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Clinical and biochemical characteristics of adults with hypophosphatasia attending a metabolic bone clinic

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ABSTRACT

Objectives: This study sought to identify the clinical and biochemical characteristics that would help distinguish hypophosphatasia (HPP) from other metabolic bone diseases in adult patients attending a metabolic bone clinic by comparing patients who have genetically confirmed HPP with a group of patients with low bone mineral density (BMD) in the osteoporotic or osteopenic range.

Methods: Data were collected from February 2016 to October 2018 for 41 patients ($n = 20$ in the HPP group, $n = 21$ in the low-BMD group) attending the metabolic bone clinic at Sheffield, United Kingdom (UK) or who were recruited via the Rare UK Diseases Study (RUDY) platform during the same period. A study questionnaire was administered to all patients, and assessments were conducted for laboratory values, physical functions, BMD, and spine imaging.

Results: Patients with HPP were characterized as being younger, more likely to have metatarsal or femoral shaft fractures, and less likely to have vertebral fractures compared with patients in the low-BMD group. The HPP group had lower total and bone-specific alkaline phosphatase, higher pyridoxal 5′-phosphate (PLP), and lower, albeit sufficient, 25-hydroxyvitamin D. Low-BMD group had lower C-terminal telopeptide and tartrate-resistant acid phosphatase 5b (61.9% were on bisphosphonates at enrollment). Dual X-ray absorptiometry (DXA) analysis found that the HPP group had higher total hip and lumbar BMD T- and *Z*-scores compared with the low-BMD group. There were no differences found between the two groups with physical functional assessments. Results of receiver operating characteristic analysis indicated strong diagnostic accuracy of these biomarkers for HPP. Thresholds of total alkaline phosphatase (ALP) activity of 43 IU/L or less and PLP level of 120 nmol/L or more were determined to be potentially clinically useful for distinguishing HPP from other metabolic bone diseases.

Conclusion: This study supported the use of ALP and PLP measurements as predictive of HPP diagnosis along with certain demographic and clinical characteristics (younger age, metatarsal or femoral fractures without low mean BMD T- and *Z*-scores on a DXA scan) that can aid in recognizing adults who should be further evaluated for HPP. The critical values identified need to be applied to an independent sample to be tested for diagnostic accuracy.

Abbreviations: 25D, 25-hydroxyvitamin D; ALP, alkaline phosphatase; *ALPL*, alkaline phosphatase, biomineralization-associated; BAP, bone-specific ALP; BMD, bone mineral density; CTX, C-terminal telopeptide; DXA, dual X-ray absorptiometry; HPP, hypophosphatasia; HRQoL, health-related quality of life; LSBMD, lumbar spine BMD; OC, osteocalcin; PINP, procollagen type I N-propeptide; PLP, pyridoxal 5′-phosphate; PTH, parathyroid hormone; RI, reference interval; ROC, receiver operating characteristic; RUDY, Rare UK Diseases Study; SD, standard deviation; SPPB, Short Physical Performance Battery; TNSALP, tissue-nonspecific alkaline phosphatase; TRACP5b, tartrate-resistant acid phosphatase 5b; UK, United Kingdom.

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1. Background

Hypophosphatasia (HPP) is a rare, inherited, metabolic disease that is caused by low activity of tissue-nonspecific alkaline phosphatase (TNSALP), due to alkaline phosphatase biomineralization-associated (*ALPL*) gene mutations [1,2]. Deficient activity of TNSALP leads to the extracellular accumulation of several TNSALP substrates such as inorganic pyrophosphate, a potent inhibitor of bone mineralization, and pyridoxal 5′-phosphate (PLP), the active form of vitamin B6. Due to the accumulation of these substrates, HPP can manifest at any age with a range of symptoms, including premature loss of teeth, HPP-related rickets (in infants and children), osteomalacia with associated fractures and pseudofractures (particularly in adults), pain, muscle weakness, and ambulatory difficulties among other symptoms (eg, vitamin B6-dependent seizures and respiratory difficulties in infants, knee pain from chondrocalcinosis, neurologic or rheumatologic complaints in adults) [2,3]. These symptoms can have a detrimental effect on the health-related quality of life (HRQoL) of adults with HPP, because of their impact on daily activities [4].

The heterogenous clinical presentation of HPP creates many challenges in diagnosing the disease. Certain signs, symptoms, or complications of HPP can change during a patient's lifetime [5]. Although HPP can be diagnosed during adulthood (age ≥ 18 years), adults may have histories of signs or symptoms before 18 years of age but their disease was not recognized during childhood. In fact, adults with HPP frequently experience substantial delays in diagnosis [3,6,7]. Moreover, HPP in adults may be misdiagnosed as osteoporosis due to similar symptoms such as frequent fractures of the lower extremities [2]. Misdiagnosis or delayed diagnosis may lead to worsening of HPP-related manifestations and the patient's general health [8,9]. In addition, initiating treatment for osteoporosis with bisphosphonates, pharmaceutical analogs of inorganic pyrophosphate, may exacerbate HPP symptoms and increase the risk of atypical femoral fractures [10–12].

