RMD Open
Rheumatic & Musculoskeletal Diseases

ORIGINAL RESEARCH

Detection of hypophosphatasia in hospitalised adults in rheumatology and internal medicine departments: a multicentre study over 10 years

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To cite: Larid G, Vix J, Preuss P, *et al*. Detection of hypophosphatasia in hospitalised adults in rheumatology and internal medicine departments: a multicentre study over 10 years. *RMD Open* 2024;**10**:e004316. doi:10.1136/rmdopen-2024-004316

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/rmdopen-2024-004316).

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Received 8 March 2024
Accepted 15 March 2024

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ABSTRACT

Introduction Hypophosphatasia (HPP) is a rare genetic disease caused by loss-of-function mutations in the ALPL gene encoding the tissue non-specific alkaline phosphatase (ALP). Mild HPP is usually misdiagnosed in adult age. While an elevated serum ALP value draws more attention than a low value, low serum ALP should be better recognised and may lead to HPP detection.

Methods Patients were selected from the records of the biochemistry department of six University Hospitals in France. Patients were hospitalised in the departments of rheumatology and internal medicine between 2007 and 2017.

Results 56 321 hospitalised patients had at least 2 serum ALP dosages and 664 of these patients had at least 2 low serum ALP≤35 UI/L. Among these 664 patients, 482 (72.6%) had fluctuating low values (mean age 62.9 years; 60% of women) and 182 patients (27.4%) had persistent low values below 35 IU/L (mean age 53.4 years; 67% of women). Among patients with persistent hypophosphatasaemia treated with bisphosphonates, 70.8% never had ALP measurement before treatment and 20.8% were treated despite an abnormal decrease of ALP. Genetic testing was performed in 18 patients and was positive in 11. Genetic diagnosis of HPP was at least 6.0% in persistent hypophosphatasaemia and at least 15.9% in patients with at least three symptoms suggestive of HPP.

Conclusion In this 10-year retrospective study, 0.32% of adult patients hospitalised in the rheumatology and internal medicine departments had persistently low serum ALP, and among them, 6% had genetically proven HPP. Reported hypophosphatasaemia represented only 3.6% of hospitalised patients.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Hypophosphatasia is a rare and often undiagnosed disorder.
⇒ Low alkaline phosphatase (ALP) values are overlooked by a majority of clinicians.

WHAT THIS STUDY ADDS

⇒ In this multicentre study, low ALPs are poorly recognised by clinicians.
⇒ 70.8% of patients treated with bisphosphonates never underwent ALP measurement before treatment initiation.
⇒ Using a combination of multiple evocative symptoms to select patients for genetic testing seems interesting as a means of increasing the diagnosis rate and control healthcare costs.
⇒ Mild to moderate adult hypophosphatasia may be more frequent than previously thought.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Sensitisation of clinicians to ALP values is needed.
⇒ ALP measurement should be mandatory in the secondary osteoporosis investigations before bisphosphonate treatment initiation.

INTRODUCTION

Hypophosphatasia (HPP) is a rare genetic skeletal disease due to an inherited metabolic disorder caused by mutations of the *ALPL* gene coding for tissue non-specific alkaline phosphatase (TNSALP).[1] Prevalence of severe forms is estimated as ranging from 1/100 000 to 1/300 00,[2] while prevalence of mild HPP was estimated at 1/6370 in Europe.[3]

Six forms of the disease have been defined: perinatal severe HPP, perinatal benign HPP, infantile HPP, childhood HPP, adult HPP and odontohypophosphatasia.[2 4]

In adults, clinical manifestations are dominated by fractures and joint disease. The most evocative fractures are localised at the metatarsals. These fractures are usually recurrent, with delayed consolidation potentially leading to pseudarthrosis. Other typical fractures affect the femoral diaphysis and occur mainly

in the lateral cortex of the subtrochanteric region. Joint disease is represented mainly by calcium pyrophosphate deposition disease.[5]

While elevated ALP is usually taken into account by clinicians, low ALP levels are easily overlooked. A monocentre study in a tertiary care hospital in France found that notification was given in only 3% of cases.[6]

The aetiologies of low ALP are multiple and differ according to hypophosphatasaemia temporality.[7 8] Furthermore, these causes are often unknown by clinicians.

The aims of this study were to estimate the recognition of hypophosphatasaemia in rheumatology and internal medicine departments, to analyse the characteristics of the population presenting persistently low ALP measurements and to estimate the number of patients highly suspected of adult HPP. Secondary analyses were performed to compare patients with persistently low ALP measurements while using or not using bisphosphonates, and those with or without an identified cause of persistently low ALP levels.

MATERIALS AND METHODS

Study design

This retrospective, descriptive and multicentre study included patients from the University hospitals of Poitiers, Nantes, Rennes, Brest, Angers and Tours. It consisted of the detection of low ALP measurement at least twice among patients hospitalised in the departments of Rheumatology and Internal Medicine between 1 July 2007 and 1 July 2017. No limit in duration between two measurements was necessary. We followed STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) instructions throughout this work.

In France, internal medicine departments are departments encompassing a combination of geriatrics, clinical immunology, infectious diseases, oncohaematological diseases and rheumatology subspecialties dealing with systemic autoimmune and autoinflammatory disorders.

Patients

The listing of patients was established from records of the Biochemistry Department of several French university hospitals by laboratory database request on the criteria of low ALP values ≤35 U/L (normal range: 40–120 IU/L). Low cut-off values were identical for men and women in the laboratories that performed the analysis. A minimum of 2 low ALP values (≤35 IU/L) was required to minimise the likelihood of an analytical error; 35 U/L was defined as it is an average between the lower bounds of adult normal values and less exclusive than previous studies have set the limit at 30 UI/L.[6 9] Patients who had previously denied or restricted access to their record for research purposes and aged less than 18 years were excluded from the study.

Once authorisations were given, paper and electronic medical records were used to search patient history, symptoms, laboratory results (basic tests, calcium phosphate metabolism and specialised blood tests (bone ALP)), bone densitometry, X-rays, CT- scan and MRI results. If done, the genetic test was notified.

Bone demineralisation was defined as a T-score <−2.5 SD.

Chondrocalcinosis and hydroxyapatite deposition disease were diagnosed based on the aspect of the calcifications visualised on X-rays.

Only non-traumatic fractures were considered in the analyses.

Scoliosis was considered as present if described in the radiologist reports of spine imaging.

