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RELIABILITY OF CALCIUM TO PHOSPHORUS (Ca/P) RATIO AS A NEW, ACCURATE AND INEXPENSIVE TOOL IN THE DIAGNOSIS OF SOME Ca-P DISORDERS

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RELIABILITY OF CALCIUM TO PHOSPHORUS (Ca/P) RATIO AS A NEW, ACCURATE AND INEXPENSIVE TOOL IN THE DIAGNOSIS OF SOME Ca-P DISORDERS

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Abstract

Purpose: The serum calcium/phosphorus (Ca/P) ratio is an accurate tool to differentiate patients with primary hyperparathyroidism (PHPT) from healthy subjects. However, other disorders of the Ca-P metabolism might impair the Ca/P ratio, such as hypophosphatemia (HypoP) not PHPT-related. The aim of this study is to examine the diagnostic value of Ca/P ratio in the diagnosis of PHPT and HypoP not PHPT-related.

Methods: Single-center, retrospective, case-control study, including 150 patients with PHPT and 306 patients with HypoP, compared with 150 controls. HypoP patients were enrolled among HIV-infected patients by selecting those with Fanconi-like syndrome due to antiretroviral treatment. Parameters which were measured were serum Ca, P, parathyroid hormone (PTH), 25-OH vitamin D, albumin and creatinine)

Results: The Ca/P ratio was significantly higher in PHPT and HypoP patients, compared to controls ($p<0.0001$). At receiver operator characteristic (ROC) curve analysis, the cut-off of 3.56 (2.75 SI) for Ca/P ratio was able to identify patients with PHPT and HypoP (sensitivity 95%; specificity 93%). Among patients with Ca/P ratio above 3.56, the thresholds of 10.3 mg/dL (2.6 mmol/L) for serum Ca (sensitivity 93%; specificity 98%) and 80.5 pg/mL for PTH (sensitivity 91%; specificity 91%) were defined for the specific diagnosis of PHPT.

Conclusions: The Ca/P ratio above 3.56 (2.75 SI) is a highly accurate tool to identify PHPT and HypoP not PHPT-related patients. Thanks to its simplicity, this index can be proposed as a screening and first-line examination in the diagnostic work-up when a disorder of Ca-P metabolism is suspected or should be ruled out.

Key words: Calcium-Phosphorus metabolism, hyperparathyroidism, hypophosphatemia, screening

Introduction

The calcium to phosphorus (Ca/P) ratio has been recently proposed for the first time as a simple and accurate tool to discriminate patients with primary hyperparathyroidism (PHPT) from healthy subjects. The reliability of this index is based on the fact that Ca and P homeostasis are closely regulated and their serum concentration is inversely correlated [1-3]. The diagnosis of PHPT is traditionally based on increased serum Ca and intact parathyroid hormone (PTH) levels [4, 5], in association with slight or mild hypophosphatemia (HypoP) [5, 6]. However, the biochemical profile of PHPT is not always so evident, making the diagnosis of PHPT challenging. For this reason, the serum Ca/P ratio could help in the diagnostic workup of PHPT, especially in asymptomatic or normocalcemic patients [3].

Nevertheless, although PHPT is the most common cause of hypercalcemia [6, 7], other disorders of the Ca-P metabolism might impair the Ca/P ratio. For example, a condition of HypoP not due to PHPT should be considered.

HypoP is a rare disease [8] that may occur by several mechanisms [9], including internal redistribution (eg, malnutrition, sepsis) or increased urinary excretion (eg, X-linked hypophoshatemic rickets, Fanconi syndrome) [10, 11]. A phosphate wasting disorder is also frequently observed in patients with Human Immunodeficiency Virus (HIV)-infection with ongoing combination antiretroviral therapy (cART) [12, 13]. In particular, among antiretroviral drugs, the use of tenofovir has been correlated with mild to severe hypophosphatemia, through a renal tubular dysfunction and chronic increased phosphaturia, resulting in a Fanconi-like syndrome [12-14].

In our previous study, the lack of a group of patients with HypoP limited the validation of serum Ca/P ratio for the differential diagnosis between PHPT and other causes of altered Ca/P ratio.[3]. For example, tenofovir-related hypophosphatemia represents a mono-factorial hypophosphatemia, making HIV-infected patients with HypoP a uniform cohort to compare with PHPT patients.

The aim of the study is to evaluate the accuracy and diagnostic value of Ca/P in the diagnosis of PHPT and HypoP related to Fanconi-like syndrome and to investigate if this index can be used for differential diagnosis.

Subjects and Methods

Study Design

A single-centre, retrospective, case-control study was carried out.

The group of cases with ascertained diseases of Ca-P metabolism was composed by two subgroups: patients with a certain diagnosis of PHPT and HIV-infected patients with HypoP. Controls consisted of subjects without any disorder of Ca-P metabolism. This trial was registered in ClinicalTrials.gov (Identifier: NCT03603444).

Subjects

A total of 150 patients followed at the Unit of Endocrinology of Modena with a diagnosis of PHPT from 2005 to January 2018 were included in the first case group (Table 1). For the other group of cases, 306 subjects with a certain diagnosis of drug-induced HypoP with reduced serum P according to the lower limit of the normal range of our laboratory (2.5 mg/dL), but normal serum Ca, were enrolled among HIV-infected patients on cART treatment from the large Modena cohort that belongs to Modena HIV Metabolic Clinic (MHMC), from 2004 to 2017. In this tertiary care teaching Hospital in Northern Italy, all individuals are screened by a multidisciplinary team for immune-metabolic disorders, antiretroviral-associated toxicities, HIV-associated non-infectious endocrine co-morbidities, geriatric syndromes and disabilities [15]. In particular, Ca, P, PTH and vitamin D are routinely checked at baseline in order to exclude Ca metabolism disorders related to osteoporosis or drug toxicity. By considering the low prevalence of hypophosphatemia in the general population and the availability of a easily-accessible large database, we chose to investigate the tenofovir-related hypophosphatemia since it allowed us to select quickly a large sample of

patients with hypophosphatemia and to have a homogeneous mono-factorial disease, such as tenofovir-related hypophosphatemia, to compare with PHPT. Tenofovir-related hypophosphatemia, however, should be considered highly representative of all other types of hypophosphatemia at least for the aim of this study since it leads to the same changes in serum such as other types of hypophosphatemia.

As a control group, we retrospectively selected 150 patients who attended the Unit of Endocrinology of Modena in the same time period, presenting serum PTH, Ca and P within the normal range. In particular, 43 (28.7%) were healthy subjects, 30 (20.0%) were male hypogonadal patients with documented normal serum testosterone during testosterone replacement therapy, 27 (18.0%) had a pituitary disease (non-functioning pituitary adenoma or empty sella with normal pituitary function), 17 (11.3%) had a non-functioning adrenocortical adenoma, 33 (22.0%) had a thyroid disorder (history of thyroidectomy, chronic thyroiditis or nodular goiter).

The following parameters were needed for the inclusion in each group: age, gender, serum Ca, P, PTH, 25-OH vitamin D and creatinine (Table 1).

Exclusion criteria for both cases and controls were: age younger than 18 or older than 90 years; severe renal and liver diseases (i.e. glomerular filtration rate (GFR) <30 mL/min); hyperparathyroidism secondary to Vitamin D deficiency; history of parathyroidectomy, even if the parathyroid disorder was considered in remission; active metabolic bone disease (e.g. Paget's disease of the bone, osteomalacia, rickets, etc); any type of cancer; malnutrition; severe obesity (BMI > 40 kg/m2); a history of gastrointestinal malabsorption; sarcoidosis; hypercortisolism, diabetes insipidus, hyperthyroidism, hypoparathyroidism and pseudohypoparathyroidism; familial hypocalciuric hypercalcemia (FHH); treatment with steroids, calcium and/or active forms of vitamin D (calcitriol, ergocalciferol, etc), thiazides, phosphate binders, lithium, cinacalcet, bisphosphonates, and denosumab.

The characteristics of patients and controls are summarized in Table 1.

Laboratory analyses

Serum Ca and P were detected using Beckman Coulter AU 680 (Beckman Coulter Italy, Cassina de' Pecchi, Milan, Italy) device by the colorimetric photometric methods based on Arsenazo III and molybdate, respectively. For both sexes, the normal reference ranges for serum Ca and P were 8.5-10.5 mg/dL and 2.5-5.1 mg/dL, respectively.

Serum Intact PTH was determined by two house-different chemiluminescence assays: a DiaSorin Liaison (DiaSorin S.p.A., Saluggia, Vercelli, Italy) from January 2005 to June 2010 and a Beckman Coulter UniCel DxI 600 Synchron Access (Beckman Coulter Italy, Cassina de' Pecchi, Milan, Italy) from June 2010 to January 2018. The normal reference range for serum PTH was 15-88 ng/L, with an intra-assay variability coefficient of 5%.