Limited disease awareness and heterogeneity of symptoms are some of the major contributing factors to the late diagnosis or misdiagnosis of HPP in adults. However, robust biomarker thresholds can assist in recognizing possible cases of HPP, and genetic testing may be useful in confirming the diagnosis. Therefore, this study utilized clinical characteristics and biochemical markers, supplemented by genetic testing, as diagnostic tools to aid in distinguishing adults with HPP from adults with other metabolic bone diseases, with the aim of raising awareness about diagnostic criteria for HPP in adults.

2. Materials and methods

2.1. Study design

This was a prospective study of adults (age ≥18 years) who attended the Metabolic Bone Centre at the Northern General Hospital, Sheffield, United Kingdom (UK), or were recruited via the Rare UK Diseases Study (RUDY) platform between February 2016 and October 2018 [13]. The study was approved by the North West-Greater Manchester East Research Ethics Committee, REC number: 16/NW/0385. All patients provided informed written consent prior to study participation.

2.2. Patients

All patients had genetic assessments by the Sheffield Children's Hospital Clinical Genetics Laboratory, using next-generation sequencing confirmed by Sanger sequencing. Two groups of patients were selected for the study: adults who had a confirmed diagnosis of HPP (HPP group) and adults who had low bone mineral density (BMD) and attended the Sheffield Metabolic Bone Centre for osteoporosis (low-BMD group).

Adult patients were eligible to be included in the HPP group if they had persistently low alkaline phosphatase (ALP) activity and the clinical presentation of musculoskeletal symptoms consistent with HPP. As part of the study protocol, the diagnosis of HPP was confirmed with genetic assessments that identified *ALPL* gene variants that were pathogenic or likely pathogenic mutations based on the American College of Medical Genetics and Genomics criteria. Patients were eligible for the low-BMD group if BMD was in the osteoporotic or the osteopenic range, if the genetic assessment identified no *ALPL* gene variants, and if ALP activity was not persistently low. The osteoporotic range was defined as T-score ≤−2.5 for lumbar spine BMD (LSBMD) or total hip BMD [14]; the osteopenic range was defined as T-score <−1.0 for LSBMD [14] or total hip BMD in patients who did not have osteoporosis. Patients in the low-BMD group were compared with patients in the HPP group (Fig. 1). Because patients with HPP are typically younger than those with osteoporosis, it was difficult to individually match patients by age using a statistical method, such as propensity score matching. Therefore, the two groups were comprised from the pool of patients who met all inclusion criteria and were available via the Sheffield Metabolic Bone Centre and RUDY platform.

At study enrollment, investigators used a standard metabolic bone questionnaire for patients to capture general information relating to bone health, such as the use of medications (including bisphosphonates), medical history, and baseline clinical characteristics. Patients in the HPP group were also assessed for pediatric-onset (aged <18 years) or adult-onset (aged ≥18 years) HPP based on the reported age of the first signs and symptoms of HPP. The specific signs and symptoms were assessed by investigators based on established literature and included premature loss of teeth [2,15,16].

2.3. Laboratory analysis

Blood samples were drawn from patients in the fasting state, and because PLP levels were being assessed, patients were required to stop taking vitamin supplements (if any) for more than 3 weeks. Samples were serum separated, stored at −70 °C, and analyzed in a single batch (at the Chemical Pathology Laboratory, Sheffield Teaching Hospital National Health Service Foundation Trust) for total ALP activity, calcium, and phosphate levels using a Roche/Hitachi Cobas 8000 e702 automated clinical chemistry analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Samples were also analyzed in the Bone Biochemistry Laboratory at the University of Sheffield for bone resorption markers and bone formation markers using an iSYS automated immunoassay system (Immunodiagnostics Ltd., Boldon, UK). The analyzed bone resorption markers were tartrate-resistant acid phosphatase 5b (TRACP5b) and C-terminal telopeptide (CTX), and the bone formation markers were bone-specific ALP (BAP), procollagen type I N-propeptide (PINP), osteocalcin (OC), 25-hydroxyvitamin D (25D), and parathyroid hormone (PTH). Serum PLP levels were measured using cation-exchange high-performance liquid chromatography (Chromsystems Instruments and Chemicals GmbH, Munich, Germany) [17,18].

