Urolithiasis
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ORIGINAL PAPER

How useful is an oral calcium load test for diagnosing recurrent calcium stone formers?

Isabelle N. Tostivint[1,2] · Vincent Castiglione[3] · Rana Alkouri[4] · Jean Philippe Bertocchio[1,5] · Rachida Inaoui[6] · Michel Daudon[7] · Marie-Paule Dousseaux[8] · Etienne Cavalier[3] · Laurence Pieroni[4,7] · Hassan Izzedine[9]

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Abstract

Hypercalciuria is the main risk factor for recurrent calcium urolithiasis. The goal of our study is to determinate how useful an oral calcium load test is for stone formers to classify different forms of hypercalciuria in pathogenetic categories defined as renal or absorptive according to the current knowledge. Between June 2013 and February 2016, a prospective study was carried out on 117 documented recurrent hypercalciuric stone formers undergoing an oral calcium load test modified from the original description by Pak. After 2 days of calcium-restricted diet, urine and blood were analyzed at baseline and 120 min after receiving orally 1 g of calcium. Total and ionized calcium, parathyroid hormone from serum and urine calcium and creatinine were assessed in order to divide patients in three groups as previously described: resorptive, absorptive, and renal hypercalciuria. This allowed the identification of 19, 39, 34 and 33 patients with normocalcemic primary hyperparathyroidism (NPHPT), renal hypercalciuria aka renal calcium leak (RCL), absorptive hypercalciuria (AH) and unidentified cause, respectively. Patients with NPHPT (who required parathyroidectomy) experienced a lower PTH decrease (41.41 ± 12.82 vs. $54.06 \pm 13.84\%$ $p < 0.01$), higher beta-crosslaps, as well as lower TmP/GFR and distal third radius bone mineral density. RCL resulted in increased fasting urine calcium-to-creatinine ratio (Uca/Cr), i.e., > 0.37 mmol/mmol), without hyperparathyroidism. AH was diagnosed by the presence of ΔUCa/Cr > 0.60 mmol/mmol between baseline and 120 min without any other anomaly. For all remaining patients, results were inconclusive due to the lack of sufficient increase in serum calcium or because the cause of lithogenesis could not be clearly identified. The oral calcium load test is useful in nearly 80% of patients by identifying the different forms of hypercalciuria causing urolithiasis and by guiding treatment, including parathyroid surgery.

Keywords Kidney stone · Hypercalciuria · Oral calcium load · Pak test · Primary hyperparathyroidism · Normocalcemic hyperparathyroidism · Calcium hyperabsorption · Renal calcium leak

Abbreviations

UL	Urolithiasis
SF	Stone-formers
CKD	Chronic kidney disease
BMD	Bone mineral density
PTH	Parathyroid hormone
HPT	Hyperparathyroidism
PHPT	Primary hyperparathyroidism
NPHPT	Normocalcemic primary hyperparathyroidism
RCL	Renal calcium leak
AH	Absorptive hypercalciuria
eGFR	Estimated glomerular filtration rate
TmP/GFR	Tubular maximal reabsorption of phosphate
U Ca/Cr	Urine calcium-to-creatinine ratio
Δ Ca/Cr	Difference between 120 min and baseline U Ca/Cr measurements
CTX	C-terminal telopeptide
25(OH)D3	25-Hydroxyvitamin D3
1.25(OH)2D3	1,25-Dihydroxyvitamin D3

Introduction

Urolithiasis (UL) is a very common and recurrent disease all around the world, and calcium is the most frequent component, present in 80% of all calculi. Recurrence can thus

✉ Isabelle N. Tostivint
isabelle.tostivint@aphp.fr

Extended author information available on the last page of the article

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reach 60% for brushite and dihydrate calcium oxalate stones, known as calcium-dependent urolithiasis [1–3]. Hypercalciuria has been described as the main risk factor involved in these stone formation. The generally accepted definition of hypercalciuria in stone-formers is 24 h urine calcium superior to 250 and 300 mg/day in women and men, respectively and/or urine calcium superior to 0.1 mmol/kg/day. However, not much literature has been published about these values [4, 5]. Metabolic evaluation of stone-formers (SF) is based on 24 h urinalysis and blood analyses in order to investigate acquired and inherited associated conditions. Nevertheless, 24 h urine analysis is dependent of many conditions (diet, collection omission) and the metabolic evaluation can be time-consuming, poorly informative or even contradictory, whereas the diagnosis of the hypercalciuria is essential to define an adequate treatment [6].

The traditional way of looking at hypercalciuria includes resorptive as in hyperparathyroidism, renal calcium leak which is an inherent kidney problem and absorptive which has increased intestinal calcium absorption. From a theoretical point of view, the distinction between renal and intestinal hypercalciuria may be correct. However, some authors consider hypercalciuria as one single disorder, in which high intestinal absorption and excretion may sustain an accelerated calcium turnover in the body. In 1975, Pak et al. published an original biological test to determine the mechanism of hypercalciuria, consisting in urine measurements before and after a calibrated oral intake of calcium [7]. This dynamic test allows classifying hypercalciuria among three main causes, namely "absorptive, resorptive, and renal hypercalciuria", the aim being to set up an adapted treatment for each of them [8]. Indeed, calcium intake have to be increased in primary renal calcium leak (RCL) because of the bone consequences and sometimes requires thiazides to correct secondary osteoporosis, or to be diminished by daily dose in the absorptive hypercalciuria (AH), respectively, whereas primary hyperparathyroidism (PHPT) requires surgical cure.