Serum 25OH-Vitamin D was measured by chemiluminescence with the LIASON® XL 1,25OH-Vitamin D assay (DiaSorin, Stillwater, MN). Normal reference range is 30-100 ng/mL and the intra- and inter-assay variability coefficient was <10%.

Plasma albumin-adjusted corrected Ca (corrected Ca) was calculated to account for falsely low serum calcium values due to hypoalbuminemia. We applied the following formula to all patients for which serum albumin was available in the record chart: corrected total calcium (mg/dL) = total serum calcium (mg/dL) + 0.8*[4 – serum albumin (g/L)] [5, 16].

Ethics

Consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used.

The Institutional Review Board of Azienda Ospedaliero-Universitaria of Modena approved the study (Protocol n. 0032443/15).

Statistical analysis

All data are shown as median and minimum-maximum. Comparisons among groups were performed by the nonparametric Mann-Whitney *U* and Kruskal-Wallis tests, followed by the Dunn's post hoc test, since variables were not normally distributed at Kolmogorov-Smirnov test.

The diagnostic accuracy of Ca/P was investigated using receiver operator characteristic (ROC) curve in order to define cut-off points that better identify affected patients according to their biochemical profile. ROC cut-offs were calculated by the Youden's index through the identification of the best pair of sensitivity and specificity.

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were evaluated.

Statistical analyses were performed using the Statistical Package for the Social Sciences' (SPSS) software for Windows (version 25.0; SPSS Inc, Chicago, IL). For all comparisons, $p<0.05$ was considered statistically significant.

Results

Age, sex and biochemical parameters of each group are summarized in Table 1.

Serum Ca was significantly higher in PHPT patients than either HypoP patients or controls ($p<0.0001$) (Table 1). As expected, even serum PTH was higher in PHPT group than others ($p<0.0001$) (Table 1).

Conversely, serum P was significantly lower in HypoP patients than PHPT patients and controls ($p<0.0001$) (Table 1).

The Ca/P ratio was significantly lower in controls compared to both PHPT ($p<0.0001$) and HypoP patients ($p<0.0001$), but it did not significantly differ among HypoP and PHPT groups ($p=0.075$) (Table 1).

Serum albumin, necessary for the calculation of corrected Ca, was available in 124 PHPT patients (79.3%), 299 HypoP patients (97.7%) and 119 controls (79.3%) (Table 1). Similarly, to

serum Ca, even serum corrected Ca was higher in PHPT patients than controls ($p<0.0001$) and HypoP patients ($p<0.0001$) (Table 1).

Vitamin D did not differ between controls and PHPT patients, but it was significantly higher in HypoP group ($p<0.0001$) (Table 1). In particular, 57 controls (38.3%), 69 PHPT patients (50.7%) and 70 HypoP patients (23.9%) had vitamin D deficiency with serum levels of 25-OH vitamin D below 20 ng/mL.

Considering renal function, serum creatinine did not differ comparing controls to PHPT group, but it was higher in HypoP group (<0.0001) (Table1). The GFR was between 30 and 60 mL/min in 8 controls (6.2%), 19 PHPT (12.8% and 15 HypoP (4.9%) patients; all other subjects had a GFR greater than 60 mL/min.

In addition, HIV-infected patients and controls were mostly male patients, while PHPT were predominantly female (Table 1). HIV-infected patients were significantly younger than PHPT patients and controls ($p<0.0001$) (Table 1).

Among PHPT patients, 38 (25.3%) were normocalcemic, while 112 (74.7%) had serum Ca above the normal range.

Table 2 shows the diagnostic values of conventional parameters used to define a condition of altered Ca-P metabolism, including either PHPT or HypoP. The Ca/P threshold of 3.56 (2.75 SI), obtained by ROC curve analysis, was identified as the best pair of sensitivity (93%) and specificity (92.7%) (Table 2, Figure 1a). In particular, by using the Ca/P ratio with a cut-off of 3.56, 424 out of the 456 HypoP and PHPT patients were correctly identified as patients with Ca-P metabolism disorder, while only 11 out of the 150 controls were falsely classified as having a disorder of Ca-P metabolism (Figure 2). When serum albumin was available, the use of serum corrected Ca/P ratio with a cut-off of 3.47 (2.68 SI) had a diagnostic power (sensitivity 93.4%, specificity 93.2%) similar to that of Ca/P (Table 2). Accordingly, PPV, NPV and accuracy of both Ca/P and corrected Ca/P were the best ones compared to the diagnostic values of PTH, serum Ca, corrected Ca and P

(Table 2). As expected, either serum Ca or PTH that were used as inclusion criteria for controls, act as confounders for the diagnostic performance (Table 2).

The combined use of Ca/P and serum Ca (which was considered positive when at least one of the two biochemical parameters was above the respective cut-offs) had the best sensitivity (95.2%) and specificity (92.7%) together with the best PPV, NPV and accuracy, all these parameters performing better than Ca/P alone to identify patients with impaired Ca-P metabolism (Table 2).

The statistical analysis performed by considering only normocalcemic patients from the PHPT cohort confirmed that 3.56 of Ca/P ratio was reliable to identify the cases (normocalcemic PHPT and HypoP) from controls. However, no valid cut-off was found to differentiate normocalcemic PHPT from HypoP patients.

Similarly, ROC curve analysis performed for patients with sufficient vitamin D and for patients with vitamin D insufficiency showed the same cut-off (Ca/P 3.56) with similar specificity and sensitivity. The same results were obtained comparing the ROC curves of patients with GFR 30-60 ml/min with those with GFR >60 ml/min.

Overall, by considering all patients with Ca-P metabolism disorder (PHPT and HypoP) with Ca/P above 3.56, the use of serum Ca and/or serum PTH alone had the best diagnostic value in discriminating PHPT from HypoP patients, as expected (Table 3, Figure 1b). In particular, the thresholds of 10.3 mg/dL (2.6 mmol/L) for serum Ca (sensitivity 92.6%; specificity 98.7%) and of 80.5 pg/mL for PTH (sensitivity 90.9%; specificity 90.9%) were defined for the specific diagnosis of PHPT (Table 3, Figure 1b). Similarly, for those patients whose albumin was measured, corrected Ca had very high diagnostic power for the threshold of 9.85 mg/dL (sensitivity 100%, specificity 99.7%) (Table 3).

On the other hand, it was not possible to identify any useful cut-off for serum P in order to discriminate PHPT from HypoP (Table 3).

Discussion

This study shows that serum Ca/P ratio has a great diagnostic power in identifying patients with PHPT and patients with HypoP not PHPT-related. In particular, a Ca/P value above 3.56 (with Ca and P measured in mg/dL) results to be an accurate, highly sensitive and highly specific tool to define a condition of impaired Ca-P metabolism due to PHPT or HypoP not PHPT-related. More importantly, both sensitivity and accuracy further increases if serum Ca and Ca/P are used in combination (Table 2).

This is in line with our previous results, since the accuracy of Ca/P to diagnose or rule out a condition of PHPT remained unaltered after adding patients with HypoP [3]. In addition to PHPT group, the inclusion of HypoP patients allowed us to investigate the accuracy of Ca/P in the differential diagnosis of other Ca-P metabolism disorders, such as HypoP not PHPT-related. Unfortunately, we did not find any valid cut-off of Ca/P ratio useful to discriminate patients with HypoP not PHPT-related from those with PHPT, either normocalcemic or hypercalcemic. Thus, up to now, we can conclude that this index is not useful to differentiate PHPT from HypoP among disorders of Ca-P metabolism.

At the same time, our results show that the use of serum Ca alone with a cut-off of 10.3 mg/dL allows identifying patients with PHPT among all patients with Ca/P ratio above 3.56. In this perspective, we recommend measuring serum Ca and P as first step in the screening of Ca-P metabolism disorders (Figure 3). Serum Ca allows to define a condition of PHPT or HypoP due to other causes, once the calculated Ca/P ratio is found above 3.56 (Figure 3). Accordingly, PHPT or HypoP not related to PHPT should be highly considered when serum Ca is above or below 10.3 mg/dL, respectively (Figure 3). As a consequence and in contrast to the current guidelines on the management of PHPT [4], not only Ca and PTH, but also P, are useful for identifying patients with PHPT. For this reason, we recommend including serum P in the clinical work-up for the diagnosis of PHPT. On the other hand, this study further demonstrates that serum P alone is unreliable in the diagnosis of PHPT, in accordance with all previous studies that evaluated this parameter alone and never in combination with serum Ca [17-20]. In the literature, there is no agreement about the use

of serum P as first-step examination in the diagnostic work-up of bone mineral diseases. Current clinical guidelines do not actually recommend to measure serum P for the diagnosis of PHPT [4] and for the management of nephrolithiasis [21-23]. On the contrary, serum P is included among first tier investigations for urolithiasis by a recent consensus statement [24] and for the management of osteoporosis by several Societies [25-30]. Again, we recommend measuring serum P together with serum total Ca allowing to calculate Ca/P as screening of Ca-P metabolism disorders, especially in the above-mentioned clinical settings, while serum PTH is not strictly needed as first-line biochemical investigation to rule out PHPT (Figure 3).