Reference intervals were established in 200 healthy women (mean age 39 years, standard deviation [SD]: 4 years, range 30 to 45 years) and 92 healthy men (mean age 47 years, SD: 21 years, range 16 to 77 years). All healthy subjects also stopped taking vitamin supplements before testing for PLP, as described above for the study population. The lower limit of the interval was established at 4.2 μg/L for BAP. The upper limit of the interval for PLP differed by sex and was 177.9 nmol/L for women and 250.7 nmol/L for men. The lower limit of the reference interval for total ALP activity was 30 IU/L. the reference interval for all laboratory assays is provided in Table 2.

2.4. Fracture and bone assessments

LSBMD and total hip BMD were assessed by dual X-ray absorptiometry (DXA) using a Discovery A densitometer (Hologic Inc., Waltham, MA). For each group, both T- and *Z*-scores were calculated based on established criteria [14]. The presence of vertebral fractures in the lateral DXA image of the spine was assessed using an algorithm-based

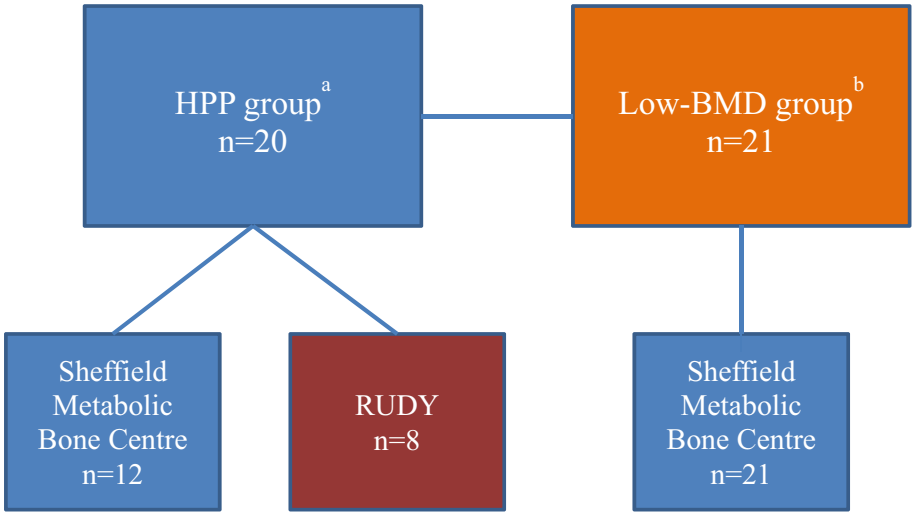

Fig. 1. Study population.
[a]Adults with a clinical diagnosis of HPP, confirmed by genetic assessments, were eligible to be enrolled in the HPP group.
[b]Patients were eligible for the low-BMD group if BMD was in the osteoporotic or osteogenic range.
BMD, bone mineral density; HPP, hypophosphatasia; RUDY, Rare UK Diseases Study.

qualitative approach [19].

2.5. Functional assessments

Patients' physical function was assessed using the grip strength test (digital hand dynamometer, Seehan Corporation, Masan, Korea) and the Short Physical Performance Battery (SPPB) [20]. Low grip strength was identified as less than 16 kg in women and less than 27 kg in men [21]. Scores for the SPPB are based on patients' performances on the chair rise, tandem balance, and the 8-foot walk tests. Patients were classified as frail (SPPB 0–6), pre-frail (SPPB 7–9), or non-frail (SPPB 10–12) based on SPPB scores [22].

2.6. Receiver operating characteristic (ROC) analysis

Sensitivity and specificity of total ALP, BAP, and PLP as diagnostic tools to recognize HPP in adult patients were calculated using ROC analysis. Maximum diagnostic likelihood ratios were also calculated using ROC analysis.

2.7. Data analysis

Wizard version 1.9.30 (Free Software Foundation, Boston, USA) and Prism for MacOS X version 7c (GraphPad Software Inc., CA, USA) statistical software were used for the data analysis. MedCalc version 16.8.4 (MedCalc, Ostend, Belgium) software was used to calculate 95% reference intervals (RIs). Variables were summarized with n, mean, and SD. Comparisons between patient groups were assessed using *P* values, which were calculated using multiple linear regression with group, age, and sex as covariates.

3. Results

3.1. Patient demographics and baseline clinical characteristics

Overall, 41 adult patients (66% women) were included in the study. The population was comprised of 21 patients in the low-BMD group and 20 patients in the HPP group. Patients in the low-BMD group were recruited via the Sheffield Metabolic Bone Centre. Of the 20 patients in the HPP group, 12 were recruited via the Sheffield Metabolic Bone Centre and 8 were recruited via the RUDY platform (Fig. 1). All patients in the HPP group had an *ALPL* pathogenic or likely pathogenic variant, as confirmed by genetic assessment. Three patients in the HPP group had compound heterozygous variants in *ALPL*. Genetic profiles of each of the 20 patients with HPP are available in Supplementary material 1. An assessment of the age of onset revealed that 7 (35%) patients in the HPP group had pediatric-onset HPP and 13 (65%) patients had adult-onset HPP.

