline phosphatase	Testis, malignant trophoblast, testicular cancer	Indicative of tissue having stem cell functions, sperm glycolytic reactions and fructose formation, tumour marker to diagnose carcinoma-*in situ* of the testis, seminoma
ALPI	Intestinal alkaline phosphatase	Gut	Intestinal tight junction integrity and maintains barrier function, attenuates inflammation, regulation of intestinal surface pH, absorption of lipids, detoxification of free nucleotides and bacterial lipopolysaccharides, possible modulation of the gut microbiota, regulation of transmucosal passage of bacteria, dephosphorylation of extracellular adenosine triphosphate

Table 3 Relative tissue activity of alkaline phosphatase in human tissue (2,8,17)

Tissue	Activity per g of wet tissue with two substrates, liver activity set to 100 as benchmark		IU/g of tissue, mean ± standard deviation
	B-glycerophosphate	Phenylphosphate	
Placenta	–	3,214	69±44
Ileum (mucosa)	1,714	2,524	38±14
Kidney	619	–	2.1±0.7
Bone	–	571	Age dependent
Colon (mucosa)	471	–	2.3±0.8
Adrenal	167	–	
Lung	129	–	2.1±0.5
Spleen	129	–	
Liver	100	100	2.6±1.4
Brain	76	–	
Stomach (mucosa)	62	–	
Heart	–	33	
Pancreas	–	10	
Skeletal muscle	–	5	
Serum (adult)	0.2	–	
Testes	–	–	0.5±0.1

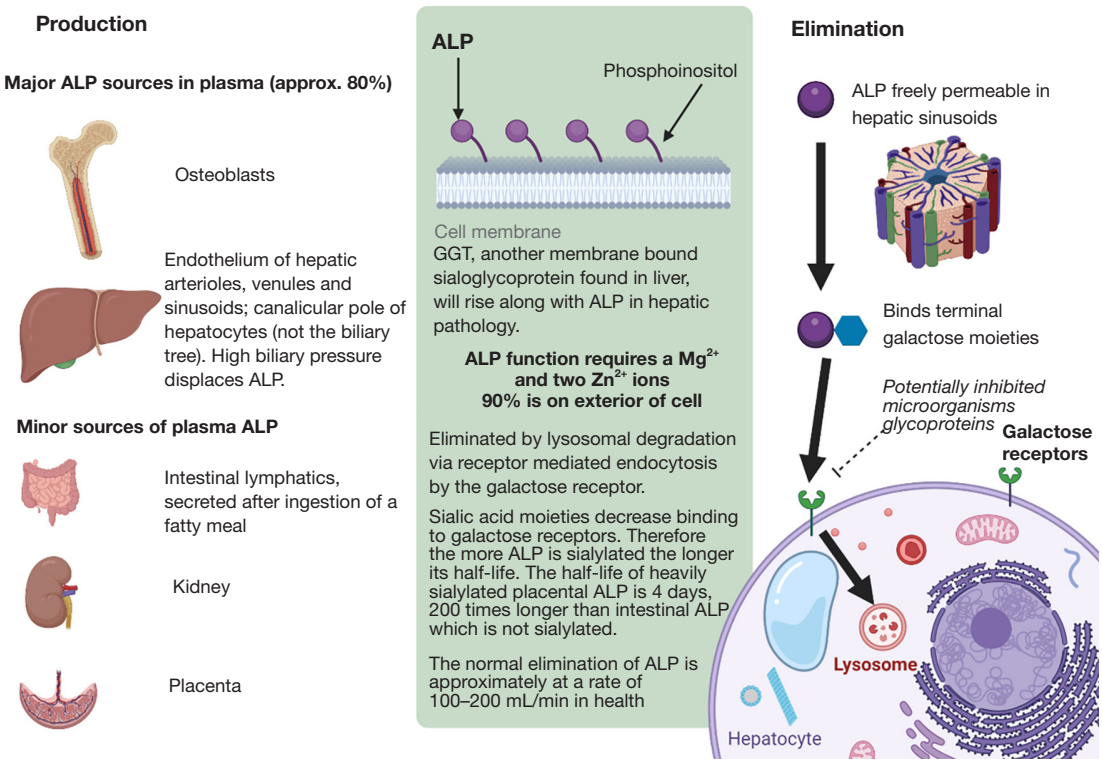

Figure 1 Summary of ALP origin, production, and elimination in the body. ALP, alkaline phosphatase; GGT, gamma glutamyl transferase; Mg, magnesium; Zn, Zinc.