In the clinical real-life, the measurement of serum Ca and serum P may be sufficient as first investigation for the diagnosis of PHPT that is one of the most frequent disorders of Ca-P metabolism [6, 7]. In addition to their worldwide availability and extreme simplicity, the use of serum Ca and P may avoid further outgoings and clinical complications due to a delayed diagnosis of PHPT, occurring in a relevant percentage (about 40%) of cases [4, 31-34].

The retrospective design represents the main limit of this study, but it assures a correct assignment to control or case (either PHPT or HypoP) groups. Another limit is represented by the lack of age- and gender-matching between patients and controls. Furthermore, vitamin D insufficiency and slight impairment of GFR seem to not influence the accuracy of Ca/P index.

In conclusion, this study further validates the serum Ca/P ratio above 3.56 (2.75 SI) as a highly accurate tool to identify patients with PHPT and HypoP not PHPT-related. Thanks to its extraordinary simplicity, together with the favourable cost-effectiveness, this index can be proposed as a screening and first-line examination in the diagnostic work-up when a disorder of Ca-P metabolism is suspected or should be ruled out, such as in patients with osteoporosis or nephrolithiasis. Moreover, both sensitivity and accuracy further increases when Ca/P ratio is taken in combination with serum total Ca. The use of a simple index, such as Ca/P, might be particularly useful especially in laboratory/medical settings relying on limited resource, in which second- or

third-generation assays for PTH are not available. Furthermore, the timely diagnosis of PHPT is useful for preventing the development of PHPT-related comorbidities.

Finally, prospective and multi-centre studies on larger sample size will be needed to confirm and validate our findings for PHPT and HypoP patients. Moreover, future studies may be performed to extent the application of Ca/P to diagnose other disorders of Ca-P metabolism, such as hypoparathyroidism, pseudohypoparathyroidism and secondary hyperparathyroidism

Declaration of interest

The authors declare that they have no conflict of interest.

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Authors contributions

B. Madeo is the Principal Investigator and conceived the study, analysed the data, provided data interpretation and wrote the manuscript. S. De Vincentis collected and analyzed the data, and wrote the manuscript. E. Kara collected clinical and biochemical data. F. Vescini contributed to conceive the study. T. Trenti provided data about laboratory assays. G. Guaraldi provided clinical and biochemical data about HypoP patients. V. Rochira conceived the study, analysed the data, provided data interpretation and wrote the manuscript. All authors approved the final version of the manuscript and fully contributed to its final version.

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Legends

Figure 1a. ROC curve analysis for Ca/P together with cut-off, sensitivity, specificity, area under the curve, standard error, and 95% confidence interval values. **Figure 1b.** ROC curve analysis for Ca and PTH among PHPT and HypoP patients with Ca/P above 3.56 (2.75 SI), together with cut-off, sensitivity, specificity, area under the curve, standard error, and 95% confidence interval values.

[footnote to Figure 1]: *Ca/P: serum calcium/phosphorus ratio (Ca and P measured in mg/dL); SI: International System of Units (Ca and P are measured in mmol/L); AUC: Area Under the Curve; SE: Standard Error; CI: Confidence Interval. Ca: serum calcium (black line in Figure 1b); PTH: serum parathyroid hormone (grey line in Figure 1b); PHPT: primary hyperparathyroidism; HypoP: hyposphoremia.*

Figure 2. Distribution of serum Ca/P ratio and serum Ca of all patients according to the thresholds of 3.56 for Ca/P ratio, to discriminate controls from PHPT and HypoP, and of 10.3 mg/dL for serum Ca, to discriminate PHPT from HypoP in patients with Ca/P > 3.56.

[footnote to Figure 2]: *Ca/P: serum calcium to phosphorus ratio; Ca: serum calcium (Ca and P measured in mg/dL); controls (marker: black diamonds); PHPT: primary hyperparathyroidism (marker: light grey circles); HypoP: hypophosphatemia (marker: dark grey triangles).*

Figure 3. Usefulness of Ca/P in the diagnostic flow-chart for the screening and diagnosis of Ca-P metabolism disorders.

[footnote to Figure 3]: *Ca: serum calcium; Ca/P: serum calcium/phosphorus ratio (Ca and P measured in mg/dL); PHPT: primary hyperparathyroidism; HypoP: hypophosphatemia; SI: International System of Units (Ca and P are measured in mmol/L)*

Table 1. Age, biochemical and hormonal differences between patients with PHPT, HypoP and controls. Measurements are expressed as median (minimum-maximum).

	Normal range	**PHPT n=150**	**HypoP n=306**	**Controls n=150**	**p-value**
Sex					
Females	-	113 (75.3%)	54 (17.6%)	64 (42.7%)	-
Males	-	37 (24.7%)	252 (82.4%)	86 (57.3%)	-
Age (yrs)	-	63.5 (23.7-90.0)*°	48.5 (27.4-77.0)^	57.1 (19.8-89.0)	<0.0001
Serum Ca (mg/dL)	8.5-10.5	10.9 (9.3-18.0)*°	9.4 (8.5-10.5)	9.4 (8.7-10.5)	<0.0001
Serum P (mg/dL)	2.5-5.1	2.5 (1.5-3.9)*°	2.3 (1.5-2.5)^	3.6 (2.5-4.9)	<0.0001
Ca/P	-	4.36 (2.38-12.00)*	4.07 (3.46-6.20)^	2.69 (2.02-4.08)	<0.0001
Serum PTH (pg/mL)	15-88	130.6 (49.9-1748.0)*°	42.4 (6.2-308.2)[#]	34.0 (14.0-88.0)	<0.0001
Serum 25-OH vitamin D (ng/mL)	30-100	19.7 (3.0-59.2)°	29.1 (4.0-100.0)^	22.7 (4.0-74.8)	<0.0001
Serum creatinine (mg/dL)	0.50-1.20	0.80 (0.50-2.58)°	0.91 (0.53-2.35)^	0.80 (0.55-1.70)	<0.0001
Serum albumin (g/dL)**	3.5-5.0	4.1 (2.6-5.0)[§°]	4.6 (3.4-5.6)^	4.3 (2.7-4.9)	<0.0001
Serum corrected Ca (mg/dL)**	-	10.9 (9.3-18.2)*°	8.8 (7.9-9.9)^	9.2 (8.6-10.7)	<0.0001
Corrected Ca/P Ratio**	-	4.57 (2.38-12.16)*°	3.84 (3.33-8.33)^	2.63 (1.99-3.98)	<0.0001

[footnote to Table 1]: *PHPT: primary hyperparathyroidism, HypoP: hypophosphatemia, Ca: serum calcium; P: serum phosphorus; Ca/P: serum calcium to phosphorus ratio; **available in 124 PHPT patients (79.3%), 299 HypoP patients (97.7%) and 119 controls (79.3%). * PHPT vs Controls: p<0.0001; ° PHPT vs HypoP: p<0.0001; ^ HypoP vs Controls: p<0.0001; [#] HypoP vs Controls: p=0.001; [§] PHPT vs Controls: p=0.002*

Table 2. Diagnostic value of several parameters for the identification of disorders of Ca-P metabolism (PHPT and HypoP) in the entire cohort. Values are expressed as percentage.