Patient demographics and characteristics of the study population are presented in Table 1. Patients in the HPP group were generally younger than those in the low-BMD group. Bisphosphonate use at study enrollment was more prevalent in the low-BMD group (61.9% of patients) than in the HPP group (0.0% of patients). In addition, patients in the HPP group were not exposed to asfotase alfa nor teriparatide or strontium, which could have affected ALP levels.

3.2. Fracture and bone assessments

Metatarsal/foot and femoral shaft fractures occurred more frequently among patients in the HPP group versus those in the low-BMD group. No patients in the low-BMD group had experienced metatarsal/foot or femoral shaft fractures, whereas 5 (25%) patients in the HPP group had experienced metatarsal/foot fractures and 2 (10%) patients experienced femoral shaft fractures (Fig. 2). In contrast, vertebral fractures were more common in the low-BMD group versus the HPP group (6 and 0, or 29% vs 0%, respectively).

Of note, one patient with compound heterozygous *ALPL* variants and pediatric-onset HPP was severely affected, reporting 9 total factures, including a right femur fracture at the age of 34 years and later experiencing the recurrence of stress fractures in the metatarsals of both feet and one radius. The other two patients with compound heterozygous *ALPL* variants, one with pediatric-onset HPP and one with adult-onset HPP, each had 1 fracture and no evidence of stress fractures.

Table 1
Patient demographics and clinical characteristics of the study population.

Characteristic	HPP group $n = 20$		Low-BMD group $n = 21$	
	Women $n = 12$	Men $n = 8$	Women $n = 15$	Men $n = 6$
Mean age, years (range)	49.1 (30.7–74.8)	52.3 (39.0–63.7)	52.2 (20.9–74.5)	60.5 (48.8–70.8)
Mean weight, kg (SD)	77.6 (27.0)	89.7 (23.1)	68.9 (15.7)	85.9 (7.7)
Mean height, cm (SD)	166.4 (9.3)	175.8 (8.1)	160.9 (5.8)	175.4 (4.0)
Treatment at enrollment, n (%)				
Bisphosphonates	0 (0.0)		13 (61.9)	

BMD, bone mineral density; HPP, hypophosphatasia; SD, standard deviation.

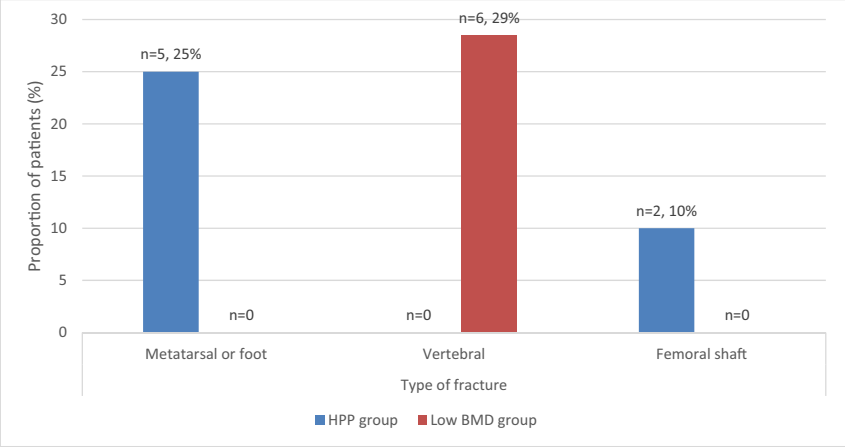

Fig. 2. Fracture prevalence among patients in the HPP and low-BMD groups. BMD, bone mineral density; HPP, hypophosphatasia.

Total hip and LSBMD T-scores were significantly higher among patients in the HPP group, and within the normal range, versus those in the low-BMD group, which were below the normal range ($P < 0.01$ and $P < 0.001$, respectively; Table 2). Total hip and LSBMD *Z*-scores were also significantly higher among patients in the HPP group versus those in the low-BMD group ($P < 0.001$ and $P < 0.001$, respectively; Table 2) but were at the expected level for their age (mean Z-score close to zero).

Table 2
Biochemistry and total hip and lumbar BMD results for patients in the HPP and low-BMD groups.