Thus, despite Pak's classification of hypercalciuria remains controversial and still debated, in our experience, Pak's test allowed us to distinguish different patterns in patients and to adapt their treatment accordingly.

Data on cut-off values are also uncommon and divergent, due to the heterogeneity of the populations studied which include patients with different types of hyperparathyroidism (HPT), patients with UL or osteopenic populations [8–11]. So, many teams have abandoned this precious tool. Oral calcium load test currently appears to have lost popularity among nephrologists and urologists as well as among endocrinologists, and its usefulness is questioned. In addition, a small number of papers regarding oral calcium load test in SF have been published in the last years [12, 13]. Here, we describe the interest of performing a Pak's test in determining the mechanism of hypercalciuria in calcium SF, changing views on appropriate therapeutic goals and patient management.

Patients and methods

Study participants

A total of 117 patients were recruited in this prospective study between June 2013 and February 2016. Patients were enrolled in our reference tertiary center for nephrolithiasis during consultations at the Nephrology Department of Pitié Salpêtrière Hospital (Paris). The study was exempted of institutional review board because the test is part of the conventional protocol for SF management. The authors are in accordance with the Helsinki Declaration. Eligibility criteria were a documented recurrent calcium urinary stone with hypercalciuria and normocalcemia. Hypercalciuria was defined as a daily urinary excretion of more than 4 mg calcium/kg body weight. Patients with evident causes of hypercalciuria (such as hyperthyroidism, sarcoidosis, hypogonadism, Paget's disease, or retinoids intake) were excluded. It was then checked by the dietitian that the patients had no dietary abuse of calcium, salt or proteins, as their excessive intake is related to higher calciuria levels. Thus it was recommended that salt and protein intake was lower than 5 g and 1.0 g/kg per day, respectively [14]. Recommended daily calcium intake was 800 to 1000 mg. Medical history of patients, medical imaging of stone disease detailed outcome as well as stone composition were recorded. As osteoporosis is commonly associated to UL, lumbar, hip and wrist bone mineral density (BMD) was measured within 6 month in case of bone demineralization risk factor [15, 16].

Calcium load test

Patients were supplemented with cholecalciferol up to the recommended level of 30 ng/mL [17, 18] within 4 months prior the calcium load test, confirmed by a blood test in our hospital. Subjects were asked to follow a calcium restricted diet (200 mg daily) during the two days before the test and to collect 24 h urine, during a dedicated nurse consultation. On the calcium load day, after a fasting period of 12 h, they underwent a first blood and urine analysis, considered as baseline, then samples were repeated 120 min after a sachet of 1000 mg of calcium carbonate dissolved in yogurt (i.e. effectively 1150 mg) was taken orally [12]. An interdisciplinary team including nephrologists, urologist, clinical chemist, rheumatologist, endocrinologist, and dietician reviewed patient's test results to establish a diagnosis and a global therapeutic management.

Analytical assessment

Urine and blood calcium and phosphate were analyzed by colorimetric method using Modular P 800 (Roche, Mannheim, Germany). Urine and blood creatinine were measured by an enzymatic IDMS-traceable method on the same analyzer. Intact parathyroid hormone (PTH) and C-terminal telopeptide (CTX) were measured by Modular E170 from Roche. 25-hydroxyvitamin D3 (25(OH) D3) and bone-specific alkaline phosphatase (BSAP) were detected by electrochemiluminescence on Liaison XL from Diasorin (Saluggia, Italy). Osteocalcin has been assessed by radioimmunoassay with Cisbio kit (Codolet, France).

GFR has been estimated by the MDRD formula [19]. The ratio of tubular maximum reabsorption of phosphate to GFR (TmP/GFR) was calculated as described by Payne et al. [20]. Calciuria was evaluated by the calcium-to-creatinine ratio (UCa/Cr). The calcium intestinal absorption is represented by the difference between UCa/Cr at baseline and 120 min timepoints (ΔCa/Cr) [8].

In our cohort, the intra-individual variability was: 1.7%, 0.2%, 2% and 4% for ionized calcium, pH, PTH, and bicarbonate, respectively.

Patient's classification

Patients were classified in several groups according to the following criteria: Initially patients with inadequate PTH inhibition were diagnosed with PHPT. A decrease above 50% of the PTH level was used to define "PTH inhibition" when the ionized calcemia on the same sample at the same moment was increased above the normal range (> 1.3 mmol/L) and/or when the ionized calcemia had raised more than + 0.1 mmol/L. The reference range for the PTH assay used was 15.0 to 65.0 pg/mL (immunochemiluminescence Roche®Cobas).