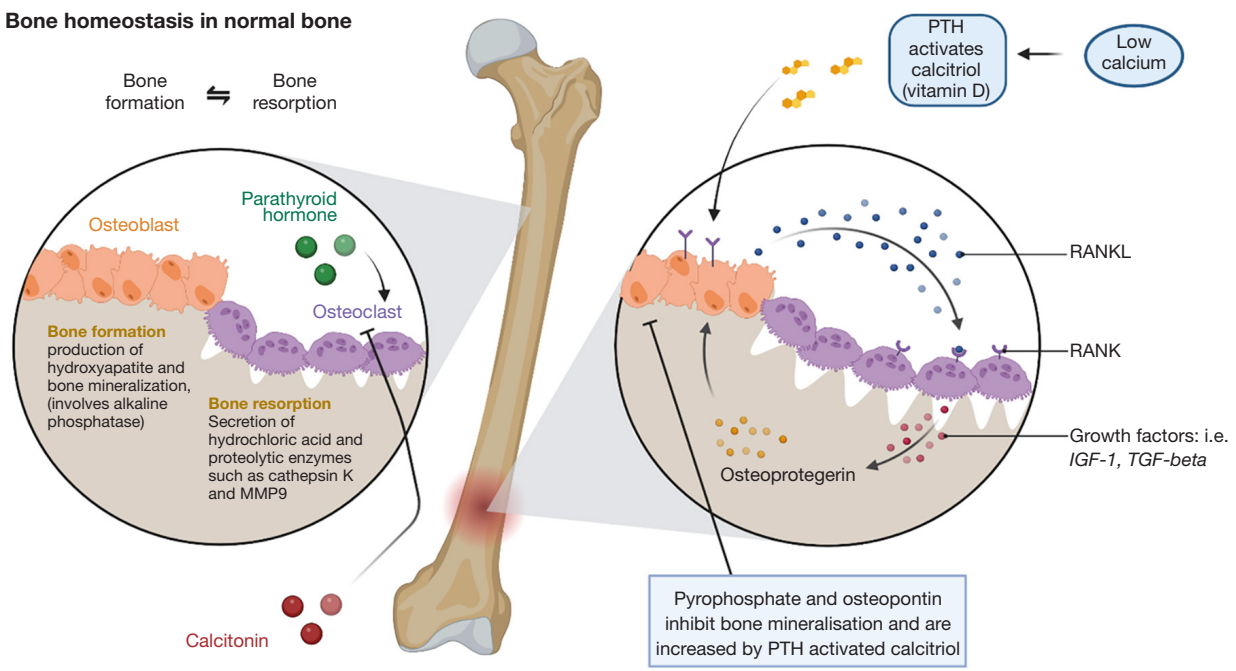

Figure 2 Bone homeostasis in normal bone. PTH, parathyroid hormone; RANKL, receptor activator of nuclear factor kappa-B ligand; RANK, receptor activator of nuclear factor kappa-B; IGF-1, insulin-like growth factor 1; TGF-beta, transforming growth factor beta.

making it a useful tool for assessment of bone turnover in the presence of chronic kidney disease (CKD) (19).

In CKD, abnormalities of mineral and bone metabolism occur with extra-skeletal calcification, called CKD-mineral bone disorder (CKD-MBD), which is distinct from the morphological diagnosis of renal osteodystrophy which is a consequence of it (20,21). Fibroblast growth factor 23 (FGF23) is raised prior to the more characteristic findings of hyperphosphataemia, high parathyroid hormone (PTH) or low activated vitamin D (1,25VitD) and is likely a key factor for renal, bone, mineral, and cardiovascular complications (22). Although FGF23 is phosphaturic, in CKD the renal response is diminished resulting in hyperphosphataemia which stimulates PTH secretion with a subsequent increase in bone turnover and measurable ALP activity. Furthermore, the impaired urinary tubular function and elevated serum FGF23 concentration lead to decreased vitamin D activation and enhanced PTH secretion under conditions of relative calcium deficiency, thereby giving rise to secondary hyperparathyroidism. BALP may be slightly superior in diagnosing bone disease in CKD-MBD but not enough to justify the additional cost for routine practice (21).

Measurement of ALP

ALP activity is dependent on zinc and activated by magnesium thereby order of blood draw is important to prevent cation chelation, and hence reduction in measured activity, by common tube additives, such as ethylenediaminetetraacetic acid (EDTA), oxalate, or citrate (23). Delayed separation of serum from the cells may very marginally increase ALP activity but analysis within 24 hours is unlikely to affect results in a clinically significant way irrespective if stored at room temperature or refrigerated (24,25).

The activity of ALP is measured by the change of absorbance at 405 nm by the yellow quinoid version of 4-nitrophenoxide formed at an alkaline pH following ALP catalysing the cleavage of phosphate from 4-nitrophenyl phosphate. The rate can be increased by including a phosphate acceptor e.g., adenosine monophosphate, which is also included in the IFCC reference method based on the above reaction (26).

Isoforms can be detected through a variety of means e.g., electrophoresis (27), differential deactivation e.g., by heat (1,28), differential response to inhibitors (1,29), affinity for lectins (30,31) and immunoassay (32,33). However, in clinical practice other routinely available tests are mostly reliable at identifying the source (as well as the marked improvement in imaging technology and availability) and the time, and cost, it may take to get these specialist tests makes them almost obsolete (34).