	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy
Ca/P	3.56 (2.75 SI)	93.0	92.7	97.5	81.3	92.9
Corrected Ca/P Ratio	3.47 (2.68 SI)	93.4	93.2	98.0	79.7	93.3
Serum PTH (pg/mL)	88.0	32.7	100	100	33.6	49.8
Serum Ca (mg/dL)	10.5 (2.63 SI)	25.5	100	100	30.4	43.2
Serum corrected Ca (mg/dL)	10.5 (2.63 SI)	20.3	99.2	98.9	25.9	37.6
Serum P (mg/dL)	2.5 (0.81 SI)	82.2	100	100	64.9	86.6
Combined Ca/P and/or serum Ca*	3.56/10.5	95.2	92.7	97.5	86.3	94.6

[footnote to Table 2]: *PHPT: primary hyperparathyroidism; HypoP: hypophosphatemia; Ca: serum calcium; P: serum phosphorus; Ca/P: serum calcium to phosphorus ratio (Ca and P measured in mg/dL); PTH: serum parathyroid hormone (measured in pg/mL); SI: International System of Units (Ca and P are measured in mmol/L); Sensitivity: number of true positives divided by the number of true positives plus the number of false negatives; Specificity: number of true negatives divided by the number of true negatives plus the number of false positives; PPV: Positive predictive value: number of true positives divided by the number of true positives plus the number of false positives; NPV: negative predictive value: number of true negatives divided by the number of true negatives plus the number of false negatives. Accuracy: number of true positives plus the number of true negatives divided by the number of true positives plus the number of true negatives plus the number of false positives plus the number of false negatives. *Combined Ca/P and/or serum Ca was considered positive when at least one of the two biochemical parameters was above the cut-off.*

Table 3. Diagnostic value of several parameters for the diagnosis of PHPT among patients with Ca/P above 3.56. Values are expressed as percentage.

	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy
Serum PTH (pg/mL)	80.5	90.9	90.9	80.9	96.0	90.9
Serum Ca (mg/dL)	10.3 (2.6 SI)	92.6	98.7	96.6	97.1	96.7
Serum corrected Ca (mg/dL)	9.6 (2.4 SI)	100	99.7	99.0	100	99.8
Serum P (mg/dL)	2.5 (0.8 SI)	57.0	0	18.5	0	16.3

[footnote to Table 3]: *PHPT: primary hyperparathyroidism; HypoP: hypophosphatemia; PTH: serum parathyroid hormone (measured in pg/mL); Ca: serum calcium; P: serum phosphorus (Ca and P measured in mg/dL); SI: International System of Units (Ca and P are measured in mmol/L); Sensitivity: number of true positives divided by the number of true positives plus the number of false negatives; Specificity: number of true negatives divided by the number of true negatives plus the number of false positives; PPV: Positive predictive value: number of true positives divided by the number of true positives plus the number of false positives; NPV: negative predictive value: number of true negatives divided by the number of true negatives plus the number of false negatives. Accuracy: number of true positives plus the number of true negatives divided by the number of true positives plus the number of true negatives plus the number of false positives plus the number of false negatives.*

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RELIABILITY OF CALCIUM TO PHOSPHORUS (Ca/P) RATIO AS A NEW, ACCURATE AND INEXPENSIVE TOOL IN THE DIAGNOSIS OF SOME Ca-P DISORDERS

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Abstract

Purpose: The serum calcium/phosphorus (Ca/P) ratio is an accurate tool to differentiate patients with primary hyperparathyroidism (PHPT) from healthy subjects. However, other disorders of the Ca-P metabolism might impair the Ca/P ratio, such as hypophosphatemia (HypoP) not PHPT-related. The aim of this study is to ~~validate~~ examine the diagnostic value~~the accuracy~~ of Ca/P ratio in the diagnosis of ~~Ca-P metabolism disorders, particularly~~ PHPT and HypoP not PHPT-related.

Methods: Single-center, retrospective, case-control study, including 150 patients with PHPT and 306 patients with HypoP, compared with 150 controls. HypoP patients were enrolled among HIV-infected patients by selecting those with Fanconi-like syndrome due to antiretroviral treatment. Parameters which were measured were serum Ca, P, parathyroid hormone (PTH), 25-OH vitamin D, albumin and creatinine)~~Main outcomes: serum Ca, P, parathyroid hormone (PTH), 25-OH vitamin D, albumin and creatinine.~~

Results: The Ca/P ratio was significantly higher in PHPT and HypoP patients, compared to controls ($p<0.0001$). At receiver operator characteristic (ROC) curve analysis, the cut-off of 3.56 (2.75 SI) for Ca/P ratio was able to identify patients with PHPT and HypoP (sensitivity 95%; specificity 93%). Among patients with Ca/P ratio above 3.56, the thresholds of 10.3 mg/dL (2.6 mmol/L) for serum Ca (sensitivity 93%; specificity 98%) and 80.5 pg/mL for PTH (sensitivity 91%; specificity 91%) were defined for the specific diagnosis of PHPT.

Conclusions: The Ca/P ratio above 3.56 (2.75 SI) is a highly accurate tool to identify PHPT and HypoP not PHPT-related patients. Thanks to its simplicity, this index can be proposed as a screening and first-line examination in the diagnostic work-up when a disorder of Ca-P metabolism is suspected or should be ruled out.

Key words: Calcium-Phosphorus metabolism, hyperparathyroidism, hypophosphatemia, screening

Introduction

The calcium to phosphorus (Ca/P) ratio has been recently proposed for the first time as a simple and accurate tool to discriminate patients with primary hyperparathyroidism (PHPT) from healthy subjects. The reliability of this index is based on the fact that Ca and P homeostasis are closely regulated and their serum concentration is inversely correlated [1-3]. The diagnosis of PHPT is traditionally based on increased serum Ca and intact parathyroid hormone (PTH) levels [4, 5], in association with slight or mild hypophosphatemia (HypoP) [5, 6]. However, the biochemical profile of PHPT is not always so evident, making the diagnosis of PHPT challenging. For this reason, the serum Ca/P ratio could help in the diagnostic workup of PHPT, especially in asymptomatic or normocalcemic patients [3].

Nevertheless, although PHPT is the most common cause of hypercalcemia [6, 7], other disorders of the Ca-P metabolism might impair the Ca/P ratio. For example, a condition of HypoP not due to PHPT should be considered.

HypoP is a rare disease [8] that may occur by several mechanisms [9], including internal redistribution (eg, malnutrition, sepsis) or increased urinary excretion (eg, X-linked hypophoshatemic rickets, Fanconi syndrome) [10, 11]. A phosphate wasting disorder is also frequently observed in patients with Human Immunodeficiency Virus (HIV)-infection with ongoing combination antiretroviral therapy (cART) [12, 13]. In particular, among antiretroviral drugs, the use of tenofovir has been correlated with mild to severe ~~reduced serum~~ hypophosphat~~a~~emia, through a renal tubular dysfunction and chronic increased phosphaturia, resulting in a Fanconi-like syndrome [12-14].

In our previous study, the lack of a group of patients with HypoP limited the validation of serum Ca/P ratio for the differential diagnosis between PHPT and other causes of altered Ca/P ratio [3]. For example, tenofovir-related hypophosphatemia represents a mono-factorial hypophosphatemia, making HIV-infected patients with HypoP a uniform cohort to compare with PHPT patients.

The aim of the study is to evaluate the accuracy and diagnostic value of Ca/P in the diagnosis of PHPT and HypoP related to Fanconi-like syndrome and to investigate if this index can be used for differential diagnosis.~~The aim of this study is to validate the accuracy and diagnostic value of Ca/P ratio in the diagnosis of Ca and P metabolism disorders such asPHPT and HypoP.~~

Subjects and Methods

Study Design

A single-centre, retrospective, case-control study was carried out.

The group of cases with ascertained diseases of Ca-P metabolism was composed by two subgroups: patients with a certain diagnosis of PHPT and HIV-infected patients with HypoP. Controls consisted of subjects without any disorder of Ca-P metabolism. This trial was registered in ClinicalTrials.gov (Identifier: NCT03603444).

Subjects

A total of 150 patients followed at the Unit of Endocrinology of Modena with a diagnosis of PHPT from 2005 to January 2018 were included in the first case group (Table 1). For the other group of cases, 306 subjects with a certain diagnosis of drug-induced HypoP with reduced serum P according to the lower limit of the normal range of our laboratory (2.5 mg/dL), but normal serum Ca, were enrolled among HIV-infected patients on cART treatment from the large Modena cohort that belongs to Modena HIV Metabolic Clinic (MHMC), from 2004 to 2017. In this tertiary care teaching Hospital in Northern Italy, all individuals are screened by a multidisciplinary team for immune-metabolic disorders, antiretroviral-associated toxicities, HIV-associated non-infectious endocrine co-morbidities, geriatric syndromes and disabilities [15]. In particular, Ca, P, PTH and vitamin D are routinely checked at baseline in order to exclude Ca metabolism disorders related to osteoporosis or drug toxicity. By considering the low prevalence of hypophosphatemia in the

general population and the availability of a easily-accessible large database, we chose to investigate the tenofovir-related hypophosphatemia since it allowed us to select quickly a large sample of patients with hypophosphatemia and to have a homogeneous mono-factorial disease, such as tenofovir-related hypophosphatemia, to compare with PHPT. Tenofovir-related hypophosphatemia, however, should be considered highly representative of all other types of hypophosphatemia at least for the aim of this study since it leads to the same changes in serum such as other types of hypophosphatemia.