Patients with fasting (UCa/Cr > 0.37 mmol/mmol) and 24 h (> 0.1 mmol/kg per day) urine hypercalciuria, under a low-calcium diet but with normal PTH inhibition (ie > 50%) after oral calcium charge, as well as induced hypercalcemia were included in RCL group. Patients with low 24 h calciuria (< 4 mmol/d) under a low-calcium diet, but with a ΔCa/Cr > 0.60 mmol/mmol, were classified in the AH group, in absence of other metabolic perturbations. When patients didn't fit in any of these groups, the test was considered as failed, and performed again later (6 months, even one or 2 years later).

Statistical analysis

Statistics were realized using Statistica version 12 (for Windows 8, StatSoft Inc. 2016). Distribution of data was tested by Shapiro–Wilk test. All data are reported as mean ± SD. Inter-group comparisons were performed using a Hotelling t-test for normally distributed continuous data, with Mann–Whitney U test for skewed continuous data, and with Pearson's chi-squared test for binary data. Comparisons of results between baseline and the second measurements were realized with paired t-test. All comparisons were considered significant when $p < 0.05$.

Results

The test was conclusive for 84 out of 117 patients divided into PHPT group (19 pts), RCL group (31 pts), and AH group (34 pts). In the remaining 33 cases, the test was not conclusive and required to be repeated in better conditions. Patients' characteristics and baseline parameters are presented in Table 1. Groups were comparable concerning eGFR, serum 25(OH) D3 and bicarbonate, as well as for urine volume and 24 h urine collection sodium, urea and phosphorus. In the RCL group a higher ($p < 0.05$) proportion of female patients was reported, whereas AH group patients were significantly younger than those of the others groups and indeed experienced their first nephritic colic earlier in life ($p < 0.01$). The RCL patients presented higher 24 h calciuria than the AH group (5.59 ± 1.82 vs 4.65 ± 1.42 mmol/d; $p < 0.05$), although not compared to PHPT group.

Stone analysis was performed in 43 patients. Stones were mainly composed of dihydrate calcium oxalate, carbapatite, monohydrate calcium oxalate by conversion of weddellite into whewellite, or brushite. There were no statistical differences among groups. There was no brushite in PHPT stone-formers, even if this constituent has been the more associated to PHPT [21]. On the other hand, crystalluria was positive at the beginning of the care with calcium dependent crystals.

Biochemical test results before and after calcium load tests are shown in Table 2. In the three groups, all measurements of serum total and ionized calcium, PTH, phosphorus and U Ca/Cr ratio at 120 min were significantly different compared to baseline ($p < 0.001$). All the PHPT patients ($n = 19$) were normocalcemic before the test, except for 3 patients who had not previously detected ionized calcium > 1.30 mmol/L. Moreover, in PHPT group, ionized calcium ($p < 0.001$) and PTH ($p < 0.0001$) were higher than in the other groups, at baseline and after calcium load. Although PHPT group had similar ionized calcium increase (ΔCa), their PTH decrease was lower than for the other groups, as shown by the calculated ΔPTH (difference between second and basal measurement), % PTH (percentage of decrease) and ΔPTH/ΔCa ($p < 0.05$). TmP/GFR was not decreased in the RCL group in comparison with the other 2 groups ($p < 0.001$). Likewise, baseline UCa/Cr (fasting calciuria) was significantly higher in RCL group

Table 1 Baseline parameters

Parameter	PHPT ($n=19$)	RCL ($n=31$)	AH ($n=34$)
Patients characteristics			
Male, *n* (%)	12 (63)	11 (35)[a]	20 (59)
Age (year)	51.7 (10.0)	50.4 (11.8)	40.2 (12.6)[b]
Age at first episode (year)	37.7 (12.3)	34.8 (13.5)	22.8 (6.5)[c]
BMI (kg/m^2)	27.1 (5.9)[a]	23.1 (2.8)	23.5 (3.8)
Serum			
Bicarbonates (mmol/l)	23.4 (1.7)	23.7 (2.1)	24.0 (2.3)
Creatinine (µmol/l)	82.6 (18.0)	71.2 (14.3)[a]	80.1 (17.1)
eGFR (MDRD) (ml/min per 1.73m^2)	80.4 (18.7)	87.9 (17.7)	86.5 (16.4)
25(OH)D3 (ng/ml)	39.7 (7.8)	37.7 (8.2)	39.5 (9.2)
24 h urine			
Volume (l/d)	1.95 (0.56)	1.95 (0.53)	1.79 (0.55)
Creatinine (mmol/d)	13.0 (4.37)	10.7 (3.22)[b] +	13.1 (3.50)[b] +
Calcium (mmol/d)	5.62 (2.03)	5.59 (1.82)[a] +	4.65 (1.42)[a] +
Phosphorus (mmol/d)	23.2 (6.81)	22.0 (6.75)	25.0 (7.21)
Sodium (mmol/d)	124 (54)	126 (65)	127 (53)
Urea (mmol/d)	323 (112)	302 (74)	320 (91)

Data are presented as mean (±SD) unless otherwise indicated

PHPT primary hyperparathyroidism, *RCL* renal calcium leak, *AH* absorptive hypercalciuria

[a] $p<0.05$

[b] $p<0.01$

[c] $p<0.001$; +, only significant between RCL and HA group

than in AH group (0.53 ± 0.21 vs. 0.28 ± 0.11 mmol/mmol; $p<0.05$) and not in PHPT group. This parameter was then similar among the three groups after calcium load. The difference between 0 and 120 min UCa/Cr (Δ Ca/Cr) was not significant among the three groups. We also performed measurements after 90 min, but they were less informative than 120 min analyses, excepted for ΔCa/Cr, that was significantly higher in AH patients at 90 min (0.12 ± 0.15 vs 0.00 ± 0.16 and 0.00 ± 0.15 mmol/mmol in AH, PHPT and AH groups, respectively; $p<0.01$).