Parameter	Reference interval[a]	HPP group $n = 20$	Low-BMD group $n = 21$
Total ALP, IU/L[b]	Adult: 30–130	23.8 (8.8)***	73.1 (32.3)
BAP, μg/L[b]	Women: 4.2–24.3	6.3 (3.1)***	18.6 (10.5)
PINP, μg/L[b]	Women: 15.5–78.6	51.3 (26.4)	38.1 (20.8)
PLP, nmol/L[c]	Women: 14.6–177.9 Men: 19.4–250.7	384.5 (196.3–608.8)**	54.2 (30.6–82.0)
TRACP5b, IU/L[b]	Women: 1.5–4.9	2.8 (1.4)**	1.8 (1.0)
CTX, ng/L[c]	Women: 50–560	102.4 (52.9–330.2)**	33.0 (33.0–76.3)
OC, μg/L[b]	Women: 5.5–29.3	11.2 (4.5)	9.2 (4.8)
PTH, ng/L[b]	7.0–69.0 [38]	23.1 (16.4)	19.4 (13.3)
25D, μg/L[b]	Sufficiency >20	27.9 (8.6)*	34.1 (9.9)
Adjusted calcium, mmol/L[b]	Adult: 2.2–2.6 [39,40]	2.4 (0.1)	2.3 (0.1)
Phosphate, mmol/L[b]	Adult: 0.8–1.5 [39,40]	1.4 (0.3)	1.2 (0.2)
LSBMD, T-score[b]	–	−0.6 (1.9)***	−2.3 (0.9)
Total hip BMD, T-score[b]	–	−0.4 (1.0)**	−1.4 (0.8)
LSBMD, *Z*-score[b]	–	0.1 (2.0)***	−1.6 (1.4)
Total hip BMD, Z-score[b]	–	0.0 (1.0)***	−0.7 (1.0)

ALP, alkaline phosphatase; BAP, bone-specific alkaline phosphatase; BMD, bone mineral density; CTX, C-terminal telopeptide; HPP, hypophosphatasia; LSBMD, lumbar spine bone mineral density; OC, osteocalcin; PINP, procollagen type I N-propeptide; PLP, pyridoxal-5-phosphate; PTH, parathyroid hormone; RI, reference interval; SD, standard deviation; TRACP5b, tartrate-resistant acid phosphatase 5b; 25D, 25-hydroxyvitamin D.

[a] Reference intervals established in the current study are listed in the table unless otherwise cited.

[b] Mean (SD) is reported and comparisons between the two groups were conducted using multiple linear regression analysis adjusted for age and sex. Significant *P* values ($P < 0.05$) are shown in the table.

[c] Median (interquartile range) is reported and comparisons between the two groups were conducted using a Mann–Whitney *U* test due to outliers in the data. Significant *P* values ($P < 0.05$) are shown in the table.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

3.3. Laboratory analysis

Consistent with the inclusion criteria, total ALP and BAP activities were significantly lower in the HPP group vs the low-BMD group (both $P < 0.001$, Table 2). The level of PLP was significantly higher among patients in the HPP group when compared with those in the low-BMD group ($P < 0.01$). Notably, total ALP was 3-fold lower and PLP was 7-fold higher in patients with HPP when compared with those in the low-BMD group. TRACP5b and CTX were significantly lower among patients in the low-BMD group when compared with those in the HPP group (both $P < 0.01$). No significant differences in the levels of PINP, OC, PTH, calcium, and phosphate were observed between the two groups. When comparing women and men of the two groups, no significant differences in biomarkers were observed (data not shown).

Additional laboratory analyses showed that a total ALP activity of 43 IU/L or less, in the presence of additional clinical features consistent with a diagnosis of HPP, could be used as an appropriate threshold to distinguish patients with HPP from control patients with osteoporosis. This threshold would classify all patients in the HPP group and 19 (of 21) patients in the low-BMD group correctly (Fig. 3). Notably, using this ALP activity threshold with an additional requirement of PLP $\geq$ 120 nmol/L would classify all patients in the HPP and low-BMD groups correctly (Fig. 3).

3.4. Functional assessments

There were no significant differences in grip strength and SPPB scores between patients in the HPP group versus those in the low-BMD group (all $P \geq 0.05$). Twelve patients ($n = 8$ women, $n = 4$ men) from the low-BMD group and 11 patients ($n = 7$ women, $n = 4$ men) from the HPP group had low grip strength classified by the predefined grip strength test thresholds. Frail, pre-frail, and non-frail SPPB scores were identified in 1, 9, and 10 patients of the HPP group and 3, 5, and 13 patients of the low-BMD group, respectively.