Aetiologies for low ALP were defined as follows:

- Corticosteroids were considered as a possible cause of hypophosphatasaemia if patients received at least a very high dose of corticosteroids (>100 mg per day) using the standardised nomenclature for glucocorticoid dosages by Buttgereit *et al*.[10]
- Severe anaemia was defined as haemoglobin (Hb) <60 g/L.
- Pernicious anaemia was considered if patients were currently not substituted.
- Hypothyroidism was considered if patients were not substituted and thyroid-stimulating hormone (TSH) was higher than 4 mUI/L.
- Hepatic insufficiency was considered if prothrombin time was lower than 50%.
- Hypervitaminosis D was considered if 25-OH vitamin D was higher than 150 ng/L.
- Hypomagnesaemia was considered if magnesium level was lower than 0.7 mmol/L.
- Vitamin C insufficiency was considered for levels lower than 2.5 mg/L.
- Zinc insufficiency was considered for levels lower than 9 µmol/L.
- Cushing disease, coeliac disease and Wilson disease were considered only if they were not currently being treated or at equilibrium.
- Intensive care stay was considered if it was an actual stay or less than 1 month before.
- Ongoing oncohaematological disease, bisphosphonate treatment, denosumab treatment, septicaemia, inflammatory disease flare and intravenous immunoglobulins were considered as potential causes of low ALP.

To determine the number of patients for whom low ALP ≤35 U/L was recognised and noted in their records, the discharge summary, the diagnosis written in the letter and/or the ICD-10 (International Classification of Diseases 10th Revision) code were used.

Patients were defined as possible HPP if they exhibited at least three symptoms evocative of HPP in addition to persistent low ALP (arthralgia, fractures, stress fractures, low bone density, dental abnormalities, chondrocalcinosis, scoliosis, high B_6 levels or high urinary phosphoethanolamine).

Biochemical assays

Both of the instruments measure ALP activity by a kinetic rate method in which a colourless organic phosphate ester substrate (nitrophenylphosphate) is hydrolysed by ALP to the yellow-coloured product p-nitrophenol and phosphate at pH of 10.3, thereby explaining the term 'alkaline'. Changes in absorbance at 410 nm are directly proportional to the enzymatic activity of ALP. A requirement of two low ALP values (≤35 U/L) was set so as to minimise the likelihood of low ALP results due to analytic error. For each selected patient, all previous ALP values against time were visually examined to determine the temporal pattern of the qualifying serum ALP values and to separate two groups of patients. When the temporal pattern of ALP values indicated a precipitous fall from usually normal values, the patient was considered to have acute hypophosphatasaemia. Diagnostic conditions and circumstances associated with acute hypophosphatasaemia were analysed. Laboratory used Glims, JMP or DXLab software.

When the temporal pattern of ALP values indicated a persistently low ALP or only 2 values, both of them under 35 U/L, the patient was considered to have persistent hypophosphatasaemia. More precise analysis was carried out to identify detailed patient history, symptoms, laboratory results (basic tests including calcium phosphate metabolism exploration and specialised blood tests such as bone ALP), bone densitometry, X-rays, CT scan and MRI results and genetic test when they had been made. To determine the number of patients in whom persistently low ALP ≤35 U/L was recognised, the discharge summary, the written diagnosis and/or the ICD-10 code (E833) were used.

Statistical methodology

Qualitative data were expressed as percentages and quantitative data as means±SD. Analysis was conducted using the Student's t-test (or Wilcoxon, as appropriate) for quantitative data and χ^2 (or Fisher's exact test, as appropriate) for qualitative data. A p value of 0.05 was considered as significant. Statistical analysis was performed by using SAS software, V.9.1 (SAS Institute) and GraphPad Prism (GraphPad Software, California).

Patient and public involvement

Patients or members of the public were not involved in the design, or conduct, or reporting, or dissemination plans of the research.

RESULTS

Population characteristics

Between 1 July 2007 and 1 July 2017, 144 242 ALP measurements were performed; 56 321 hospitalised patients had at least 2 serum ALP measurements. Inclusion period differed according to the centres, with mean inclusion time of 8.42 years (±2.478). A total of 664 patients hospitalised in the rheumatology and internal medicine departments of the University Hospitals of Poitiers, Nantes, Rennes, Brest, Angers and Tours had at least two ALP values below or equal to 35 IU/L (figure 1 and table 1). There was a difference in the sex ratio with 57.8% of female patients (208/360) in internal medicine departments vs 66.8% in rheumatology departments (203/304) (p=0.017). Prevalence of all-cause hypophosphatasaemia was 1.18%. Among the patients, 182 (27.4 %) had persistently low serum ALP levels, representing a general prevalence of 0.32% for persistent hypophosphatasaemia (182/56321), while 482 patients (72,6%) had fluctuating serum ALP values, at least two of which were below or equal 35 IU/L, which representing prevalence of 0.86%.

All in all, 38.1% of patients were male. In only 24 cases (3.61%) was hypophosphatasaemia reported in the patient's records.

Reasons for hospitalisations were various. In rheumatology departments, the top 10 reasons for hospitalisations were lumbosacral radiculopathy, haemopathy, osteoporosis, arthritis, fractures, rheumatoid arthritis, polyarthralgia, low back pain, spondyloarthritis and suspicion of rheumatic disease. In internal medicine departments, the top 10 reasons for hospitalisations were infectious disease, chronic myeloid leukaemia or lymphoma or multiple myeloma, polyarthralgia, severe anaemia/haemorrhage, autoimmune cytopenia, vasculitis, inflammatory myositis, intravenous immunoglobulin infusions, systemic lupus erythematosus, undernutrition or severe anorexia or hydro electrolytic disorders.

Initial comparisons of characteristics of patients with transient versus persistent hypophosphatasaemia

Clinical characteristics of the patients with transient and persistent hypophosphatasaemia were compared (table 2). Patients with persistent hypophosphatasaemia were younger (53.36 vs 62.93 years/old), were less heavy (64.01 vs 68.82 kg) and were more frequently treated in the rheumatology department (74.2% vs 35.1%). Their mean ALP values were significantly lower than in the transient group (28.0 vs 30.1 UI/L respectively).

In terms of recognition, persistent hypophosphatasaemia was more frequently identified than transient hypophosphatasaemia (12.6% vs 0.2%).

Among the 182 patients with persistent hypophosphatasaemia, 70 patients (38.4%) had no joint imaging and 49 patients (26.9%) had no spinal imaging. Only 12 patients in the transient hypophosphatasaemia group had peripheral joint X-rays and nine had spinal X-rays (table 2).

Patients with persistent hypophosphatasaemia experienced pain more frequently (90.1% vs 22.8%). Stress fractures were present only in patients with persistent hypophosphatasaemia.

As regards medical history, chondrocalcinosis, hydroxyapatite deposition disease, dental abnormalities, early tooth loss, childhood rickets, family HPP and convulsion were found only in patients with persistent hypophosphatasaemia.