BMD and bone markers measurements are presented in Table 3. The amount of patients suffering from osteoporosis or osteopenia did not differ between groups. No significant difference in Z-score and T-score of BMD could be shown neither, excepted for distal third (33%) radius T-score between PHPT and AH group (− 1.43 ± 0.91 vs. − 0.58 ± 1.12 $p<0.05$). Similarly, osteocalcin was higher in PHPT group than in AH patients (28.5 ± 9.7 vs. 22.8 ± 14.1 $p<0.05$). CTX was also higher in PHPT group compared to both the other groups ($p<0.05$).

Discussion

We report a cohort of well-documented recurrent calcium SF who underwent oral calcium load. A wide range of biological markers has been measured in order to identify differences between groups in the absence of established threshold values (Fig. 1).

Resorptive hypercalciuria

PHPT: the first goal of the test is the diagnosis of normocalcemic PHPT which is a frequent condition, particularly associated with nephrolithiasis and osteoporosis [22, 23].

We show how the Pak's test is efficient in the case of normocalcemic PHPT based on impaired PTH inhibition despite calcemia elevation [23, 25]. We also show that ionized calcium measurement is necessary to diagnose PHPT. Total serum calcium was normal before the test for all patients, and didn't overcome thresholds even after calcium load, excepted for two patients. In contrast, 66 patients (79%) had ionized hypercalcemia at 120 min. This is why we recommend measuring ionized calcium instead of total calcium [26].

Two previous studies showed the interest of PTH inhibition rate for selecting primary, secondary, and other forms of HPT [9, 27]. However, their cut-off values based on intravenous calcium load as opposed to oral calcium load in the Pak's test are inappropriate.

In the present study, there was an overlap in the PTH variations (% and ΔPTH) as well as in ΔCa among groups but not in ΔPTH/ΔCa; this is explained by the simple fact that some patients in RCL group have low calcemia increase,

Table 2 Oral calcium load test

Parameters	**PHPT** (n=19)			**RCL** (n=31)			**AH** (n=34)		
Serum analyses									
Calcium 0min (mmol/l)	2.32	(0.08)		2.28	(0.09)		2.30	(0.08)	
Calcium 120min (mmol/l)	2.46	(0.09)	*f*	2.42	(0.10)	*f*	2.44	(0.12)	*f*
Ionized calcium 0min (mmol/l)	1.28	(0.03)	*c*	1.24	(0.03)		1.24	(0.03)	
Ionized calcium 120min (mmol/l)	1.37	(0.05)	*b,f*	1.32	(0.04)	*f*	1.33	(0.04)	*f*
PTH 0min (pg/ml)	75.38	(22.66)	*d*	42.92	(14.51)		39.39	(13.00)	
PTH 120min (pg/ml)	43.71	(13.9)	*d,f*	20.05	(8.18)	*f*	16.69	(5.45)	*f*
Δ Ca	0.08	(0.04)		0.07	(0.05)		0.09	(0.04)	
% PTH	41.41	(12.82)	b	52.99	(11.67)		55.03	(15.7)	
Δ PTH	31.7	(14.5)	*a*	22.9	(10.5)		22.7	(11.7)	
ΔPTH/ΔCa	396	(252)	*a*	239	(235)		241	(208)	
Phosphate 0 min (mmol/l)	0.82	(0.14)		0.96	(0.11)	*b*	0.86	(0.17)	
Phosphate 120min (mmol/l)	0.78	(0.13)		0.89	(0.11)	*c,e*	0.76	(0.12)	*e*
TmPi/GFR (mmol/l)	0.64	(0.18)		0.80	(0.11)	*b*	0.66	(0.18)	
Urine analyses									
U Ca/Cr 0min (mmol/mmol)	0.45	(0.22)		0.53	(0.21)		0.28	(0.11)	*c*
U Ca/Cr 120min (mmol/mmol)	1.08	(0.67)	*f*	1.16	(0.33)	*f*	1.03	(0.34)	*f*
Δ Ca/Cr (mmol/mmol)	0.63	(0.52)		0.63	(0.35)		0.75	(0.34)	

Data are presented as mean (± SD) unless otherwise indicated

PHPT primary hyperparathyroidism, *RCL* renal calcium leak, *AH* absorptive hypercalciuria

Δ Ca, % PTH, Δ PTH, ΔPTH/ΔCa

U Ca/Cr, urinary calcium on creatinine, Δ Ca/Cr, difference between second and baseline measurement of U Ca/Cr