3.5. ROC analysis

Results of the ROC analysis are presented in Fig. 4. The area under the ROC curves for total ALP, BAP, and PLP were 0.991 (95% confidence interval [CI]: 0.971–1.000), 0.914 (0.813–1.000), and 0.988

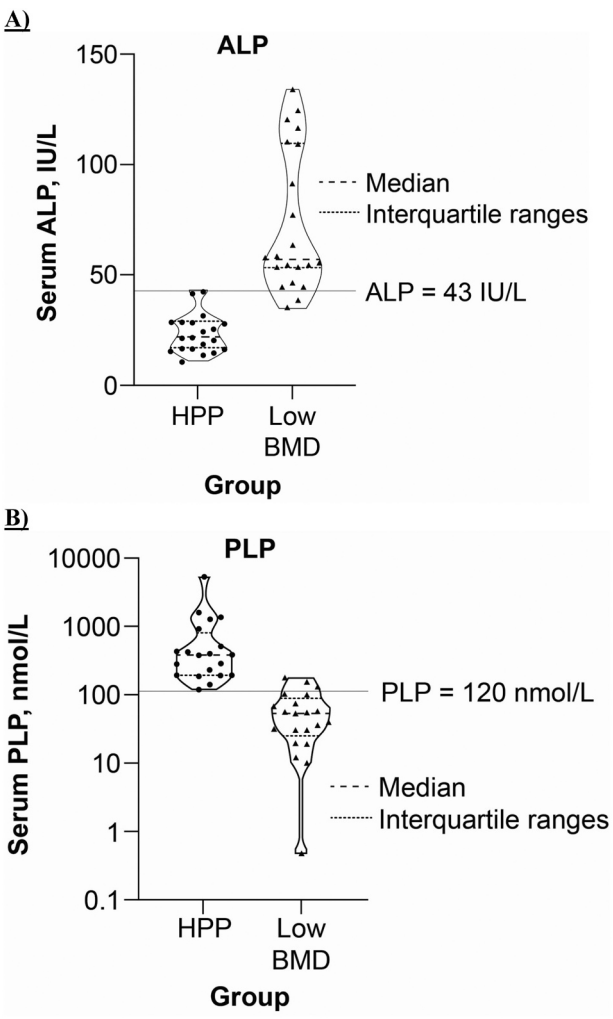

Fig. 3. Violin plots of laboratory thresholds in the HPP and low-BMD groups.
A) All the patients with HPP had a total ALP activity of 43 IU/L or lower; 2 patients in the low-BMD group were below this threshold.
B) All patients with HPP had PLP above 120 nmol/L; 18 of 21 patients in the low-BMD group had PLP below 120 nmol/L.
ALP, alkaline phosphatase; BMD, bone mineral density; HPP, hypophosphatasia; PLP, pyridoxal-5-phosphate.

(0.965–1.000), respectively, indicating strong diagnostic accuracy of these biomarkers for HPP. In addition, the maximum diagnostic likelihood ratio values were 36.5 IU/L, 10.1 μg/L, and 182 nmol/L for total ALP, BAP, and PLP, respectively.

4. Discussion

Adults with HPP may present with a wide range of clinical manifestations, including fractures, pain, muscle weakness, and gross motor difficulties that often overlap with other metabolic bone diseases [2,15,16,23]. Consequently, adults with HPP have commonly been undiagnosed or misdiagnosed for osteomalacia related to other causes, osteopenia, and/or osteoporosis [2,24]. This results in a delay of initiating treatment or even incorrect management of HPP, which can impact quality of life [3,4]. The present analysis has described and quantified clinical and biochemical indicators of HPP that could be used in clinical practice to assist in distinguishing and diagnosing patients who have HPP from patients who do not have HPP but present with similar symptoms.

In the current study, we identified several demographic and clinical characteristics that were common in adults with HPP. Specifically, patients with HPP were younger and had more metatarsal and femoral shaft fractures, but fewer vertebral fractures, when compared with patients in the low-BMD group. The main sites of fracture were metatarsal and femoral, which is consistent with the findings from other studies [3,23,25]. Chondrocalcinosis may also be helpful in distinguishing HPP from osteoporosis [26]; however, in this study, no patients had evidence of chondrocalcinosis (knee pain/soreness and/or radiographic features). DXA results indicated higher BMD T- and *Z*-scores for the total hip and lumbar spine in patients with HPP when compared with patients who had low BMD. Other studies have found BMD T-scores to be either at or above the expected level for age, with high LSBMD being one of the risk factors for femoral fractures [23], or only slightly reduced in patients with HPP [27,28]. These results should alert a clinician to a diagnosis of HPP if a younger patient demonstrates clinically increased bone fragility in the absence of low BMD.

This study also evaluated biochemical markers that could be used to distinguish patients with HPP from those with low BMD. Reduced serum ALP activity, elevated levels of PLP, and reduced BAP activity are well-known biomarkers for HPP [25,29,30]. The challenge of using these biomarkers for diagnosis arises from imprecise thresholds.