There are several caveats to consider when interpreting ALP results:

- Race and sex can impact ALP activity: positive correlation with increases in mean body mass index (BMI) of populations, seen in those of Hispanic descent greater than those of African American, which were greater than those of Caucasian. Males have higher activities than females within populations, which is also positively correlated to BMI (35).
- People who smoke have activity 10% higher than those who do not (36).
- Activity fluctuates approximately 6% from week to week in a healthy individual (37).

ALP can become bound to immunoglobulin, called macro-ALP, which prevents it being cleared as quickly (38,39). This is analytically correct, i.e., there is an increase in ALP activity in the serum, but does not represent increased tissue turnover and therefore a potential cause of spurious results.

Low ALP activity

In a study of unselected male patients, the causes of a low ALP, which was rare, occurring in only 0.2% of almost 70,000 samples, were:

- Cardiac surgery and cardiopulmonary bypass (26.5%), mean pre-surgical ALP was 71 U/L which fell to 20 U/L, corresponding magnesium concentration fell from a mean of 0.98 to 0.54 mmol/L.
- Malnutrition (12.0%), mean ALP of 18 U/L, secondary to decreased activity of both bone and hepatic ALP.
- Severe magnesium deficiency (mean concentration 0.48 mmol/L) affected 4.8% with a mean ALP activity of 21 U/L.
- Hypothyroidism (2.4%), ALP activity returning to normal once euthyroid.
- Severe anaemia attributed to iron deficiency (1.2%) (40).

In an audit of a year's cases another group identified blood transfusion, cardiopulmonary bypass, and chemotherapy as causes, but a few cases had no identifiable cause (41). This led the team to conclude that the lower limit of ALP activity is too arbitrary to be useful to pick up important pathology. The causes of low ALP activity will be discussed below with *Figure 3* providing a diagnostic algorithm aimed at helping