Highlighted in yellow: what is very different for HPT and therefore resorptive hypercalciuria = ionized calcemia significantly higher than the 2 other groups, PTH brakes less than the 2 other groups in absolute value and in %, ΔPTH/ΔCa ratio much higher than the 2 other groups = the most discriminating

Highlighted in green: what characterizes primary renal hypercalciuria compared to the 2 other groups: Phosphate is significantly higher from base and especially at 120 min, TmPi/GFR is normal, not lowered

Highlighting in pink: specificity of the profile of hypercalciuria by primary renal calcium leak: The fasting urinary Ca/creat ratio is significantly higher than the other 2 groups

[a] $p < 0.05$

[b] $p < 0.01$

[c] $p < 0.001$

[d] $p < 0.0001$ vs. each of the two other groups

[e] $p < 0.01$ vs. baseline

[f] $p < 0.0001$ vs. baseline

and some patients in AH groups have normal baseline PTH levels. The PTH cannot decrease much further, resulting in lower values of PTH (%) and ΔPTH/ΔCa, while there is no relation to an impaired PTH inhibition. Concerning PHPT diagnosis, there also were an increase in 24 h calciuria, fasting calciuria and ΔCa/Cr due to consecutive hyperabsorption of calcium in PHPT secondar to the induced hypercalcitrolemia. On the other side, TmP/GFR mean was lower and this can be explained by the PTH effect on phosphate renal excretion. Higher CTX levels and lower 33% radius BMD are associated with increased bone turnover. This observation is in line with the fact that radius could

Table 3 Bone mineral density and bone biomarkers

Parameter	**PHPT** (n=10)			**RCL** (n=23)		**AH** (n=18)		
Age (yrs)	**51.2(11.5)**			**50.2 (10.3)**		**40.5 (8.9)**		
Gender male/female	**7/3**			**5/18**		**10/8**		
Menopausal status	**2/3**			**17/18**		**3/8**		
No BMD loss, n (%)	5	(50%)		9	(39%)	8	(45%)	
Osteopenia, n (%)	4	(40%)		10	(44%)	6	(33%)	
Osteoporosis, n (%)	1	(10%)		4	(17%)	4	(22%)	
Lumbar spine, T-score	-0.87	(0.92)		-0.55	(1.53)	-0.30	(1.36)	
Lumbar spine, Z-score	-0.73	(0.98)		-0.23	(1.41)	-0.48	(1.23)	
Femoral neck, T-score	-0.68	(1.09)		-0.42	(1.01)	-0.77	(1.12)	
Femoral neck, Z-score	-0.13	(0.75)		0.12	(0.94)	-0.55	(0.97)	
Total femoral, T-score	-0.43	(1.20)		-0.22	(1.26)	-0.45	(1.03)	
Total femoral, Z-score	-0.07	(0.97)		0.23	(1.09)	-0.42	(0.94)	
Ultra-distal Radius, T-score	-0.10	(0.17)		-1.20	(1.72)	-1.09	(1.39)	
Ultra-distal Radius, Z-score	0.33	(0.40)		-0.67	(1.41)	-1.09	(1.36)	
Radius 33%, T-score	-1.43	(0.91)	b	-0.82	(1.58)	-0.58	(1.12)	b
Radius 33%, Z-score	-1.08	(0.89)		-0.41	(1.29)	-0.56	(1.23)	
CTX (ng/ml)	0.684	(0.259)	*a*	0.554	(0.204)	0.538	(0.171)	
OST (ng/ml)	28.5	(9.7)	*b*	23.8	(9.0)	22.8	(7.0)	*b*
TAP (U/L)	74.3	(38.6)		60.9	(14.8)	55.7	(14.1)	
BSAP (µg/ml)	14.0	(11.9)		10.1	(3.8)	9.9	(2.6)	

Data are presented as mean (± SD) unless otherwise indicated

PHPT primary hyperparathyroidism, *RCL* renal calcium leak, *AH* absorptive hypercalciuria, *BMD* bone mineral density, *CTX* β-C-terminal telopeptide; *OST* osteocalcin; *TAP* total alkaline phosphatase, *BSAP* bone-specific alkaline phosphatase

Osteopenia definition: T-score between −1 and −2.5

Osteoporosis definition: T-score < −2.5

Highlighted in yellow: characteristic of HPT compared to the 2 other groups: more resorption reflected by greater increase in serum CTX

[a] $p < 0.05$ vs both of the other groups

[b] $p < 0.05$ between PHTP and HA groups

be an accurate tool for early osteopenia diagnostic in PHPT patients and also, because the content in cortical and trabecular bone is more sensitive to PTH effect [28]. It is essential for diagnostic decision to detect PHPT patients, even when normocalcemic, UL being an indication for selective parathyroidectomy. The surgery is successful in reducing calciuria and nephrolithiasis recurrence rate, and also in improving BMD [29, 30].