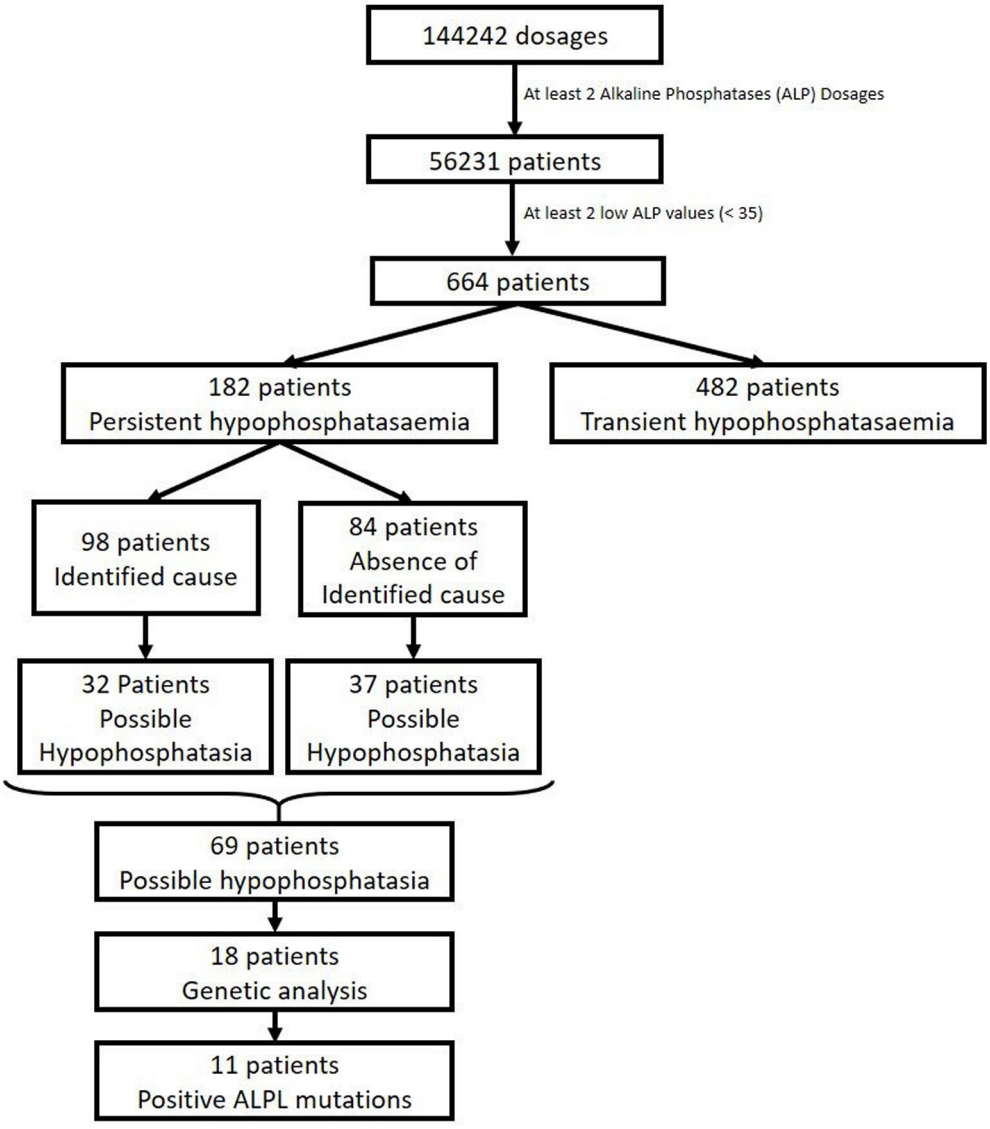

Figure 1 Flow chart.

A total of 48 patients with persistent hypophosphatasaemia had a bone mineral density (BMD) measurement. However, values were not always available and sometimes only the conclusion appeared. Osteopenia was diagnosed in 20 patients, and osteoporosis in 15, while 13 patients presented with normal BMD. Details are found in online supplemental table 1. Only four patients in the transient hypophosphatasaemia group had a BMD measurement, with three normal BMD and one osteopenia.

Further comparisons of aetiologies in patients with transient versus persistent hypophosphatasaemia

Potential aetiologies of hypophosphatasaemia were compared (table 3).

Considering possible causes of hypophosphatasaemia, severe anaemia, intensive care unit stay, active onco-haematological disease, ongoing bisphosphonate treatment, sepsis, inflammatory disease flare and intravenous immunoglobulin treatment were more frequently found in transient hypophosphatasaemia, while corticosteroid intake was more frequent in persistent hypophosphatasaemia. In those patients, HPP was possible in 69 patients, in 37 of whom there was no identified cause.

Documentation of low ALP values in patients with persistent hypophosphatasaemia with bisphosphonate treatment

Since bisphosphonate treatment is contraindicated in HPP, we analysed whether patients with low ALP measurements were tested before bisphosphonate initiation. Among the 24 patients treated with bisphosphonates, 19 (79.2%) had never undergone ALP measurement before treatment, while in 5 patients (20.8%), this treatment had been initiated despite an abnormal decrease of ALP. Details for those patients are in online supplemental table 2.

Table 1 Population characteristics (n=664)

Age (years; mean±SD) (n=660)	60.3 (±19.4)
Male sex	253 (38.1%)
Weight (n=446) (kg; mean±SD)	67.4 (±17.4)
Height (n=338) (cm; mean±SD)	164.4 (±10.7)
Mean value of ALP (IU; mean±SD)	29.5 (±4.0)
Transient hypophosphatasaemia	482 (72.6%)
Persistent hypophosphatasaemia	182 (27.4%)
Inclusions by departments	
Internal medicine	360 (54.2%)
Rheumatology	304 (45.8%)
Notification	
Total	24 (3.61%)
Internal medicine	3 (0.8% of patients)
Rheumatology	21 (6.9% of patients)
Inclusions by centre (n; inclusion period)	
Brest	71 (8.5 years)
Angers	35 (9 years)
Nantes	149 (9.5 years)
Tours	34 (3.5 years)
Poitiers	226 (10 years)
Rennes	149 (10 years)

ALP, alkaline phosphatase; n, number of observations.

Comparisons of clinical and radiological features of patients with persistent hypophosphatasaemia with and without identified cause

Out of the 182 'persistent' patients, 84 cases had an identified cause and 98 did not (table 4). There were no differences in ALP measurements between groups.