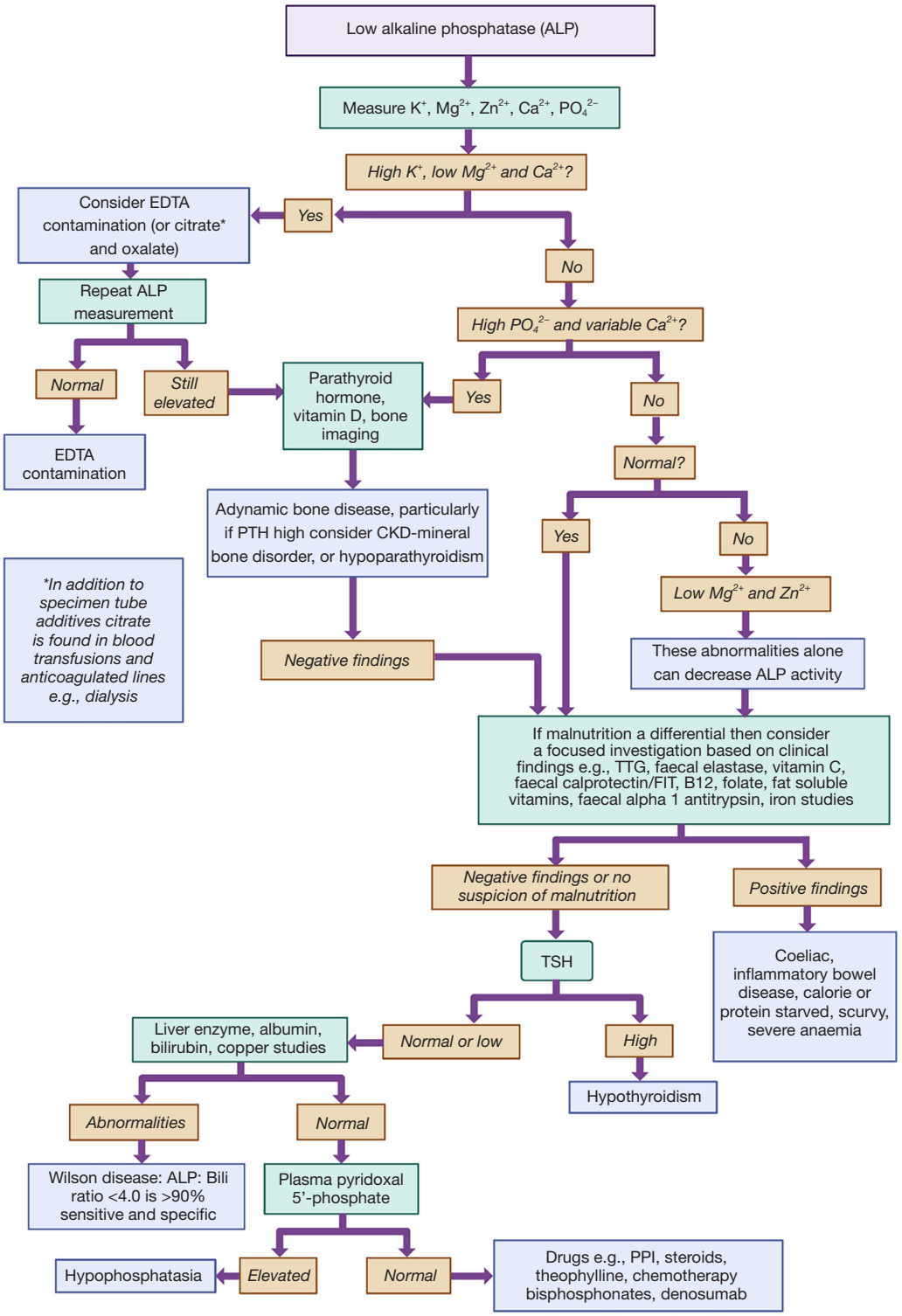

Figure 3 Algorithm and supporting information for the laboratory investigation of low ALP. ALP, alkaline phosphatase; K, potassium; Mg, magnesium; Ca, calcium; PO, phosphate; EDTA, ethylenediaminetetraacetic acid; PTH, parathyroid hormone; CKD, chronic kidney disease; TTG, tissue transglutaminase; FIT, faecal immunochemical test; B12, vitamin B12; TSH, thyroid stimulating hormone; PPI, protein pump inhibitor.

the clinician approach the investigation.

Pseudo hypophosphatasia

For an unexpected isolated low ALP activity EDTA, or other tube additives such as oxalate or citrate, contamination should be considered. Serum ALP activity decreased significantly at EDTA concentrations of >1.86 mmol/L (unexpected hyperkalaemia and hypocalcaemia are further clues) but this degree of contamination is not commonly reached in most cases of contamination (42,43). EDTA, citrate and oxalate bind cations and hence reduce ALP activity which requires both zinc and magnesium ions. Citrate is also found in blood transfusions and anticoagulated lines, e.g., in haemodialysis.

Primary hypophosphatasia

Primary low ALP activity, called hypophosphatasia, is due to genetic mutations in the *ALPL* gene (TNSALP) and clinical symptoms can include rickets and osteomalacia, epilepsy, myopathy, respiratory difficulties, hypercalcaemia, nephrocalcinosis, and tooth loss (16). At its most severe it can be fatal in infancy, but others will present as adults (16). Despite the wide phenotype all will have dental and skeletal mineralization symptoms, with high PPi in the bone matrix (16). Deformed and painful bones in infancy or painless, early, tooth loss (with roots attached) may indicate hypophosphatasia (16). ALP deactivates active B6, PLP, to form pyridoxal, and the accumulation of pyridoxal may account for the seizures seen in some babies with hypophosphatasia, occurring a few days after birth. Although not demonstrated in humans, restoring ALP activity in deficient mice resolves the seizures (16). High urinary PEA is another useful screening tool however genetic diagnostics (more than 300 mutations have been described in the *ALPL* gene but not all are disease causing) and enzyme replacement therapy is now available (16). Hyperphosphataemia can be seen, and later hypercalcaemia, as the phosphate and calcium homeostasis mechanisms are normal promoting renal excretion which can lead to renal complications such as renal failure and nephrocalcinosis (16).

Malnutrition and malabsorption

Malnutrition can lead to low ALP activity. This may be caused by metal deficiencies such as zinc or magnesium (44,45). In one study all those with a low ALP, compared to controls, were deficient in either zinc (47.6%) or magnesium (52.4%) (46). Nutritional rickets can reduce ALP activity, however if vitamin D deficient with low dietary calcium intake (47), ALP may be elevated (although not reliably) (48-50). A low protein state may lead to a low ALP activity (51,52) but again this observation is not consistent in kwashiorkor (53).

Vitamins C and B12 have been shown to promote bone growth, and deficiencies have a negative impact on bone growth (54) and increase the rate of osteoporosis (55). ALP is a marker for bone turnover therefore any decrease in turnover will affect the activity of ALP (55). However, it is unusual to find isolated nutritional deficiencies with, for example, a case report of scurvy demonstrating elevated ALP due to hypovitaminosis D, and coeliac disease which is often associated with elevated ALP activity (56,57).

Endocrine and metabolic links to ALP

Wilson disease, an inborn error of copper metabolism, has been associated with low ALP activity, particularly in the initial stages (58). It is believed that copper competition with zinc causes the suppression in ALP activity (25). Conditions associated with low bone turnover, such as hypoparathyroidism and hypothyroidism, may reduce ALP activity, likely not enough to cause low ALP activity commonly (16,59). In cardiopulmonary bypass ALP is presumed to be consumed by dephosphorylating inflammatory chemicals and the reduction in activity correlates with worsening outcomes (60).

Drugs and toxins

Clofibrate reduces ALP activity but is no longer used (61,62), having been replaced by other drugs in the fibrate class due to side effects. ALP activity is inhibited by theophylline, sulphonamides, arsenates, molybdates, and other agents, with active development of ALP inhibitors underway to attempt to treat ectopic calcification which are not yet on the market (8,63-65). Inhibition by cation chelation by citrate can cause a transiently low ALP, e.g., in neonates or massive transfusions (41). ALP production is reduced by bisphosphonates (66,67), denosumab (68), and proton pump inhibitors (69) although they may not result in ALP activity beneath the reference range (70) (see *Table 4*).