As a control group, we retrospectively selected 150 patients who attended the Unit of Endocrinology of Modena in the same time period, presenting serum PTH, Ca and P within the normal range. In particular, 43 (28.7%) were healthy subjects, 30 (20.0%) were male hypogonadal patients with documented normal serum testosterone during testosterone replacement therapy, 27 (18.0%) had a pituitary disease (non-functioning pituitary adenoma or empty sella with normal pituitary function), 17 (11.3%) had a non-functioning adrenocortical adenoma, 33 (22.0%) had a thyroid disorder (history of thyroidectomy, chronic thyroiditis or nodular goiter).

The following parameters were needed for the inclusion in each group: age, gender, serum Ca, P, PTH, 25-OH vitamin D and creatinine (Table 1).

Exclusion criteria for both cases and controls were: age younger than 18 or older than 90 years; severe renal and liver diseases (i.e. glomerular filtration rate (GFR) <30 mL/min); hyperparathyroidism secondary to Vitamin D deficiency; history of parathyroidectomy, even if the parathyroid disorder was considered in remission; active metabolic bone disease (e.g. Paget's disease of the bone, osteomalacia, rickets, etc); any type of cancer; malnutrition; severe obesity (BMI > 40 kg/m2); a history of gastrointestinal malabsorption; sarcoidosis; hypercortisolism, diabetes insipidus, hyperthyroidism, hypoparathyroidism and pseudohypoparathyroidism; familial hypocalciuric hypercalcemia (FHH); treatment with steroids, calcium and/or active forms of vitamin D (calcitriol, ergocalciferol, etc), thiazides, phosphate binders, lithium, cinacalcet, bisphosphonates, and denosumab.

The characteristics of patients and controls are summarized in Table 1.

Laboratory analyses

Serum Ca and P were detected using Beckman Coulter AU 680 (Beckman Coulter Italy, Cassina de' Pecchi, Milan, Italy) device by the colorimetric photometric methods based on Arsenazo III and molybdate, respectively. For both sexes, the normal reference ranges for serum Ca and P were 8.5-10.5 mg/dL and 2.5-5.1 mg/dL, respectively.

Serum Intact PTH was determined by two house-different chemiluminescence assays: a DiaSorin Liaison (DiaSorin S.p.A., Saluggia, Vercelli, Italy) from January 2005 to June 2010 and a Beckman Coulter UniCel DxI 600 Synchron Access (Beckman Coulter Italy, Cassina de' Pecchi, Milan, Italy) from June 2010 to January 2018. The normal reference range for serum PTH was 15-88 ng/L, with an intra-assay variability coefficient of 5%.

Serum 25OH-Vitamin D was measured by chemiluminescence with the LIASON® XL 1,25OH-Vitamin D assay (DiaSorin, Stillwater, MN). Normal reference range is 30-100 ng/mL and the intra- and inter-assay variability coefficient was <10%.

Plasma albumin-adjusted corrected Ca (corrected Ca) was calculated to account for falsely low serum calcium values due to hypoalbuminemia. We applied the following formula to all patients for which serum albumin was available in the record chart: corrected total calcium (mg/dL) = total serum calcium (mg/dL) + 0.8*[4 – serum albumin (g/L)] [5, 16].

Ethics

Consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used.

The Institutional Review Board of Azienda Ospedaliero-Universitaria of Modena approved the study (Protocol n. 0032443/15).

Statistical analysis

All data are shown as median and minimum-maximum. Comparisons among groups were performed by the nonparametric Mann-Whitney *U* and Kruskal-Wallis tests, followed by the Dunn's post hoc test, since variables were not normally distributed at Kolmogorov-Smirnov test.

The diagnostic accuracy of Ca/P was investigated using receiver operator characteristic (ROC) curve in order to define cut-off points that better identify affected patients according to their biochemical profile. ROC cut-offs were calculated by the Youden's index through the identification of the best pair of sensitivity and specificity.

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were evaluated.

Statistical analyses were performed using the Statistical Package for the Social Sciences' (SPSS) software for Windows (version 25.0; SPSS Inc, Chicago, IL). For all comparisons, $p<0.05$ was considered statistically significant.

Results

Age, sex and biochemical parameters of each group are summarized in Table 1.

Serum Ca was significantly higher in PHPT patients than either HypoP patients or controls ($p<0.0001$) (Table 1). As expected, even serum PTH was higher in PHPT group than others ($p<0.0001$) (Table 1).

Conversely, serum P was significantly lower in HypoP patients than PHPT patients and controls ($p<0.0001$) (Table 1).

The Ca/P ratio was significantly lower in controls compared to both PHPT ($p<0.0001$) and HypoP patients ($p<0.0001$), but it did not significantly differ among HypoP and PHPT groups ($p=0.075$) (Table 1).

Serum albumin, necessary for the calculation of corrected Ca, was available in 124 PHPT patients (79.3%), 299 HypoP patients (97.7%) and 119 controls (79.3%) (Table 1). Similarly, to

serum Ca, even serum corrected Ca was higher in PHPT patients than controls (p<0.0001) and HypoP patients (p<0.0001) (Table 1).

~~Serum creatinine and v~~Vitamin D did not differ between controls and PHPT patients, but ~~they were~~it was significantly higher in HypoP group (p<0.0001) (Table 1). In particular, 57 controls (38.3%), 69 PHPT patients (50.7%) and 70 HypoP patients (23.9%) had vitamin D deficiency with serum levels of 25-OH vitamin D below 20 ng/mL.

Considering renal function, serum creatinine did not differ comparing controls to PHPT group, but it was higher in HypoP group (<0.0001) (Table1). The GFR was between 30 and 60 mL/min in 8 controls (6.2%), 19 PHPT (12.8% and 15 HypoP (4.9%) patients; all other subjects had a GFR greater than 60 mL/min.

In addition, HIV-infected patients and controls were mostly male patients, while PHPT were predominantly female (Table 1). HIV-infected patients were significantly younger than PHPT patients and controls (p<0.0001) (Table 1).

Among PHPT patients, 38 (25.3%) were normocalcemic, while 112 (74.7%) had serum Ca above the normal range.

Table 2 shows the diagnostic values of conventional parameters used to define a condition of altered Ca-P metabolism, including either PHPT or HypoP. The Ca/P threshold of 3.56 (2.75 SI), obtained by ROC curve analysis, was identified as the best pair of sensitivity (93%) and specificity (92.7%) (Table 2, Figure 1a). In particular, by using the Ca/P ratio with a cut-off of 3.56, 424 out of the 456 HypoP and PHPT patients were correctly identified as patients with Ca-P metabolism disorder, while only 11 out of the 150 controls were falsely classified as having a disorder of Ca-P metabolism (Figure 2). When serum albumin was available, the use of serum corrected Ca/P ratio with a cut-off of 3.47 (2.68 SI) had a diagnostic power (sensitivity 93.4%, specificity 93.2%) similar to that of Ca/P (Table 2). Accordingly, PPV, NPV and accuracy of both Ca/P and corrected Ca/P were the best ones compared to the diagnostic values of PTH, serum Ca, corrected Ca and P

(Table 2). As expected, either serum Ca or PTH that were used as inclusion criteria for controls, act as confounders for the diagnostic performance (Table 2).

The combined use of Ca/P and serum Ca (which was considered positive when at least one of the two biochemical parameters was above the respective cut-offs) had the best sensitivity (95.2%) and specificity (92.7%) together with the best PPV, NPV and accuracy, all these parameters performing better than Ca/P alone to identify patients with impaired Ca-P metabolism (Table 2).

The statistical analysis performed by considering only normocalcemic patients from the PHPT cohort confirmed that 3.56 of Ca/P ratio was reliable to identify the cases (normocalcemic PHPT and HypoP) from controls. However, no valid cut-off was found to differentiate normocalcemic PHPT from HypoP patients.

Similarly, ROC curve analysis performed for patients with sufficient vitamin D and for patients with vitamin D insufficiency showed the same cut-off (Ca/P 3.56) with similar specificity and sensitivity. The same results were obtained comparing the ROC curves of patients with GFR 30-60 ml/min with those with GFR >60 ml/min.

Overall, by considering all patients with Ca-P metabolism disorder (PHPT and HypoP) with Ca/P above 3.56, the use of serum Ca and/or serum PTH alone had the best diagnostic value in discriminating PHPT from HypoP patients, as expected (Table 3, Figure 1b). In particular, the thresholds of 10.3 mg/dL (2.6 mmol/L) for serum Ca (sensitivity 92.6%; specificity 98.7%) and of 80.5 pg/mL for PTH (sensitivity 90.9%; specificity 90.9%) were defined for the specific diagnosis of PHPT (Table 3, Figure 1b). Similarly, for those patients whose albumin was measured, corrected Ca had very high diagnostic power for the threshold of 9.85 mg/dL (sensitivity 100%, specificity 99.7%) (Table 3).