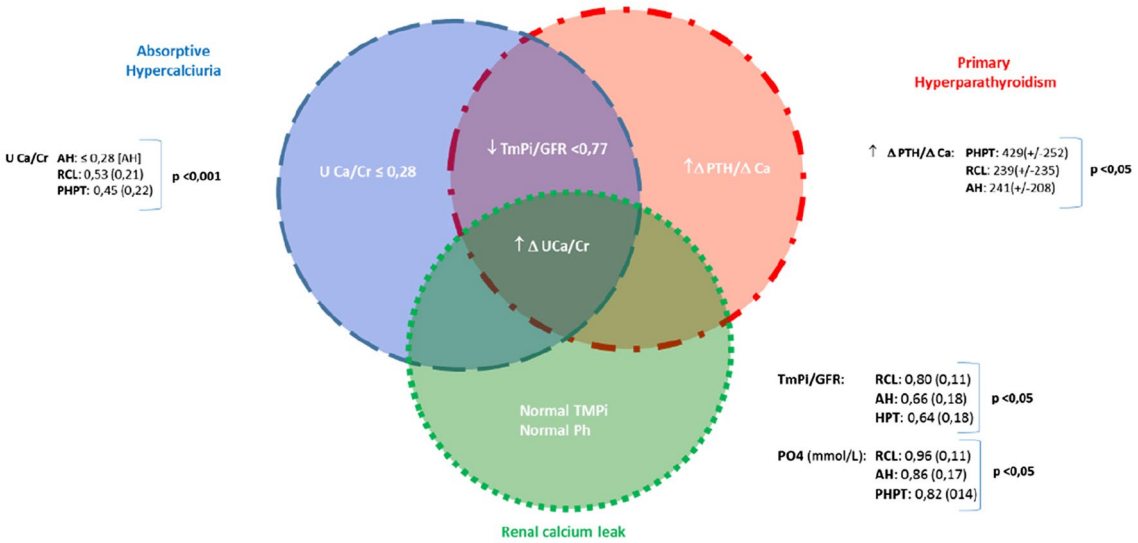

Fig. 1 Specificity of biochemical profiles repartition according to the mechanism of hypercalciuria. ΔUCa/Cr marker is increased during hypercalciuria, regardless of its mechanism, and is therefore non-discriminating. The lowering of TmPi less than 0.86 is not discriminating in our cohort: it is found in cases of primary hyperparathyroidism (PHPT) and absorptive hypercalciuria (AH) mainly linked to cases of renal phosphate leak. The significant increase in the ΔPTH/ΔCa level is specific for PHPT cohort because it is a marker of the pathologic relationship between iCa and PTH secretion. Fasting UCa/Cr ≤ 0.28 mmol/mmol only concerned cases of AH and no other hypercalciuria cases (PHPT or RCL). Serum phosphate (PO4) level is normal and the TmPi/GFR not lowered only in RCL cases

Renal calcium leak

Once PHPT has been excluded, the second step is to discriminate RCL from AH because the objectives of calcium intakes and bone management are different.

In renal calcium leak, hypercalciuria is present on fasting urine, even relatively high during calcium restricted diet. However 24 h hypercalciuria evidence was inconsistent. The 24 h urine volume, sodium and urea in RCL group were similar to other groups ($p > 0.05$) proving that higher 24 h calciuria was due to differences in diet. In this group, calcemia can be low due to renal calcium loss, leading to secondary HPT. However, higher PTH levels were correctly inhibited [31].

The higher number of women in our RCL group could explain the lower levels of 24 h UCr, and could be a bias since osteoporosis can cause hypercalciuria independently of a direct calcium leak. In any case, no differences for osteoporosis among all groups were observed.

Renal calcium leak is associated to monogenetic tubulopathies (Bartter Syndrome, claudin mutations, calcium-sensing receptor activating mutation) as well as Cacchi-Ricci and others. It requires an increased calcium intake, up to 1.0 or 1.2 g per day to compensate the leak and avoid bone demineralization. In addition, thiazides use can be discussed, as they increase diuresis, reduce calciuria, although decreasing kalemia and urine citrate [32]. Literature on required doses of diuretics is variable [33] and therapy by potassium and calcium citrate was shown to be useful in preventing calcium oxalate saturation and bone resorption. These results are due to calcium intake which decrease CTX level and to citrate which prevents urine calcium precipitation and increases pH [34].

Therefore, we recommend the evaluation of BMD because fasting UCa/Cr elevation has been related to increased bone resorption independently from PTH levels. Arrabal-Polo et al. have determined a direct correlation between 8 h fasting UCa/Cr and lumbar spine T-score densitometry, as well as CTX in calcium SF [35]. In the paper from Pak et al., fasting UCa/Cr was considered normal when lower than 0.37 mmol/mmol (0.11 mg/mg) (7). In the male stone-formers population described by Letavernier et al., UCa/Cr was higher in osteopenic patients, and UCa/Cr > 0,25 mmol/mmol was a reliable cut-off in discriminating patients with bone loss [10].

Absorptive hypercalciuria

In the absence of PHPT and fasting hypercalciuria, the third and last step is to identify absorptive hypercalciuria. It should be envisaged in the presence of ΔUCa/Cr > 0.60 mmol/mmol. The mean ΔU Ca/Cr was not significantly different in the AH group, as a hyperabsorption of calcium is also present in the two other groups. The 90 min urinary measurements were also performed after calcium load (data not shown). The ΔUCa/Cr at 90 min was significantly higher in the AH group as compared to

the others (0.12 ± 0.15 vs 0.00 ± 0.16 in PHPT group, and 0.00 ± 0.15 mmol/mmol in RCL group, $p < 0.01$).