Patients with unidentified cause of hypophosphatasaemia were more likely to have mechanical pain (70.5% vs 44.7%), diffuse pain (26.9% vs 15.3%) and knee chondrocalcinosis history (66.7% vs 11.1%), while they less frequently had pain in the limbs (28.2% vs 47.1%), fracture history (16.7% vs 29.9%), mixed pattern pain (10.3% vs 28.2%), low BMD (10.7% vs 37.1%) and radiographic vertebral fractures (10.7% vs 31.2%).

HPP among patients with persistent hypophosphatasaemia

Among all patients with persistent hypophosphatasaemia, 69 presented at least three symptoms evocative of HPP in addition to persistent low ALP and were classified as possible HPP (table 3). Among them, 18 underwent genetic analysis in search of ALPL gene mutation, and 11 patients presented with genetically proven HPP (61.1%). The diagnosis of genetic HPP was thereby confirmed in at least 1.7% of our total population (11/664). Among those 11 patients, 3 had another potential cause of low ALP (2 had taken corticosteroids, and 1 had a vitamin C deficiency). Selection of patients with persistently decreased ALP rendered genetic analysis more cost-effective, with a positive diagnosis ranging from at least 1.7% (11/664) to at least 6% (11/182), and even higher than 15.9% (11/69) if they were classified as possible HPP. Pyridoxal phosphate (PLP) measurements had been performed in only 6 patients out of the 664 patients included (mean±SD: 58.33±18.26 nmol/L (normal range: 30–100 nmol/L)). All of them had persistent low ALP: five were genetically tested, among whom three were positive

DISCUSSION

In our study, the prevalence of all-cause hypophosphatasaemia among patients hospitalised in the internal medicine and rheumatology departments was 1.18% while that of persistent hypophosphatasaemia was 0.32%. This proportion was higher than in the study by Maman *et al* in which 0.13% of hospitalised patients (every department except the emergency department) had persistently low values with a less stringent threshold of 40 UI/L,[6] as in the study of Hepp *et al*, in which prevalence of 0.20% was found in adults admitted to an endocrinological outpatient clinic in Denmark,[11] or in the study by García-Fontana *et al* with prevalence of 0.12% in a Spanish university hospital.[12] A German study retrospectively analysing 6 918 126 subjects with a measurement of ALP between 2011 and 2016 in a single laboratory identified prevalence of ALP values below 30 of 8.46% and 9.47% between 30 and 40 UI/L, respectively, thereby underscoring the need to focus on persistent low ALP levels since transient hypophosphatasaemia is quite common.[9] McKiernan *et al* identified 1.1% of patients with at least two values under 40 UI/L among consultants in a multidisciplinary centre, and 0.06% of ALP level persistently below 30 UI/L.[8] This is concordant with a German study, which found 1.31% of patients treated in rheumatology at the University Hospital of Bonn from 2017 to 2019 showed persistently low serum ALP levels (<35 UI/L).[13] As regards the proportion of patients with persistent hypophosphatasaemia, it was 33.3% in the study by McKiernan *et al*,[8] and 39% in a study by Vieira *et al*,[14] which is concordant with our result of 27.6%. Similarly, Feurstein *et al* found 5.5% of patients with at least one low ALP value under 40 UI/L, with only 13.9% of patients presenting persistent low ALP levels and musculoskeletal symptoms; they represented 0.8% of the whole population from a rheumatology outpatient clinic in Vienna specialised in rheumatology and rare bone diseases.[15]

In terms of notification, reporting of low ALP values was found in 3.61% in our population, which is close to the 3% noted by Maman *et al*.[6] Low ALP is clearly not sufficiently recognised, even if rheumatologists seem to better identify this abnormality with a reported 6.91% vs 0.83% in internal medicine. As a result, adult HPP is highly underdiagnosed. A few years ago, some laboratories only indicated the 'high' cut-off and, in the absence of personal knowledge of the lower normal cut-off, the ALP drop was not always noticed,[16] and therefore, easily overlooked.[17] In our study, many patients had not

Table 2 Clinical and radiological features of patients with transient hypophosphatasaemia and persistent hypophosphatasaemia

	Transient (N=482)	Persistent (N=182)	P value
Age	62.9 (±19.3) N=478	53.4 (±18.0)	**<0.0001**
Female sex	289 (60%)	122 (67%)	0.094
Weight (kgs)	68.8 (±18.4) N=314	64.0 (±14.5) N=132	**0.0122**
Height (cm)	164.4 (±11.3) N=218	164.4 (±9.6) N=120	0.8045
Department of inclusion			
Internal medicine	313	47	**<0.0001**
Rheumatology	169	135	
Alkaline phosphatase measurement			
Mean (±SD)	30.1 (±3.5)	28.0 (±4.9)	**<0.0001**
Detection			
Coding	1 (0.2%)	23 (12.6%)	**<0.0001***
Pain			
Pain	110 (22.8%)	164 (90.1%)	**<0.0001**
Mechanical pain	54 (49.1%)	94 (57.3%)	0.180
Inflammatory pain	54 (49.1%)	38 (23.2%)	**<0.0001**
Mixed pain	2 (1.8%)	32 (19.5%)	**<0.0001**
Pain location			
Spine	66 (60%)	70 (42.7%)	**0.005**
Appendicular skeleton	40 (36.4%)	61 (37.2%)	0.889
Both	4 (3.6%)	33 (20.1%)	**<0.0001**
Fractures history			
Fractures	3 (27.3%) N=11	43 (23.8%) N=181	–
Spine	2 (66.7%)	19 (44.2%)	–
Pelvis	0	4 (9.3%)	–
Femur	0	2 (4.7%)	–
Upper limb	1 (33.3%)	6 (14.0%)	–
Ankle, feet	0	4 (9.3%)	–
Multiple	0	8 (18.6%)	–
Numbers (mean±SD)	1±0	2.4±2.2	–
Stress fractures			
Stress fractures	0 N=10	14 (7.7%) N=182	–
Feet	–	2 (14.3%)	–
Lower limb	–	3 (21.4%)	–
Upper limb	–	1 (7.1%)	–
Unspecified locations	–	8 (57.1%)	–
Numbers (mean±SD)	0	1.6±0.7	–
Chondrocalcinosis history			
Chondrocalcinosis	0 N=9	18 (9.9%) N=182	–
Wrist	–	5 (27.8%)	–