High ALP

The commonest tissue origins of an elevated ALP activity are liver and bone (see *Table 5*). The key differentials will

Table 4 Drugs that can lower measured ALP activity in humans (8,71,72)

Drugs	Mechanism
Proton pump inhibitors	Inhibit osteoblasts, decreases bone turnover
Steroids	Effects variable on bone turnover and can also raise ALP
Theophylline, aminophylline	Inhibits ALP activity and so test is falsely low
Bisphosphonates	Suppress osteoclast and so negative feedback to osteoblast
Denosumab	Suppress osteoclast and so negative feedback to osteoblast
Sulfonamides	Inhibits ALP activity and so test is falsely low
Cimetidine and ranitidine	Inhibits ALP activity and so test is falsely low
Imidazole and levamizole	Inhibits ALP activity and so test is falsely low
Nitrofurantoin, cyanides, arsenals	Inhibits ALP activity and so test is falsely low

ALP, alkaline phosphatase.

Table 5 Relative tissue activity of alkaline phosphatase in human pathology (2,73-77)

Disorder	Patients with abnormal ALP (%)	Mean ALP (multiple of upper reference limit)
Primary liver cancer	92	5.5
Tumour, metastatic liver	88	5.5
Extrahepatic obstruction	94	4.9
Intrahepatic obstruction	82	2.8
Acute viral hepatitis	80	2.5
Inactive cirrhosis	75	2.1
Alcoholic hepatitis	77	1.8
Chronic active hepatitis	78	1.7
Primary biliary cholangitis	>95	1.67[†]
Osseous metastases	74	2
Pregnancy	100	Depends on stage, up to 4
Osteomalacia	80	1.5
Low vitamin D	0	–
Hyperthyroidism	44	Up to 5
Secondary hyperparathyroidism	75	–

[†], wide range of values, this figure, and above, is the trigger for treatment with ursodeoxycholic acid. ALP, alkaline phosphatase.

differ depending on how well the person is and, potentially, the degree of elevation (*Table 5*). In hospitalised patients ALP is commonly raised [causes include pyelonephritis, malignancy, congestive heart failure and renal failure (14,78,79)]; in those with underlying conditions (who are well) elevations tend to resolve within 3 months (14). The causes of a rise in ALP activity are discussed below. A diagnostic algorithm is provided as a systematic framework that can be used to guide rather than be followed proscriptively, see *Figure 4* (80,81). It is important to note in mild elevations of ALP activity other laboratory tests may not be helpful in identifying the cause, particularly in asymptomatic patients, and there may need to be a strategy of watchful waiting to determine if the elevation