In this study, intestinal absorption of calcium seemed to occur more rapidly in AH patients compared to the others. This hyperabsorption can be the result of calcitriol elevation due to excessive supplementation in vitamin D, sarcoidosis, phosphate renal leak or *CYP24A1* mutation [36–39]. Mutations of VDR have also been widely described [40]. Hence, evaluation of 1,25(OH)2D3 is useful in these cases.

All serum phosphate and TmP/GFR measurements are significantly higher in RCL group ($p < 0.01$). This rely on PTH provoking the decrease of phosphate reabsorption in PHPT group, while AH group include patients with phosphate renal waste ($n = 9$) in our cohort. These patients present decreased phosphate tubular reabsorption, defined by a TmP/GFR < 0.80 mmol/L [41]. Nevertheless, phosphate leak patients do not present increased 24 h phosphaturia as compared to other groups.

AH patients should be initially treated with moderate decreased daily calcium intake (around 800 mg/d), and more importantly, by dividing calcium intake during the day, avoiding intakes exceeding 300 mg at once. Vitamin D supplementation should also be adapted to vitamin D concentration in serum and its metabolites ideally every day and not monthly. Bone is also a concern in this population, as hypercalciuria can be reduced but persistent during calcium restricted diet, and BMD seems to be correlated to intestinal absorption of calcium [42–44].

Causes of test failure

In 33 patients out of 117, the test did not lead to clear diagnosis, due to different identifiable causes. First of all, avoidance of calcium, salt, and protein restriction diet can be suspected thanks to 24 h urine chemistry, and confirmed by patient's interrogation. Incorrect 24 h urine collection is suspected when diuresis volume is extremely low or high. Moreover, 24 h urine creatinine should not vary too much between two measurements. As already mentioned, chronic vitamin D deficiency promote secondary HPT and can also impair the intestinal calcium absorption required for test interpretation [45–47]. It appears that even long-term vitamin D supplementation (100.000 IU/week for minimum 4 month) was not enough to replete every patient ($n = 7$). In 14 cases, despite the vitamin D repletion, calcemia did not increase enough after the load test to exclude PHPT. These patients are candidate for an i.v. calcium load test. Lastly, in few cases, the Pak's test was not interpretable because of the lack of concordance between two measurements or for the absence of metabolic perturbation ($n = 12$).

In order to avoid these issues, multidisciplinary collaboration among clinicians, nurses, and laboratory, is the cornerstone to optimize the test efficiency. This teamwork is mandatory for determining the best pre-analytical conditions, for making an appropriate diagnosis and therefore for making the best therapeutic decision [26].

Our cohort include a high number of patients having normocalcemic hyperparathyroidism, not recorded in previous works, and a large number of patients having renal hypercalciuria which usually has low frequency at least in the works of C. Pak [7]. This is explained by the specificity of our reference tertiary UL center caring for very high recurrences UL patients coming from the all country.

We provide a modified calcium load test from the original version by Pak et al.

We provide 1150 mg calcium, primarily in the form of calcium carbonate vs original description (1000 mg calcium, primarily liquid calcium).

Samples are taken within the two hours following calcium intake (vs 4 h in the original "Pak" test). In the literature, up to 2 weeks of calcium restricted diet were recommended [46] or on the contrary others consider 8 h fasting during the night enough to interpret morning calciuria [35].

The 7 days period of calcium restricted diet is reduced to 2 days, which was considered sufficient to perform the calcium load test [10];

Calcium intake lower than 400 mg/d in order to interpret the 24 h urinalysis and to exclude dietary causes of hypercalciuria that frequently occurs.

The Pak test becomes more relevant when the evolution of the PTH assay is integrated into its interpretation. We can then, as a clinician, best specify the mechanism of hypercalcemia by integrating the resorptive mechanism of normocalcemic primary hyperparathyroidism and to guide therapy according to the 3 mechanisms.

We propose a diagnostic approach of hypercalciuria in a flowchart fashion (Fig. 2).

Conclusions

Calcium load test is a useful diagnostic tool in recurrent hypercalciuric SF. Even if it is complex to set up or, in some cases, difficult to interpret, Pak test allows to discriminate normocalcemic HPT, renal or absorptive hypercalciuria when patients are properly selected and prepared. It also brings information about patient's calcium metabolism and lithogenesis pathway, allowing to adapt treatment and to prevent recurrences. We believe this test should be used in daily practice in association with evaluation of BMD that is inherent in this population.