Continued

Table 2 Continued

	Transient (N=482)	Persistent (N=182)	P value
Symphysis	–	2 (11.1%)	–
Knee	–	7 (38.9%)	–
Others	–	4 (22.2%)	–
Unspecified locations	–	5 (27.8%)	–
Medical history			
Hydroxyapatite deposition disease	0 N=9	24 (13.2%) N=182	–
Dental abnormalities	0 N=6	10 (5.5%) N=182	–
Early tooth loss	0 N=3	4 (28.6%) N=14	–
Bone demineralisation	3 (23.1%) N=13	45 (24.9%) N=181	–
Rickets in childhood	0 N=7	5 (7.9%) N=63	–
Craniosynostosis in childhood	0 N=4	1 (2.5%) N=40	–
Hypophosphatasia in family	0 N=4	4 (10.5%) N=38	–
Convulsion history	0 N=5	6 (15%) N=40	–
Intracranial hypertension history	0 N=4	0 N=38	–
Radiological findings			
Scoliosis	1 (11.1%) N=9	51 (38.3%) N=133	–
Vertebral fractures	1 (11.1%) N=9	30 (22.6%) N=133	–
Number of vertebral fractures	1	1.9±1.6	–
Ligament and tendon calcifications	5 (41.7%) N=12	62 (55.4%) N=112	–
Wrist/hands	3 (60%)	12 (19.4%)	–
Knee	1 (20%)	9 (14.5%)	–
Shoulder	2 (40%)	17 (27.4%)	–
Elbow	1 (20%)	3 (4.8%)	–
Feet/ankle	2 (40%)	4 (6.4%)	–
Pelvis	1 (20%)	13 (21.0%)	–
Trochanter/hips	1 (20%)	21 (33.9%)	–
Unspecified locations	0	13 (21.0%)	–
Multiple locations	4 (80%)	21 (33.9%)	–
Intervertebral disc calcification	3 (33.3%) N=9	16 (12.0%) N=133	–
Paravertebral calcification	4 (44.4%) N=9	16 (12.0%) N=133	–

Significant values are in bold.
*Fisher's exact test.

Table 3 Comparisons between patients with transient or persistent hypophosphatasaemia

	Transient (N=482)	Persistent (N=182)	P value
Possible cause of hypophosphatasaemia			
Wilson disease	0 (0%) N=387	1 (0.6%) N=160	0.292*
Cushing disease	4 (1.0%) N=419	0 (0%) N=146	0.577*
Hypothyroidism	21 (5.1%) N=411	9 (7.0%) N=129	0.419
Hepatic insufficiency	14 (3.1%) N=458	3 (1.7%) N=179	0.421*
Severe anaemia	110 (23.3%) N=473	21 (11.8%) N=178	**0.001**
Coeliac disease	4 (1.37%) N=291	0 (0%) N=116	0.581*
Hypomagnesaemia	20 (15.5%) N=129	4 (6.0%) N=67	0.066*
Hypervitaminosis D	1 (0.34%) N=294	1 (1.0%) N=98	0.438*
Vitamin C deficiency	18 (14.8%) N=122	5 (8.2%) N=61	0.207
Zinc deficiency	13 (27.1%) N=48	1 (5%) N=20	0.050*
Vitamin B_{12} deficiency	15 (14.7%) N=102	1 (3.4%) N=29	0.121*
Fibrate treatment	42 (9.3%) N=451	16 (8.9%) N=179	0.884
Intensive care unit stay	92 (19.1%) N=481	3 (1.6%) N=182	**<0.0001***
Active oncohaematological disease	116 (24.1%) N=481	23 (12.6%) N=182	**0.001**
Ongoing bisphosphonate treatment	132 (27.5%) N=480	24 (13.2%) N=182	**0.0001**
Ongoing denosumab treatment	4 (0.8%) N=480	1 (0.5%) N=182	>0.999*
Sepsis	70 (14.5%) N=481	6 (3.3%) N=182	**<0.0001**
Inflammatory disorder flare	141 (29.3%) N=481	17 (9.3%) N=182	**<0.0001**
Corticosteroids intake	64 (13.6%) N=469	37 (20.4%) N=181	**0.032**
Intravenous immunoglobulin treatment	41 (8.5%) N=480	6 (3.3%) N=182	**0.019**
Identification of the cause of hypophosphatasaemia			
Identified cause	445 (92.3%)	98 (53.8%)	**<0.0001**
Absence of identified cause	37 (7.7%)	84 (46.2%)	
Possible hypophosphatasia (at least 2 signs of hypophosphatasia+persistent low ALP values)			
Possible hypophosphatasia	–	69 (37.9%)	–
Identified origin	–	32 (46.4%)	–
Non-identified origin	–	37 (63.6%)	–

Significant values are in bold.
*Fisher's exact test.
ALP, alkaline phosphatase.

Table 4 Clinical and radiological features of persistent hypophosphatasaemia with and without identified cause

	Identified cause (N=98)	No identified cause (N=84)	P value
Alkaline phosphatase measurement			
Mean (±SD)	28.3 (±4.3)	27.6 (±5.5)	0.827
Pain			
Pain	85 (86.7%)	78 (92.9%)	0.178
Mechanical pain	38 (44.7%)	55 (70.5%)	**0.0003**
Inflammatory pain	23 (27.1%)	15 (19.2%)	0.353
Mixt pain	24 (28.2%)	8 (10.3%)	**0.008**
Pain location			
Spine	32 (37.6%)	35 (44.9%)	0.208
Appendicular skeleton	40 (47.1%)	22 (28.2%)	**0.038**
Both	13 (15.3%)	21 (26.9%)	**0.043**
Fractures history			
Fractures	29 (29.9%) N=97	14 (16.7%) N=84	**0.037**
Spine	15 (51.7%)	4 (28.6%)	0.199*
Pelvis	3 (10.3%)	1 (7.1%)	1*
Femur	0	2 (14.3%)	0.101*
Upper limb	5 (17.2%)	1 (7.1%)	0.645*
Ankle, feet	1 (3.4%)	3 (21.4%)	0.094*
Multiple	5 (17.2%)	3 (21.4%)	1*
Numbers (mean±SD)	2.3±2.2	2.5±2.3	0.908
Stress fractures			
Stress fractures	7 (7.1%) N=98	7 (8.3%) N=84	0.764
Feet	1 (14.3%)	1 (14.3%)	1*
Lower limb	–	3 (42.9%)	0.192*
Upper limb	–	1 (14.3%)	1*
Unspecified locations	6 (85.7%)	2 (28.6%)	0.103*
Numbers (mean±SD)	1.8±1.0	1.4±0.5	0.762
Chondrocalcinosis history			
Chondrocalcinosis	9 (9.2%) N=98	9 (10.7%) N=84	0.730
Wrist	4 (44.4%)	1 (11.1%)	0.294*
Symphysis	2 (22.2%)	0	0.470*
Knee	1 (11.1%)	6 (66.7%)	**0.050***
Others	–	3 (33.3%)	0.206*
Unspecified locations	3 (33.3%)	2 (22.2%)	1*
Medical history			
Hydroxyapatite deposition disease	12 (12.2%) N=98	12 (14.3%) N=84	0.685
Dental abnormalities	2 (2.0%) N=98	8 (9.5%) N=84	**0.046***
Early tooth loss	1 (14.3%) N=7	3 (42.9%) N=7	–
Bone demineralisation	36 (37.1%) N=97	9 (10.7%) N=84	**<0.0001**