On the other hand, it was not possible to identify any useful cut-off for serum P in order to discriminate PHPT from HypoP (Table 3).

Discussion

This study shows that serum Ca/P ratio has a great diagnostic power in identifying patients with PHPT and patients with HypoP not PHPT-related. In particular, a Ca/P value above 3.56 (with Ca and P measured in mg/dL) results to be an accurate, highly sensitive and highly specific tool to define a condition of impaired Ca-P metabolism due to PHPT or HypoP not PHPT-related. More importantly, both sensitivity and accuracy further increases if serum Ca and Ca/P are used in combination (Table 2).

This is in line with our previous results, since the accuracy of Ca/P to diagnose or rule out a condition of PHPT remained unaltered after adding patients with HypoP [3]. In addition to PHPT group, the inclusion of HypoP patients allowed us to investigate the accuracy of Ca/P in the differential diagnosis of other Ca-P metabolism disorders, such as HypoP not PHPT-related. Unfortunately, we did not find any valid cut-off of Ca/P ratio useful to discriminate patients with ~~PHPT from those with~~ HypoP not PHPT-related from those with PHPT, either normocalcemic or hypercalcemic. Thus, up to now, we can conclude that this index is not useful to differentiate PHPT from HypoP among disorders of Ca-P metabolism.

At the same time, our results show that the use of serum Ca alone with a cut-off of 10.3 mg/dL allows identifying patients with PHPT among all patients with Ca/P ratio above 3.56. In this perspective, we recommend measuring serum Ca and P as first step in the screening of Ca-P metabolism disorders (Figure 3). Serum Ca allows to define a condition of PHPT or HypoP due to other causes, once the calculated Ca/P ratio is found above 3.56 (Figure 3). Accordingly, PHPT or HypoP not related to PHPT should be highly considered when serum Ca is above or below 10.3 mg/dL, respectively (Figure 3). As a consequence and in contrast to the current guidelines on the management of PHPT [4], not only Ca and P~~H~~THH, but also P, are useful for identifying patients with PHPT. For this reason, we recommend including serum P in the clinical work-up for the diagnosis of PHPT. On the other hand, this study further demonstrates that serum P alone is unreliable in the diagnosis of PHPT, in accordance with all previous studies that evaluated this parameter alone and never in combination with serum Ca [17-20]. In the literature, there is no

agreement about the use of serum P as first-step examination in the diagnostic work-up of bone mineral diseases. Current clinical guidelines do not actually recommend to measure serum P for the diagnosis of PHPT [4] and for the management of nephrolithiasis [21-23]. On the contrary, serum P is included among first tier investigations for urolithiasis by a recent consensus statement [24] and for the management of osteoporosis by several Societies [25-30]. Again, we recommend measuring serum P together with serum total Ca allowing to calculate Ca/P as screening of Ca-P metabolism disorders, especially in the above-mentioned clinical settings, while serum PTH is not strictly needed as first-line biochemical investigation to rule out PHPT (Figure 3).

In the clinical real-life, the measurement of serum Ca and serum P may be sufficient as first investigation for the diagnosis of PHPT that is one of the most frequent disorders of Ca-P metabolism [6, 7]. In addition to their worldwide availability and extreme simplicity, the use of serum Ca and P may avoid further outgoings and clinical complications due to a delayed diagnosis of PHPT, occurring in a relevant percentage (about 40%) of cases [4, 31-34].

The retrospective design represents the main limit of this study, but it assures a correct assignment to control or case (either PHPT or HypoP) groups. Another limit is represented by the lack of age- and gender-matching between patients and controls. Furthermore, vitamin D insufficiency and slight impairment of GFR seem to not influence the accuracy of Ca/P index.

In conclusion, this study further validates the serum Ca/P ratio above 3.56 (2.75 SI) as a highly accurate tool to identify patients with PHPT and HypoP not PHPT-related. Thanks to its extraordinary simplicity, together with the favourable cost-effectiveness, this index can be proposed as a screening and first-line examination in the diagnostic work-up when a disorder of Ca-P metabolism is suspected or should be ruled out, such as in patients with osteoporosis or nephrolithiasis. Moreover, both sensitivity and accuracy further increases when Ca/P ratio is taken in combination with serum total Ca. The use of a simple index, such as Ca/P, might be particularly useful especially in laboratory/medical settings relying on limited resource, in which second- or

third-generation assays for PTH are not available. Furthermore, the timely diagnosis of PHPT is useful for preventing the development of PHPT-related comorbidities.

Finally, prospective and multi-centre studies on larger sample size will be needed to confirm and validate our findings for PHPT and HypoP patients. Moreover, future studies may be performed to extent the application of Ca/P to diagnose other disorders of Ca-P metabolism, such as hypoparathyroidism, pseudohypoparathyroidism and secondary hyperparathyroidism

Declaration of interest

The authors declare that they have no conflict of interest.

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Authors contributions

B. Madeo is the Principal Investigator and conceived the study, analysed the data, provided data interpretation and wrote the manuscript. S. De Vincentis collected and analyzed the data, and wrote the manuscript. E. Kara collected clinical and biochemical data. F. Vescini contributed to conceive the study. T. Trenti provided data about laboratory assays. G. Guaraldi provided clinical and biochemical data about HypoP patients. V. Rochira conceived the study, analysed the data, provided data interpretation and wrote the manuscript. All authors approved the final version of the manuscript and fully contributed to its final version.

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Legends

Figure 1a. ROC curve analysis for Ca/P together with cut-off, sensitivity, specificity, area under the curve, standard error, and 95% confidence interval values. **Figure 1b.** ROC curve analysis for Ca and PTH among PHPT and HypoP patients with Ca/P above 3.56 (2.75 SI), together with cut-off, sensitivity, specificity, area under the curve, standard error, and 95% confidence interval values.

[footnote to Figure 1]: *Ca/P: serum calcium/phosphor~~o~~us ratio (Ca and P measured in mg/dL); SI: International System of Units (Ca and P are measured in mmol/L); AUC: Area Under the Curve; SE: Standard Error; CI: Confidence Interval. Ca: serum calcium (black line in Figure 1b); PTH: serum parathyroid hormone (grey line in Figure 1b); PHPT: primary hyperparathyroidism; HypoP: hyposphoremia.*

Figure 2. Distribution of serum Ca/P ratio and serum Ca of all patients according to the thresholds of 3.56 for Ca/P ratio, to discriminate controls from PHPT and HypoP, and of 10.3 mg/dL for serum Ca, to discriminate PHPT from HypoP in patients with Ca/P > 3.56.

[footnote to Figure 2]: *Ca/P: serum calcium to phosphor~~o~~us ratio; Ca: serum calcium (Ca and P measured in mg/dL); controls (marker: black diamonds); PHPT: primary hyperparathyroidism (marker: light grey circles); HypoP: hypophosph~~o~~at~~r~~emia (marker: dark grey triangles).*

Figure 3. Usefulness of Ca/P in the diagnostic flow-chart for the screening and diagnosis of Ca-P metabolism disorders.

[footnote to Figure 3]: *Ca: serum calcium; Ca/P: serum calcium/phosphor~~o~~us ratio (Ca and P measured in mg/dL); PHPT: primary hyperparathyroidism; HypoP: hypophosphat~~or~~emia; SI: International System of Units (Ca and P are measured in mmol/L)*

Table 1. Age, biochemical and hormonal differences between patients with PHPT, HypoP and controls. Measurements are expressed as median (minimum-maximum).