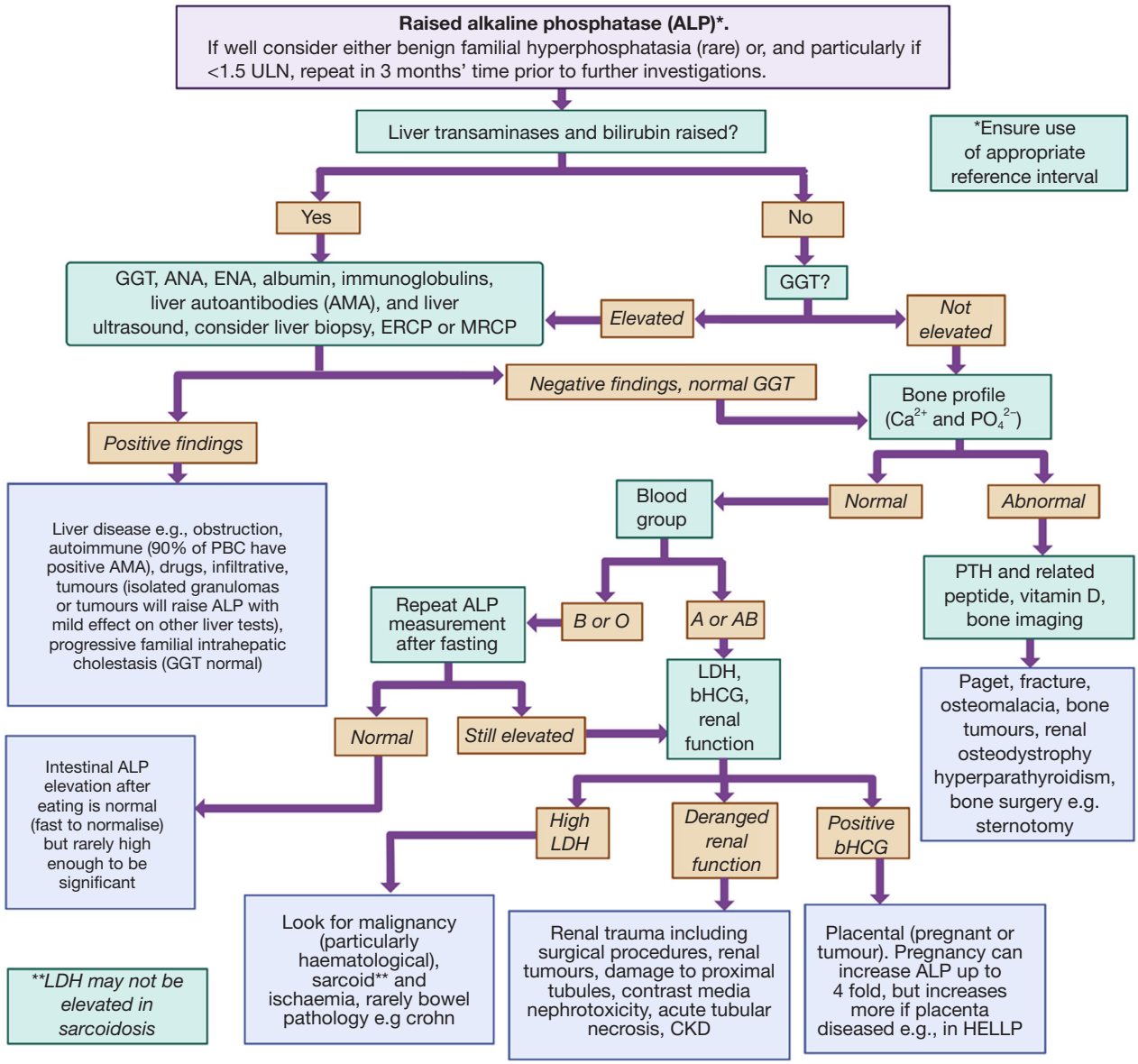

Figure 4 Algorithm and supporting information for the laboratory investigation of raised ALP. ALP, alkaline phosphatase; ULN, upper limit of normal; GGT, gamma-glutamyl transferase; ANA, anti-nuclear antibody; ENA, extractable nuclear antigen antibody; AMA, anti-mitochondrial antibody; Ca, calcium; PO, phosphate; ERCP, endoscopic retrograde cholangiopancreatography; MRCP, endoscopic retrograde cholangiopancreatography; PBC, primary biliary cholangitis; PTH, parathyroid hormone; LDH, lactate dehydrogenase; bHCG, beta human chorionic gonadotrophin; CKD, chronic kidney disease; HELLP, HELLP syndrome.

is persistent and the organ source [if less than 1.5 times the upper limit of normal (ULN) for example consider repeating at 3 months if isolated and patient is well] (82).

ALP and its association with the liver and biliary tract

Most test panels for liver function include the enzymes ALT and ALP. A larger rise in ALT activity (compared with the ULN) may indicate hepatocellular damage whereas a relatively more significant ALP elevation (compared with the ULN) likely indicates a cholestatic pathology. Gamma-glutamyl transferase (GGT) elevation supports the diagnosis of a liver aetiology (GGT should be normal in bone causes of high ALP, unless there is

more than one pathology) (83-85). An exception is the rare familial intrahepatic cholestasis which has raised ALP and normal GGT activities (86). This can present in a benign form that occurs at any age, and lasts for several weeks to months, or a progressive form that causes severe cholestasis before 6 months of age and progressing to cirrhosis, liver failure and death, unless a liver transplant is provided (86).

When reviewing raised ALP results, an elevation of ALP of approximately four times the ULN or greater occurs in up to 75% of the patients with cholestasis, either intrahepatic or extrahepatic in one study (87). Liver diseases that principally effect parenchymal cells, such as infectious hepatitis, typically show only moderately elevated or even normal ALP activity but this depends upon study (see *Table 5*). As per the European Association for the Study of the Liver (EASL), activity thresholds for serum requiring diagnostic work-up are >1.5 times ULN (88).

This review will concentrate on the cholestatic liver diseases as the hepatocellular diseases will be covered in more detail in the companion article to this (on investigation of elevated transaminases). Cholestatic liver diseases include autoimmune e.g., primary biliary cholangitis or cirrhosis (PBC) (89). PBC is diagnosed by the presence of anti-mitochondrial antibodies (AMAs) (90) and increased concentrations of immunoglobulins [mainly immunoglobulin M (IgM)] (91). Anti-nuclear antibodies (ANAs) and anti-smooth muscle antibodies (SMAs) are found in nearly 50% of PBC patients (92). ANA, anti-glycoprotein 210 and/or anti-sp100 (nuclear membrane proteins) may be present in those who are AMAs negative (93).

Primary sclerosing cholangitis (PSC) and secondary sclerosing cholangitis are differentials for PBC (94,95). Up to 80% of people with PSC are diagnosed with inflammatory bowel disease (IBD), primarily ulcerative colitis (88). In PSC immunoglobulins are often elevated with IgM (96) and IgG raised in 45% and 61% respectively, with IgG typically exceeding 1.5 times the ULN (97). Immunoglobulin G Subclass 4 (IgG4) was found to be elevated in 9% of PSC patients in another study and has been suggested to be a disease subtype (98,99), with those with raised IgG4 tending to progress more rapidly without treatment (98,100). Other autoantibodies present include perinuclear antineutrophil cytoplasmic antibodies (pANCAs) (26–94%), ANA (8–77%), and SMAs (0–83%) (101).