Continued

Table 4 Continued

	Identified cause (N=98)	No identified cause (N=84)	P value
Rickets in childhood	2 (5.6%) N=36	3 (8.1%) N=27	–
Craniosynostosis in childhood	1 (4.2%) N=24	0 N=16	–
Hypophosphatasia in family	2 (10%) N=20	2 (11.1%) N=18	–
Convulsion history	3 (13.0%) N=23	3 (17.6%) N=17	–
Intracranial hypertension history	0 N=20	0 N=18	–
Radiological findings			
Scoliosis	30 (39.0%) N=77	21 (37.5%) N=56	0.864
Vertebral fractures	24 (31.2%) N=77	6 (10.7%) N=56	**0.005**
Number of vertebral fractures	2.0±1.8	1.5±0.8	0.520
Ligament and tendon calcifications	33 (50.8%) N=65	29 (61.7%) N=47	0.251
Wrist/hands	9 (27.3%)	3 (10.3%)	0.116*
Knee	3 (9.1%)	6 (20.7%)	0.283*
Shoulder	9 (27.3%)	8 (27.6%)	1*
Elbow	2 (6.1%)	1 (3.4%)	1*
Feet/ankle	3 (9.1%)	2 (6.9%)	1*
Pelvis	5 (15.2%)	7 (24.1%)	0.521*
Trochanter/hips	10 (30.3%)	11 (37.9%)	0.596*
Unspecified locations	5 (15.2%)	6 (20.7%)	0.741*
Multiple locations	11 (33.3%)	10 (34.5%)	0.923
Intervertebral disc calcification	11 (14.3%) N=77	11 (19.6%) N=56	0.411
Paravertebral calcification	10 (13.0%) N=77	12 (21.4%) N=56	0.196

Significant values are in bold.
*Fisher's exact test.

been explored, and the final report never mentioned low ALP. Indeed, it 'normal liver test' was often noted without details, even though the ALP levels were lower than 35 IU/L. That is why hypophosphatasaemia was not coded and did not result in further explorations.

The difficulty of diagnosing HPP led several teams to propose algorithms to enhance the rate of diagnosis. The first strategy is based on the adjunction of PLP measurement to ALP so as to better stratify the likelihood of HPP diagnosis with high PLP and low ALP as features of HPP.[18 19] Another team added BMD measurement by Dual-energy X-ray absorptiometry to generate a strategy of rationalised mutational analysis in resource-limiting conditions.[20] While these approaches are interesting, PLP is not performed in daily practice, thereby limiting its usefulness if low ALP has not been previously identified. In our study, PLP measurements had been performed in only 6 out of the 664 patients included. All of them had persistent low ALP; five were genetically tested, among whom three were positive. This lack of data is not surprising since low ALP is poorly recognised in daily practice. Therefore, physicians would not dose PLP since they did not take low ALP into account. Moreover, the algorithm mentioning PLP measurement in order to better screen patients was published after our inclusion period, which may be another, though less important, explanation.

Another approach is to focus on populations with highly suggestive features of HPP. Tsiantouli *et al* analysed ALP values in a population of 72 patients with atypical

femur fractures (AFF) with at least 1 ALP value available. There was no difference in the median value of ALP compared with the control group with hip fracture, and no difference in the titre of ALP if they were treated with antiresorptive agent. Moreover, none of the patients with AFF without antiresorptive drugs in this single-centre study presented with low ALP levels.[21] Similarly, Marini *et al* performed ALPL genotyping in patients with AFF or other biochemical or clinical signs of adult HPP.[22] This led them to identify three rare variants of ALPL (2.8%) in this population. Monozygotic ALPL common variants were found in 11.3% of the patients, with a higher proportion of 22% of patients with normal ALP values, 30.8% of patients with AFF, 16.7% of patients with normal ALP and high PLP levels and also, unfortunately, in 13.5% of non-HPP controls. Those results should draw the attention of clinicians to the need to carefully consider the possibility that some variants have no detrimental effect on the ALP protein and that different kinds of disease severity or carrying a nonpathogenic mutation can be encountered.[23] Since metatarsal fractures are suggestive of HPP, Koehler *et al* focused on this population and found 0.12% prevalence of pathogenic ALPL variants in a population of 1611 metatarsal fractures, a proportion that rose to 15% when low ALP measurement was associated.[24] In our study, the same approach using clinical, biological and imaging features identified 69 patients with at least three evocative symptoms of HPP in addition to persistently low ALP values (possible HPP) among which 11 were found to have genetically proven HPP, representing a diagnosis rate of at least 15.9%. This value is probably underestimated since only 18 patients benefited from genetic testing, corresponding to a diagnosis rate of 61.1% of the tested patients. The combination of at least three signs in addition to persistent hypophosphatasaemia should, therefore, be tested in a larger population to evaluate its cost-effectiveness.

As expected, possible causes of hypophosphatasaemia were more frequently found in transient cases. Interestingly, corticosteroids were more frequently found in persistent hypophosphatasaemia. Since patients with persistent hypophosphatasaemia more frequently had crystal arthropathy history as well as pain, we may hypothesise that this difference is the result of its use to treat arthritis flares or pain as well as long-term treatments in systemic inflammatory disease. Indeed, patients with chronic low dose corticosteroid use were frequently treated with bisphosphonates in order to prevent corticosteroid-induced osteoporosis.

Pain in itself is also an important point to consider insofar as more than 90% of patients with persistent hypophosphatasaemia in our study reported pain. Pain also represents the greatest burden in HPP patients, as shown in the global HPP registry.[25] Moreover, there is multiple evidence in the literature that TNSALP exerts a role in the biosynthesis of adenosine, a key molecule with antinociceptive effect with TNSALP, prostatic acid phosphatase and ecto-5'-nucleotidase playing crucial roles in determining the overall sensitivity of the nociceptive circuits, as reviewed extensively by Street and Sowa.[26]

The relationship between tissue-nonspecific ALP and inflammation is an increasing source of interest. A recent review article by Graser *et al*[27] affirms that TNSALP deficiency contributes to inflammatory reactions. TNSALP is implicated in the balance between proinflammatory ATP effects and anti-inflammatory effects of adenosine. Moreover, TNSALP's ectophosphatase activity is involved in the modulation of TLR ligands like LPS and double-stranded RNA mimic poly-inosine:cytosine. TNSALP is also a T-cell activity modulator. In synthesis, TNSALP is now known to exert an anti-inflammatory effect. Furthermore, ALP levels are higher in case of systemic inflammation.[28 29] In our study, the two main reasons in which inflammatory disease flare were associated with low ALP were corticosteroids, intravenous immunoglobulin treatments or bisphosphonate therapy for prevention of corticosteroid-induced osteoporosis. In Internal Medicine departments, patients were often hospitalised for sepsis or active onco-haematological disease, which can also explain low ALP.