	Normal range	PHPT n=150	HypoP n=306	Controls n=150	p-value
Sex					
Females	-	113 (75.3%)	54 (17.6%)	64 (42.7%)	-
Males	-	37 (24.7%)	252 (82.4%)	86 (57.3%)	-
Age (yrs)	-	63.5 (23.7-90.0)*°	48.5 (27.4-77.0)^	57.1 (19.8-89.0)	<0.0001
Serum Ca (mg/dL)	8.5-10.5	10.9 (9.3-18.0)*°	9.4 (8.5-10.5)	9.4 (8.7-10.5)	<0.0001
Serum P (mg/dL)	2.5-5.1	2.5 (1.5-3.9)*°	2.3 (1.5-2.5)^	3.6 (2.5-4.9)	<0.0001
Ca/P	-	4.36 (2.38-12.00)*	4.07 (3.46-6.20)^	2.69 (2.02-4.08)	<0.0001
Serum PTH (pg/mL)	15-88	130.6 (49.9-1748.0)*°	42.4 (6.2-308.2)[#]	34.0 (14.0-88.0)	<0.0001
Serum 25-OH vitamin D (ng/mL)	30-100	19.7 (3.0-59.2)°	29.1 (4.0-100.0)^	22.7 (4.0-74.8)	<0.0001
Serum creatinine (mg/dL)	0.50-1.20	0.80 (0.50-2.58)°	0.91 (0.53-2.35)^	0.80 (0.55-1.70)	<0.0001
Serum albumin (g/dL)**	3.5-5.0	4.1 (2.6-5.0)[§]°	4.6 (3.4-5.6)^	4.3 (2.7-4.9)	<0.0001
Serum corrected Ca (mg/dL)**	-	10.9 (9.3-18.2)*°	8.8 (7.9-9.9)^	9.2 (8.6-10.7)	<0.0001
Corrected Ca/P Ratio**	-	4.57 (2.38-12.16)*°	3.84 (3.33-8.33)^	2.63 (1.99-3.98)	<0.0001

[footnote to Table 1]: *PHPT: primary hyperparathyroidism, HypoP: hypophosphatemia, Ca: serum calcium; P: serum phosphor~~o~~us; Ca/P: serum calcium to phosphor~~o~~us ratio; **available in 124 PHPT patients (79.3%), 299 HypoP patients (97.7%) and 119 controls (79.3%). * PHPT vs Controls: p<0.0001; ° PHPT vs HypoP: p<0.0001; ^ HypoP vs Controls: p<0.0001; [#] HypoP vs Controls: p=0.001; [§] PHPT vs Controls: p=0.002*

Table 2. Diagnostic value of several parameters for the identification of disorders of Ca-P metabolism (PHPT and HypoP) in the entire cohort. Values are expressed as percentage.

	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy
Ca/P	3.56 (2.75 SI)	93.0	92.7	97.5	81.3	92.9
Corrected Ca/P Ratio	3.47 (2.68 SI)	93.4	93.2	98.0	79.7	93.3
Serum PTH (pg/mL)	88.0	32.7	100	100	33.6	49.8
Serum Ca (mg/dL)	10.5 (2.63 SI)	25.5	100	100	30.4	43.2
Serum corrected Ca (mg/dL)	10.5 (2.63 SI)	20.3	99.2	98.9	25.9	37.6
Serum P (mg/dL)	2.5 (0.81 SI)	82.2	100	100	64.9	86.6
Combined Ca/P and/or serum Ca*	3.56/10.5	95.2	92.7	97.5	86.3	94.6

[footnote to Table 2]: *PHPT: primary hyperparathyroidism; HypoP: hypophosphatemia; Ca: serum calcium; P: serum phosphor~~o~~us; Ca/P: serum calcium to phosphor~~o~~us ratio (Ca and P measured in mg/dL); PTH: serum parathyroid hormone (measured in pg/mL); SI: International System of Units (Ca and P are measured in mmol/L); Sensitivity: number of true positives divided by the number of true positives plus the number of false negatives; Specificity: number of true negatives divided by the number of true negatives plus the number of false positives; PPV: Positive predictive value: number of true positives divided by the number of true positives plus the number of false positives; NPV: negative predictive value: number of true negatives divided by the number of true negatives plus the number of false negatives. Accuracy: number of true positives plus the number of true negatives divided by the number of true positives plus the number of true negatives plus the number of false positives plus the number of false negatives. *Combined Ca/P and/or serum Ca was considered positive when at least one of the two biochemical parameters was above the cut-off.*

Table 3. Diagnostic value of several parameters for the diagnosis of PHPT among patients with Ca/P above 3.56. Values are expressed as percentage.

	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy
Serum PTH (pg/mL)	80.5	90.9	90.9	80.9	96.0	90.9
Serum Ca (mg/dL)	10.3 (2.6 SI)	92.6	98.7	96.6	97.1	96.7
Serum corrected Ca (mg/dL)	9.6 (2.4 SI)	100	99.7	99.0	100	99.8
Serum P (mg/dL)	2.5 (0.8 SI)	57.0	0	18.5	0	16.3

[footnote to Table 3]: *PHPT: primary hyperparathyroidism; HypoP: hypophosphatemia; PTH: serum parathyroid hormone (measured in pg/mL); Ca: serum calcium; P: serum phosphor~~o~~us (Ca and P measured in mg/dL); SI: International System of Units (Ca and P are measured in mmol/L); Sensitivity: number of true positives divided by the number of true positives plus the number of false negatives; Specificity: number of true negatives divided by the number of true negatives plus the number of false positives; PPV: Positive predictive value: number of true positives divided by the number of true positives plus the number of false positives; NPV: negative predictive value: number of true negatives divided by the number of true negatives plus the number of false negatives. Accuracy: number of true positives plus the number of true negatives divided by the number of true positives plus the number of true negatives plus the number of false positives plus the number of false negatives.*

Figure 1a. ROC curve analysis for Ca/P together with cut-off, sensitivity, specificity, area under the curve, standard error, and 95% confidence interval values. Figure 1b.

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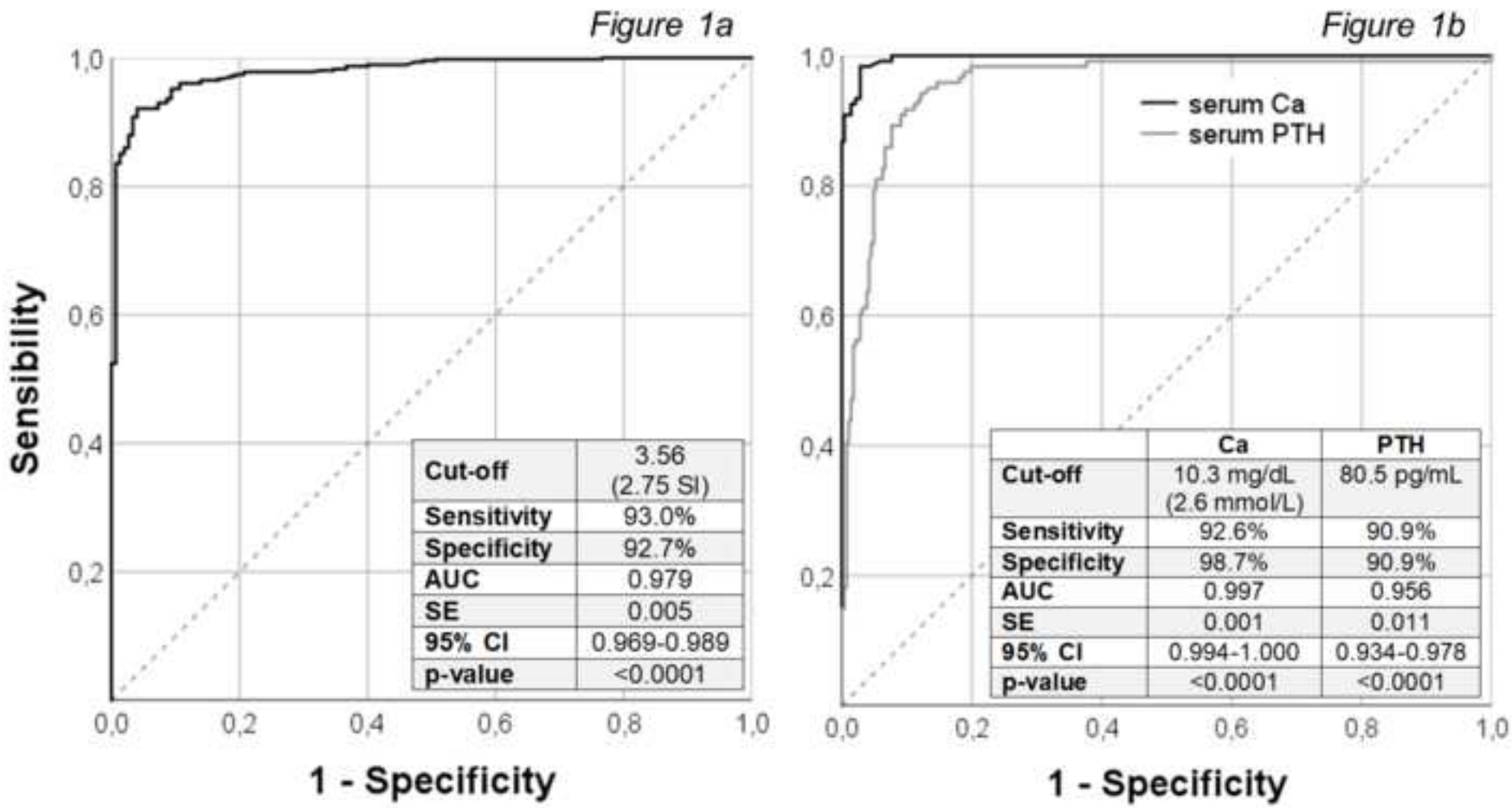