Imaging is relevant for all patients in whom cholestasis is suspected, cholangiography, preferentially with non-invasive magnetic resonance imaging (MRI), or endoscopically, to exclude PSC. Transient elastography is another non-invasive tool that has shown high degree of accuracy in diagnosing advanced fibrosis in patients with PBC (102). A liver biopsy is rarely required.

Obstruction of the biliary tree, or cholestasis, will lead to an elevation of the ALP and causes for this include gallstone, biliary strictures and tumours, infiltrative processes, and medication (see *Table 6*) (82,108-111). For example, colestipol, a bile acid sequestrant, increases ALP activity but is not widely used anymore (61).

Bone

Increasing bone turnover, particularly osteoblast activity, will elevate ALP (5). Other tests including vitamin D, calcium, phosphate and PTH may be required to distinguish some of the following disease entities, and this is further illustrated in *Figure 4*. There are other, less commonly requested tests, which might also be available for the clinician to distinguish bone diseases (see sister algorithms in this special series on calcium and phosphate).

Common causes of bone pathology that increases ALP are periods of increased skeletal growth, such as during adolescence (4), fracture recovery (112), cancer including osteosarcoma or bone metastasis, hyperparathyroidism, CKD and vascular calcification (113).

Rickets, a rare condition in the UK, is the clinical consequence of impaired mineralization of the growth plate cartilage and spongiosa in the metaphysis of children and adolescents (114) due to calcium and/or vitamin D deficiency (calcipenic rickets), renal tubule dysfunction and disturbances of chondrocytes and osteoblasts (*Table 7*). Bones are resorbed to release calcium, under PTH action, which also results in hypophosphataemia (116,117). Osteomalacia is rickets occurring after the growth plates fuse, i.e., the adult version (114), and biochemically there will be raised PTH with deficiencies in phosphate and vitamin D. In osteomalacia vitamin D supplementation will return ALP activity to normal after about 6 months of treatment, therefore there is no need to remeasure activity prior to this time point (34).

Paget disease is an increasingly rare condition of disordered bone turnover with no clear single cause (118,119). Markers of bone turnover are raised including type I procollagen N-terminal peptide (P1NP), N-terminal telopeptide of type I collagen and bone ALP (120), with ALP activity used to measure treatment efficacy (121).

Table 6 Drugs that can cause an elevation in measured alkaline phosphatase activity in humans—excluding hepatotoxic medications (82,103-107)

Drugs	Mechanism
Antibiotics	
Penicillin derivatives	Intrahepatic cholestasis
Erythromycin	Intrahepatic cholestasis
Aminoglycosides	Enzyme induction
Sulfa drugs	Intrahepatic cholestasis
Antiepileptic drugs	
Carbamazepine	Intrahepatic cholestasis
Phenobarbital	Enzyme induction
Phenytoin	Enzyme induction
Sodium valproate	Enzyme induction
Antihistamines	
Cetirizine	Intrahepatic cholestasis
Cardiovascular drugs	
Captopril	Intrahepatic cholestasis
Diltiazem	Enzyme induction
Felodipine	Enzyme induction
Verapamil	Intrahepatic cholestasis
Disease modifying agents	
Penicillamine	Intrahepatic cholestasis
Sulfa drugs	Intrahepatic cholestasis
Allopurinol	Causes a granulomatous hepatitis
Polycyclic aromatic hydrocarbons	
Oral contraceptive pill (oestrogen)	Enzyme induction
Anabolic and corticosteroids	Enzyme induction but variable and can lead to low ALP
Psychotropic drugs	
Monoamine oxidase inhibitors	Intrahepatic cholestasis
Phenothiazines and chlorpromazine	Intrahepatic cholestasis
Lipid lowering	
Statins	Enzyme induction

ALP, alkaline phosphatase.

However, there is an argument that ALP normalization is an inappropriate measure of treatment success as only occurred in ~25% of those treated with bisphosphonates, though ALP activity overall did reduce by ~41% (122). Treating to normalise ALP has no reported differences in fracture rate, pain relief, hearing, need for orthopaedic surgery or quality of life when compared to treating to alleviate bone pain solely (123,124). Imaging is used to confirm a diagnosis of Paget disease (125).

Osteoporosis is not associated with an elevation of ALP, unless there is a secondary fracture, however BALP can be used to monitor the effect of treatment on bone turnover (126). Osteomyelitis does not raise ALP either. A femoral neck fracture elevates serum ALP activity by approximately 30% and trochanteric by 100% (14,127).

Note that oestrogens reduce BALP in post-menopausal women which might theoretically disguise subtle elevations in women on hormone replacement therapy (128). Steroids have an unpredictable effect on BALP as although they may be osteogenic in exogenous or endogenous hypercortisolism there may be no noticeable effects or mild suppression (66,129,130). Hyperthyroidism and thyrotoxicosis cause an elevation in ALP, likely by effects on bone metabolism (131,132).