Concerning the patients with persistent hypophosphatasaemia, patients under bisphosphonate treatment were analysed separately to identify differentiating features. The observed differences all seem to be related to osteoporosis with frequent history of fractures, bone deformities, bone demineralisation and vertebral fractures.

In terms of bone frailty, vertebral fractures were numerically less frequent in patients with persistent hypophosphatasaemia without identified cause. In the study by Hepp *et al*,[30] none of the HPP patients had vertebral fractures. The study by Genest *et al* found a significant correlation between low ALP levels and high spine BMD in a cohort of HPP patients.[31] In the literature review by Sadhukhan *et al*, vertebral fractures were not observed in HPP patients and high lumbar spine BMD was more likely.[20]

This study has some limitations. First of all, this study has a retrospective design which induced differences in the number of observations of the different parameters, and therefore, may wane some of our conclusions. There was a bias regarding the variability of the number of patients included per centre, and it did not allow us to be completely exhaustive. Indeed, 10 years of inclusion was not possible in all centres. The listing established through the cooperation with the laboratory technicians of each centre was not always complete and the extraction of data by the resident at each site was time-consuming. This difference can come from the diversity of software laboratories, which did not always lead to online results for a number of years. This required a longer duration of analysis with a risk of error and limitation of the data to a more restricted period. In addition, some centres underwent software changes during the inclusion period, generating difficulty in returning to previous data. Moreover, the time interval between the two measurements was variable, depending on each patient. The need for two measurements to limit the risk of analytical error may

also be at the origin of a bias since body weight could be different at each time point. Since this condition is poorly recognised, genetic testing was performed in only a few cases in which physicians suspected HPP. In this study, long-term low-dose glucocorticoid treatment was not taken into account as a cause of low ALP levels which is another limitation. However, literature is scarce and conflicting about this point which led us not to consider it as a possible cause of low ALP levels. Indeed, in a study by LoCascio *et al* about 23 patients treated with 10–25 mg a day of glucocorticoid for various immune diseases, no significant decline of ALP levels were found after 1–2 months, 5–7 months or 12 months glucocorticoid treatment.[32] Moreover, in 13 patients treated for chronic glomerulonephritis with a mean dose of 43.8 mg a day of GC progressively tapered, Sasaki *et al* demonstrated that ALP levels decreased significantly at 1, 3 and 6 months endpoints compared with baseline and bone-specific ALP levels decreased significantly only at 3 and 6 months of follow-up but each measurement remained in the normal range.[33] Pearce *et al* showed that GC doses of 10 mg and less for polymyalgia rheumatica resulted in higher bone specific ALP levels during a 27-month follow-up.[34] Finally, Korczowska *et al* showed that ALP levels increased significantly at 12 months after glucocorticoid initiation in patients with rheumatoid arthritis, while there was no difference in patients already treated before the beginning of the follow-up.[35]

Although this study could not be exhaustive (missing data, impossibility to carry out genetic research or to perform PLP measurements in all the patients suspected of HPP in a retrospective study), it has the advantage of inventorying our practice in view of improvement, and the multicentre character over 10 years reinforces our conclusions. The results suggest that moderate forms of genetic HPP in adults are certainly more frequent than previously thought and highlight the need for special attention to the value of ALP.

The situation is further complicated by the hypothesis that some variants could lead to low density osteopathy, without HPP-disease criteria.[36] Indeed, the presence of heterozygosity in some patients with suggestive symptoms suggests that other mechanisms are involved in the phenotypic expression of adult HPP.[37] For very mild adult forms and exclusive dental forms, mutations may be heterozygous.[38] In their proposal of genetic-based nosology of HPP, Mornet *et al* described a mild HPP form with adult onset of unspecific symptoms caused by an autosomal dominant haploinsufficiency with prevalence of 1/508.[23] However, the existence of such an entity is still controversial.

As a conclusion, hypophosphatasaemia was recognised only in 3.61% of the patients presenting this biological abnormality and hospitalised in rheumatology and internal medicine departments. At least 15.9% of patients with three or more evocative symptoms of HPP in addition to persistent hypophosphatasaemia had HPP. This multicentre retrospective study shows that adult HPP remains underdiagnosed. The prevalence of moderate forms of adult HPP appears to be higher than previously thought and highlights the need for according special attention to ALP values.

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Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval The study was approved by the local institutional ethics committee and conducted in accordance with the Declaration of Helsinki. It was likewise approved by the CNIL (French Data Protection Commission). Written consent was not required according to the MR-004 French legislation.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

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REFERENCES

1 Whyte MP. Hypophosphatasia — Aetiology, Nosology, pathogenesis, diagnosis and treatment. *Nat Rev Endocrinol* 2016;12:233–46.
2 Mornet E. Hypophosphatasia. *Metabolism* 2018;82:142–55.
3 Mornet E, Yvard A, Taillandier A, *et al*. A molecular-based estimation of the prevalence of Hypophosphatasia in the European population: prevalence of Hypophosphatasia. *Ann Hum Genet* 2011;75:439–45.
4 Bloch-Zupan A. Hypophosphatasia: diagnosis and clinical signs – a dental surgeon perspective. *Int J Paediatr Dent* 2016;26:426–38.
5 Briot K, Roux C. Adult Hypophosphatasia. *Archives de Pédiatrie* 2017;24:5S71–3.
6 Maman E, Borderie D, Roux C, *et al*. Absence of recognition of low alkaline phosphatase level in a tertiary care hospital. *Osteoporos Int* 2016;27:1251–4.
7 McKiernan FE, Shrestha LK, Berg RL, *et al*. Acute Hypophosphatasemia. *Osteoporos Int* 2014;25:519–23.
8 McKiernan FE, Berg RL, Fuehrer J. Clinical and radiographic findings in adults with persistent Hypophosphatasemia:

hypophosphatasemia; clinical and radiographic findings. *J Bone Miner Res* 2014;29:1651–60.
9 Schmidt T, Schmidt C, Amling M, *et al*. Prevalence of low alkaline phosphatase activity in laboratory assessment: is Hypophosphatasia an Underdiagnosed disease *Orphanet J Rare Dis* 2021;16:452.
10 Buttgereit F, da Silva JAP, Boers M, *et al*. Standardised nomenclature for glucocorticoid dosages and glucocorticoid treatment regimens: Current questions and tentative answers in rheumatology. *Ann Rheum Dis* 2002;61:718–22.
11 Hepp N, Frederiksen AL, Duno M, *et al*. Biochemical, clinical and genetic characteristics in adults with persistent Hypophosphatasaemia; data from an endocrinological outpatient clinic in Denmark. *Bone Rep* 2021;15:101101.
12 García-Fontana C, Villa-Suárez JM, Andújar-Vera F, *et al*. Epidemiological, clinical and genetic study of Hypophosphatasia in A Spanish population: identification of two novel mutations in the Alpl gene. *Sci Rep* 2019;9:9569.
13 Karakostas P, Dolscheid-Pommerich R, Hass MD, *et al*. Prävalenz der Hypophosphatasie BEI Adulten Patienten in der Rheumatologie. *Z Rheumatol* 2022;81:513–9.
14 Vieira LHR, Peixoto KC, Flósi CL, *et al*. Active search of adult patients with persistently low serum alkaline phosphatase levels for the diagnosis of Hypophosphatasia. *Arch Endocrinol Metab* 2021;65:289–94.
15 Feurstein J, Behanova M, Haschka J, *et al*. Identifying adult Hypophosphatasia in the rheumatology unit. *Orphanet J Rare Dis* 2022;17:435.
16 McComb RB, Bowers GN, Posen S. *alkaline Phosphatase*. In: *Clinical Utilization of Alkaline Phosphatase Measurements. Alkaline Phosphatase*. Boston, MA: Springer, 1979: 525–786.
17 Whyte MP. *Primer on the metabolic bone diseases and disorders of mineral metabolism*. Wiley-Blackwell, n.d.: 838–42.
18 Garcia-Carretero R, Olid-Velilla M, Perez-Torrella D, *et al*. Predictive modeling of Hypophosphatasia based on a case series of adult patients with persistent Hypophosphatasemia. *Osteoporos Int* 2021;32:1815–24.
19 González-Cejudo T, Villa-Suárez JM, Ferrer-Millán M, *et al*. Mild Hypophosphatasia may be twice as prevalent as previously estimated: an effective clinical algorithm to detect Undiagnosed cases. *Clin Chem Lab Med* 2024;62:128–37.
20 Sadhukhan S, Mehta P, Rajender S, *et al*. Proposing a clinical algorithm for better diagnosis of Hypophosphatasia in resource-limiting situations. *Osteoporos Int* 2022;33:2479–93.
21 Tsiantouli E, Biver E, Chevalley T, *et al*. Prevalence of low serum alkaline phosphatase and Hypophosphatasia in adult patients with atypical Femur fractures. *Calcif Tissue Int* 2022;110:703–11.
22 Marini F, Masi L, Giusti F, *et al*. ALPL Genotypes in patients with atypical Femur fractures or other biochemical and clinical signs of Hypophosphatasia. *J Clin Endocrinol Metab* 2022;107:e2087–94.
23 Mornet E, Taillandier A, Domingues C, *et al*. Hypophosphatasia: a genetic-based Nosology and new insights in genotype-phenotype correlation. *Eur J Hum Genet* 2021;29:289–99.
24 Koehler K, Atway S, Pipes J, *et al*. Diagnosis of Hypophosphatasia in adults presenting with Metatarsal stress fracture: Proof-Of-Concept for a Case-Finding strategy. *JBMR Plus* 2021;5:e10495.
25 Dahir KM, Seefried L, Kishnani PS, *et al*. Clinical profiles of treated and untreated adults with Hypophosphatasia in the global HPP Registry. *Orphanet J Rare Dis* 2022;17:277.
26 Street SE, Sowa NA. TNAP and pain control. In: Fonta C, Négyessy L, eds. *Neuronal Tissue-Nonspecific Alkaline Phosphatase (TNAP)*. Dordrecht: Springer Netherlands, 2015: 283–305.
27 Graser S, Liedtke D, Jakob F. TNAP as a new player in chronic inflammatory conditions and metabolism. *Int J Mol Sci* 2021;22:919.
28 Lai Z, Liu Y, Huang M, *et al*. Associations between Atherosclerosis and elevated serum alkaline phosphatase in patients with coronary artery disease in an inflammatory state. *Heart Lung Circ* 2023;32:1096–106.
29 Presbitero A, Mancini E, Brands R, *et al*. Supplemented alkaline phosphatase supports the immune response in patients undergoing cardiac surgery: clinical and computational evidence. *Front Immunol* 2018;9:2342.
30 Hepp N, Frederiksen AL, Duno M, *et al*. Biochemical and clinical manifestations in adults with Hypophosphatasia: a national cross-sectional study. *Osteoporos Int* 2022;33:2595–605.
31 Genest F, Claußen L, Rak D, *et al*. Bone mineral density and fracture risk in adult patients with Hypophosphatasia. *Osteoporos Int* 2021;32:377–85.
32 LoCascio V, Bonucci E, Imbimbo B, *et al*. Bone loss in response to long-term glucocorticoid therapy. *Bone Miner* 1990;8:39–51.
33 Sasaki N, Kusano E, Ando Y, *et al*. Changes in Osteoprotegerin and markers of bone metabolism during glucocorticoid treatment in patients with chronic glomerulonephritis. *Bone* 2002;30:853–8.
34 Pearce G, Ryan PF, Delmas PD, *et al*. The deleterious effects of low-dose corticosteroids on bone density in patients with Polymyalgia Rheumatica. *Br J Rheumatol* 1998;37:292–9.
35 Korczowska I, Olewicz-Gawlik A, Trefler J, *et al*. Does low-dose and short-term glucocorticoids treatment increase the risk of osteoporosis in rheumatoid arthritis female patients *Clin Rheumatol* 2008;27:565–72.
36 Nielson CM, Zmuda JM, Carlos AS, *et al*. Rare coding variants in ALPL are associated with low serum alkaline phosphatase and low bone mineral density. *J Bone Miner Res* 2012;27:93–103.
37 Taillandier A, Domingues C, Dufour A, *et al*. Genetic analysis of adults heterozygous for ALPL mutations. *J Bone Miner Metab* 2018;36:723–33.
38 Taillandier A, Domingues C, De Cazanove C, *et al*. Molecular diagnosis of Hypophosphatasia and differential diagnosis by targeted next generation sequencing. *Mol Genet Metab* 2015;116:215–20.