Although ALP is routinely measured in metabolic bone clinics, low ALP levels may not be flagged as abnormal and could be disregarded [25,26,31]. While persistently low ALP activity is a clear diagnostic indicator for HPP (supported by our ROC analysis and other data) [25], alternative causes of low ALP activity, such as Cushing syndrome, hypothyroidism, multiple myeloma, osteogenesis imperfecta type II, or treatments such as glucocorticoids and antiresorptive drugs, should be excluded [25,32]. In addition, a recent study investigating patients with osteoporosis and low-serum ALP activity (16/3285 or 0.5% of bone clinic patients) found a relatively high prevalence of pathogenic variants in *ALPL* (87.5%) among these patients [33]. However, unlike patients with HPP, those with osteoporosis and low-serum ALP activity were not found to have any distinguishing clinical or biochemical characteristics [33].

The reliability of low ALP activity alone as an indicator of HPP has been found to be inconsistent in a larger, general population [34]. Therefore, clinical presentation and other biochemical markers alongside low ALP activity are more likely to make the diagnosis of HPP more robust. Notably, in this study, when used together, total ALP activity of 43 IU/L or less and PLP levels above 120 nmol/L were able to fully discriminate patients with HPP from those with low BMD. It is of interest that the PLP threshold value of 120 nmol/L determined in this study is close to the estimate for the upper limit of the reference interval for PLP established in the large National Health and Nutrition Examination Survey (NHANES) that was recently published [35]. It is also important to note that PLP is not always elevated in HPP. Other studies have reported that only 50% of patients with HPP have elevated PLP levels [34,36].

This study also evaluated laboratory markers other than ALP and PLP that may assist in distinguishing patients who have HPP from those who do not have HPP but present with similar symptoms. BAP was assessed in this study; however, its use as a diagnostic marker was not as strong as ALP and PLP. In addition, bone resorption markers, such as CTX and TRACP5b, were assessed in this study, but were within normal limits among patients with HPP and were significantly lower in the low-BMD group. This difference may be due to bisphosphonate therapy, as 61.9% of the patients in the low-BMD group were taking bisphosphonate therapy for osteoporosis. Phosphoethanolamine (PEA) was not assessed in this study, because other studies have found PEA to be a less reliable diagnostic marker of HPP than PLP. Although inorganic pyrophosphate (PPi) is a reliable diagnostic marker of HPP, it was not assessed in this study, because it is currently assessed only in research settings [25,37].

Functional assessments showed that patients in the HPP group had similar physical function and strength based on the SPPB and hand grip strength tests when compared with patients in the low-BMD group. Although functional burdens due to HPP in adults are comparable with