Tumour placental ALP

In cancer there can be significant production of ALP which cannot be related to the tissue involvement and therefore represents paraneoplastic production of foetal proteins (particularly placental type ALP) from the tumour. Regan isoform, a rare variant of placental ALP, is one example; in one series (133) of 239 people with malignant disease 25.5% had elevated Regan isoenzyme detectable in the serum. Tumour types reported to demonstrate elevations of Regan isoform include kidney, stomach, uterine, lung, cervical, endometrial, testicular, ovarian, medullary thyroid, haematopoietic, prostate and germ cell (15). Other examples include Kasahara a foetal intestinal ALP isoform (134) e.g., in renal cell carcinoma (135), and Nagao a placental-like ALP isoform (134,136).

Miscellaneous

Transient hyperphosphatasaemia (TH) is a benign condition, of unknown aetiology, characterized by marked elevation of serum ALP activity, such as an increase of four-fold ULN. There should be a notable absence of associated diseases such as liver, bone, or kidney pathology and it

Table 7 Typical biochemical features of various forms of osteomalacia/rickets (115)

Cause	Calcium	Vitamin D	PTH	Phosphate	Other
Hypovitaminosis D	L	L	H	L	H ALP
Low 1,25D (renal failure, vitamin D dependent rickets type I caused by loss of function in 1α hydroxylase)	L	N	H	L/H	H ALP, L 1,25D
Vitamin D dependent rickets type II (loss of function of vitamin D receptor)	L	N	H	L	H ALP, H 1,25D
Low phosphate (multiple causes see later slide)	N	N	N	L	H ALP

PTH, parathyroid hormone; L, low; H, high; N, normal; ALP, alkaline phosphatase.

settles within weeks or months of initial observation (137). However, ALP activity can remain elevated for extended periods of time, for greater than 4 months, in approximately 20% of cases (138). Diagnosis of TH is linked to the 'fast' α_2 band which is detected on agarose gel electrophoresis (139) but testing may not be necessary if the person is asymptomatic. Prevalence of TH is suggested to be around 2.8% (ALP >1,000 U/L) (140) in the classically affected population, children younger than 5 years old, but has also been described in transplant patients (141,142).

Bacterial and fungal glycoproteins can compete with the receptors involved in the excretion and elimination of ALP from the serum (14). Therefore, ALP elevations can be seen in infections without any obvious liver toxicity or cholestasis (143).

Raised ALP has also been identified in a range of other disorders including rheumatoid arthritis (144,145), atherosclerosis (146), and axial spondylarthritis (147). However, the links between elevated ALP and the diseases have not been fully established, or the mechanisms understood.

In individuals with blood groups O and B, ALP concentration increases by about 20% after consuming a fatty meal, due to contribution from the intestinal tract isoenzyme (14,148). It was found that red cells of blood group A bind almost all intestinal ALP (149), which is not replicated in those with types O or B. As this elevation can persist for up to 14 hours in the serum, the recommendation is to check the serum enzyme activity in a fasting state (150). In one case a patient's intestinal ALP activity was measured as high as 140 U/L, with a normal range suggested to be <18 U/L (151). There are also cases of benign familial conditions causing elevated intestinal ALP. Rosalki *et al.* presents examples of patients with persistent and unexplained raised ALP, above the reference range, that had a genetic component, with an autosomal dominant pattern of inheritance suggested as the cause (152).

Special states

Serum ALP varies with age and gender. There are two peaks of ALP activity during infancy and puberty, which fall mid-childhood and towards the end of adolescence respectively (153,154). A German study of over 300,000 paediatric plasma samples demonstrated that at birth ALP activity is low. Activity increases quickly, peaking at 20 days and then decreasing again until 4 years of age with an increase during adolescence to reach adult ranges (155). For a reference source for paediatric values the CALIPER database is a useful source (156). Gender will also influence these peaks, with females shown to peak 2 years earlier than males in keeping with growth rates (112,138).

Gender also influences expected BALP activity in later life, significantly higher activities were found in postmenopausal women when compared to premenopausal (157). During pregnancy, bone turnover increases (158) contributing to an increase in BALP plus an additional increase in placental ALP activity in serum resulting in an ALP activity threefold higher at the end of term (159). Therefore, ALP activity should be reviewed against appropriate reference ranges to avoid over diagnosing or missing pathology.

Conclusions

Serum ALP activity can be affected by normal physiological states and diseases. Pathological causes of high activity commonly are attributable to bone or liver disorders. Unexpectedly low activity may be due to pre-analytical contamination with cation chelators. A set of diagnostic algorithms have been created to guide the reader's approach and provide a systematic route of testing. Each patient is unique however and the clinical picture may direct the

reader to skip steps or refer to other algorithms within the series or in the literature. These algorithms are not a replacement for experience, expert opinion or local guidelines and should instead act as a diagnostic aid.

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