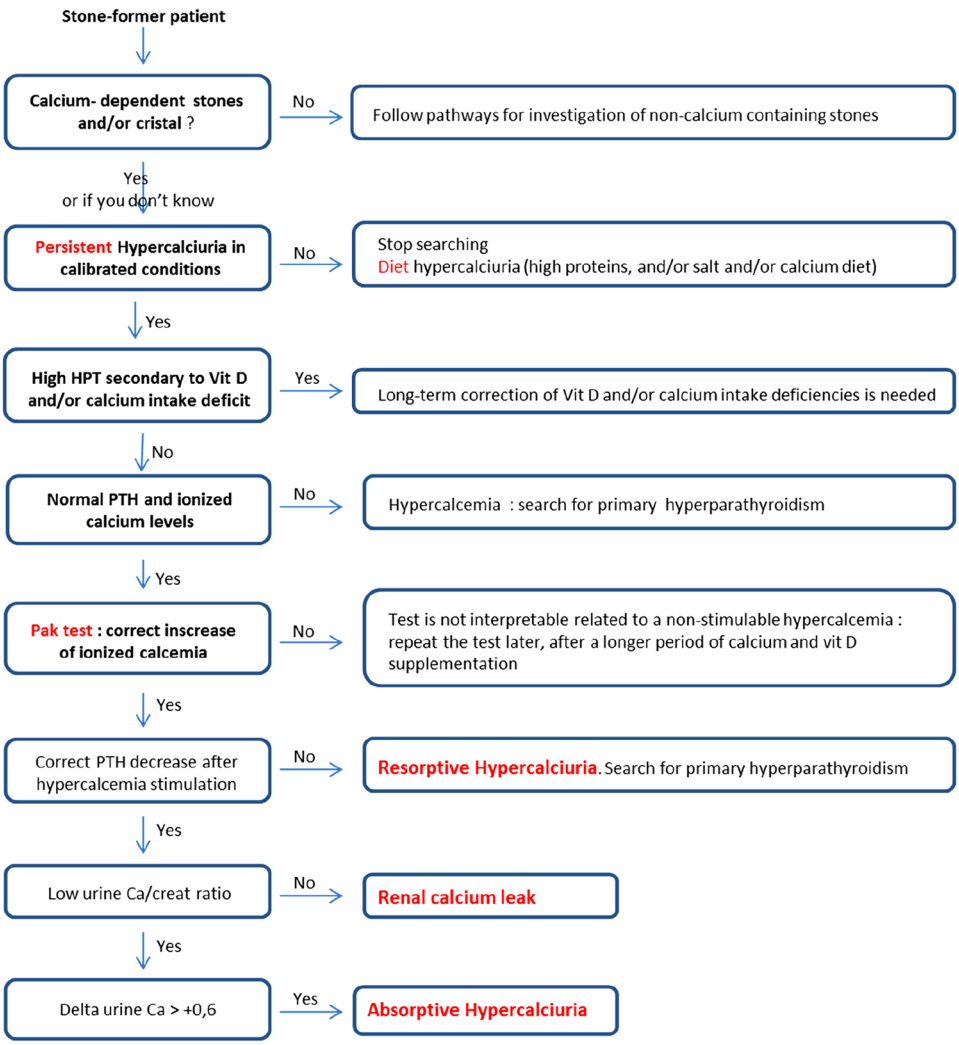

Fig. 2 When should the Pak test be performed in a stone-former patient?

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All studies conducted by the investigators involving human participants were in accordance with the ethical standards of the Institutional Review Boards.

Informed consent Informed consent was obtained from all participants in studies conducted by the investigators.

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Authors and Affiliations

Isabelle N. Tostivint[1,2] · Vincent Castiglione[3] · Rana Alkouri[4] · Jean Philippe Bertocchio[1,5] · Rachida Inaoui[6] · Michel Daudon[7] · Marie-Paule Dousseaux[8] · Etienne Cavalier[3] · Laurence Pieroni[4,7] · Hassan Izzedine[9]

Vincent Castiglione
v.castiglione@student.ulg.ac.be

Rana Alkouri
rana.alkouri@aphp.fr

Jean Philippe Bertocchio
jean-philippe.bertocchio@aphp.fr

Rachida Inaoui
rachidainaoui@wanadoo.fr

Michel Daudon
daudonmichel24@gmail.com

Marie-Paule Dousseaux
mariepaule.dousseaux@free.fr

Etienne Cavalier
etienne.cavalier@chu.ulg.ac.be

Laurence Pieroni
laurence.pieroni@aphp.fr

Hassan Izzedine
h.izzedine@ramsaygds.fr

1 Department of Nephrology, AP-HP, Pitie Salpetriere Hospital, 48 Boulevard de l'Hôpital, 75013 Paris, France

2 GRC 20 SORBONNE UNIVERSITY Clinical multidisciplinary Research Group on Kidney Stones, Sorbonne University Tenon Hospital, Paris, France

3 Department of Clinical Chemistry, University Hospital of Liege, Liege, Belgium

4 Department of Metabolic Biochemistry, AP-HP, Pitie Salpetriere Hospital, Paris, France

5 Rare Diseases Network OSCAR, Center for Excellence in Rare Calcium and Phosphate Disorders, Paris, France

6 Department of Rheumatology, AP-HP, Pitie Salpetriere Hospital, Paris, France

7 Department of Biochemistry, Cristal Laboratory, AP-HP, Tenon Hospital, Paris, France

8 Department of Nutrition and Dietetics, AP-HP, Pitie Salpetriere Hospital, Paris, France

9 Department of Nephrology, Peupliers Private Hospital, Paris, France