Cut-off	3.56 (2.75 SI)
Sensitivity	93.0%
Specificity	92.7%
AUC	0.979
SE	0.005
95% CI	0.969-0.989
p-value	<0.0001

	Ca	PTH
Cut-off	10.3 mg/dL (2.6 mmol/L)	80.5 pg/mL
Sensitivity	92.6%	90.9%
Specificity	98.7%	90.9%
AUC	0.997	0.956
SE	0.001	0.011
95% CI	0.994-1.000	0.934-0.978
p-value	<0.0001	<0.0001

Figure 2. Distribution of serum Ca/P ratio and serum Ca of all patients according to the thresholds of 3.56 for Ca/P ratio, to discriminate controls from PHPT and HypoP, and

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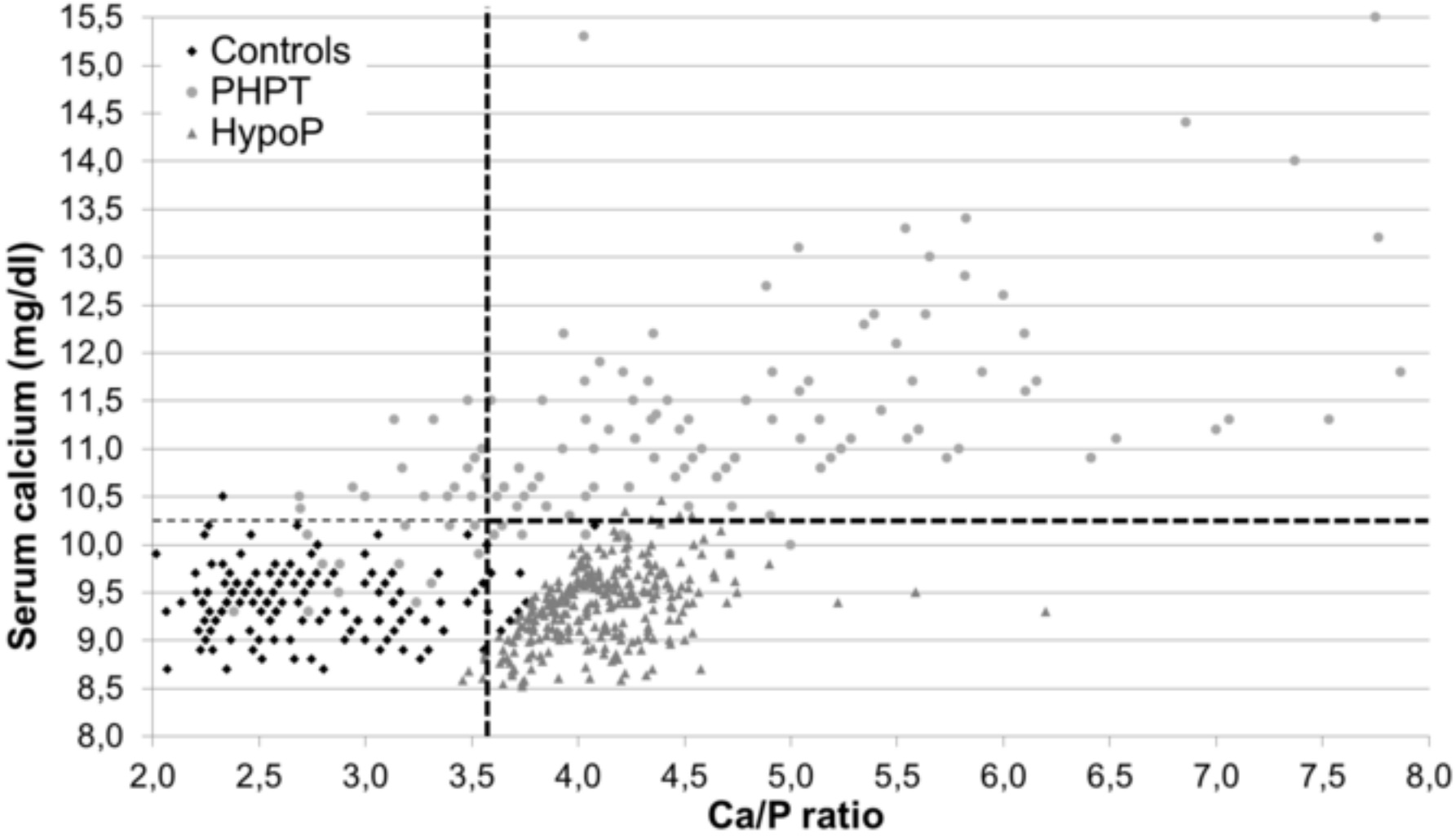
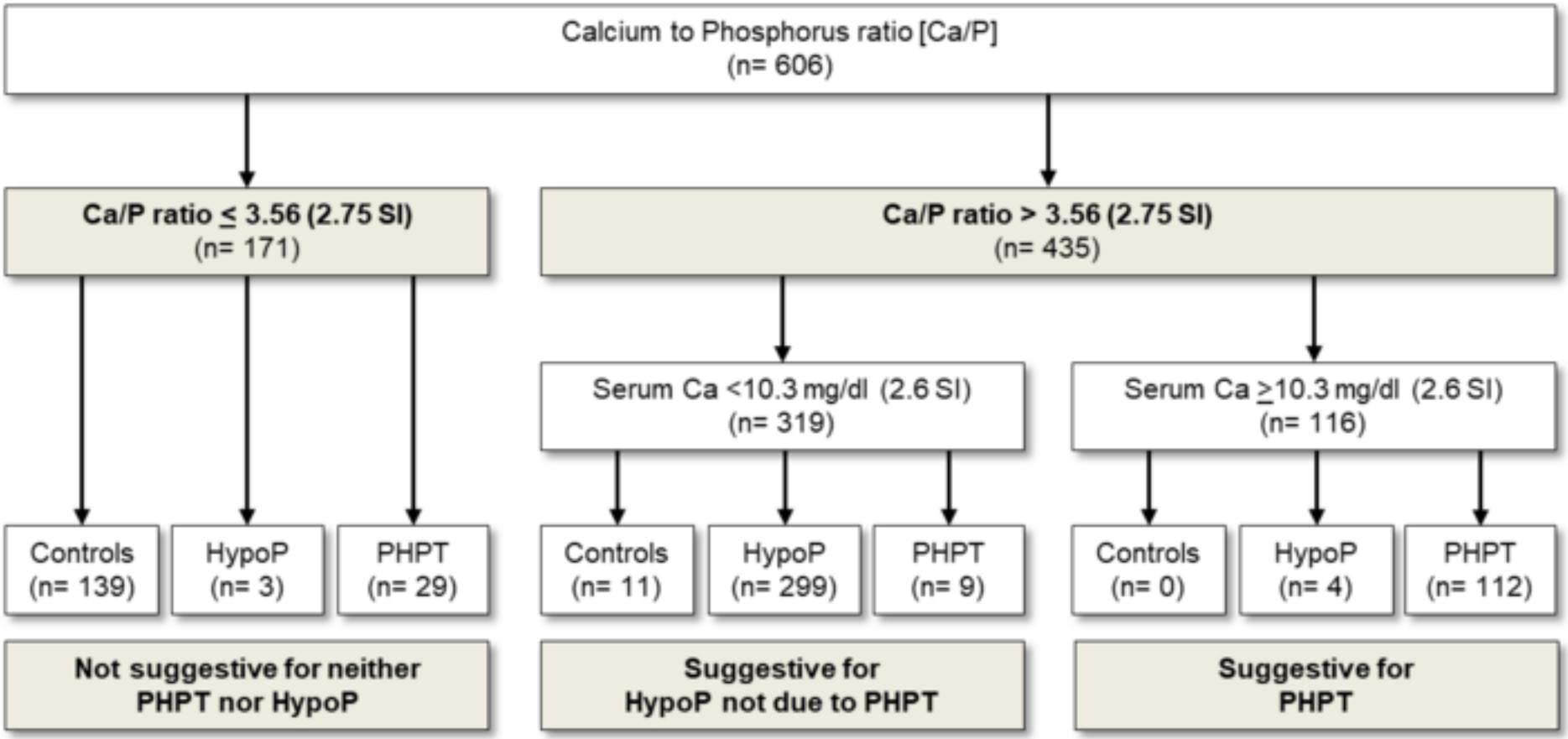


Figure 3. Usefulness of Ca/P in the diagnostic flow-chart for the screening and diagnosis of Ca-P metabolism disorders.

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