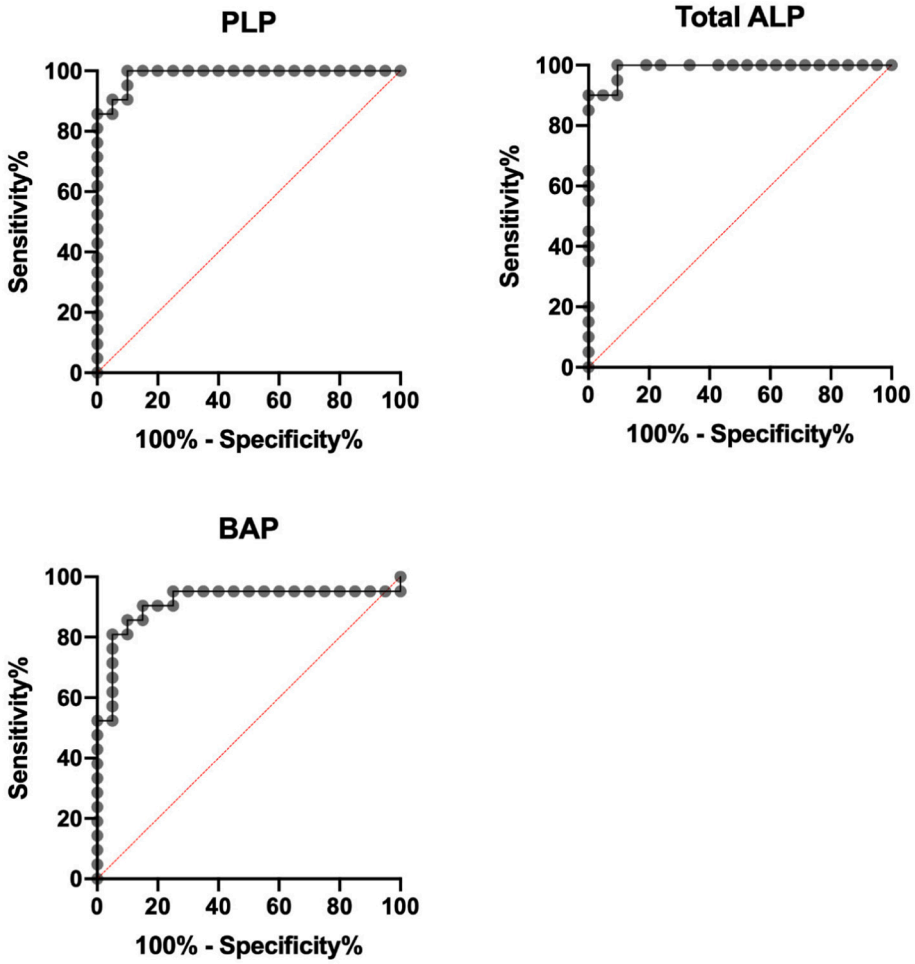

Fig. 4. ROC analysis for PLP, total ALP, and BAP.
ALP, alkaline phosphatase; BAP, bone-specific ALP; PLP, pyridoxal-5-phosphate; ROC, receiver operating characteristic.

the burdens caused by osteoporosis, adult patients with HPP do not have as much support or the benefit of wide public health awareness as patients with osteoporosis do.

The resemblance of HPP to rheumatological and bone fragility disorders such as osteoporosis suggests that osteoporosis clinics and metabolic bone centers may have a notable prevalence of undiagnosed HPP. An observational study found that patients with suspected, unrecognized HPP presented with a higher prevalence of rheumatologic conditions, including chondrocalcinosis, calcific periarthritis, enthesopathy, and diffuse idiopathic skeletal hyperostosis when compared with controls who had normal serum ALP activity (defined as 40 to 125 IU/L) [26].

Biochemical characteristics in patients with confirmed HPP may vary. Therefore, the identification of threshold values for ALP and PLP and the precise quantitation of these biomarkers in this study may be useful for screening patients in general, especially for screening patients who attend a metabolic bone clinic, as there may be an increased prevalence of HPP in this population due to misdiagnosis or missed diagnosis.

This study is limited by the single-center nature of the study design. Because patients with HPP are typically younger than those with osteoporosis, it was difficult to individually match patients by age; thus, the groups were comprised from the pool of patients who were available via the Sheffield Metabolic Bone Centre and RUDY platform. In addition, the small sample size may limit generalizability of these findings to other settings or patient populations. We appreciate, in particular, that the thresholds are based only on 41 patients and so the estimates are subject to a large degree of uncertainty and need to be confirmed by future studies. Despite these limitations, findings of this study will hopefully contribute to improvements in accurate and timely diagnosis of adult patients with HPP, thereby reducing the likelihood of misdiagnosis and potential harm from bisphosphonate use in these patients.

5. Conclusion

The lack of disease awareness and the overlap of HPP symptoms with other, similar and more well-known disorders is a major contributing factor to the underdiagnosis or misdiagnosis of HPP in adults. This study showed that biochemical tests for PLP and total ALP performed well at distinguishing HPP from osteoporosis in patients attending a metabolic bone clinic. Total ALP and PLP values were found to be the most suitable diagnostic biomarkers for HPP in adult patients. These biochemical markers, along with key clinical features, such as the presence or history of lower-extremity fractures, showed promise for assisting in the timely and accurate identification and diagnosis of HPP in patients attending metabolic bone clinics.

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CRediT authorship contribution statement

Robert Desborough: Writing – review & editing. **Philip Nicklin:** Data curation, Formal analysis. **Fatma Gossiel:** Writing – review & editing. **Meena Balasubramanian:** Formal analysis, Writing – review &

editing. **Jennifer S. Walsh:** Writing – review & editing. **Anna Petryk:** Conceptualization, Writing – review & editing. **Megan Teynor:** Conceptualization, Writing – review & editing. **Richard Eastell:** Conceptualization, Methodology, Formal analysis, Supervision, Writing – review & editing.

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Declaration of competing interest

Anna Petryk and Megan Teynor are employees of, and may own stock/options in, Alexion Pharmaceuticals, Inc. Richard Eastell receives consultancy funding from IDS, Roche Diagnostics, GSK Nutrition, FNIH, Mereo, Lilly, Sandoz, Nittobo, AbbVie, Samsung and Haoma Medica, and grant funding from Nittobo, IDS, Roche, Amgen and Alexion. Jennifer S Walsh receives speaker's honoraria from Eli Lilly and Sandoz, grant funding from Alexion and Immunodiagnostic Systems, and consulting fees from Shire, Mereo BioPharma, Kyowa Kirin, UCB Pharma and Pharmacosmos. Robert Desborough, Philip Nicklin, Meena Balasubramanian, and Fatma Gossiel have nothing to disclose.

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