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SYMPOSIUM PAPER

Composition and morphology of phosphate stones and their relation with etiology

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Abstract Calcium phosphate (CaP) stones account for about 15% of all urinary stones, with a marked female preponderance, and reflect a wide diversity of etiology. Variation of the relative prevalence of CaP urolithiasis over time is disputed, and relevance of CaP stone analysis for etiologic diagnosis is underestimated or even negated. Based on the analysis of more than 50,000 stones over the past three decades, we evaluated the changes in the relative proportion of CaP stones between 1980–1989 (period 1) and 2000–2009 (period 2). In addition, using morphologic examination combined with Fourier-transform infrared analysis, we assessed the associations between CaP stone analysis and etiopathogenic factors. Between periods 1 and 2, the overall proportion of struvite-free stones remained essentially unchanged (11.6 vs. 11.1%), with a decreasing proportion of carbapatite stones (10.6 vs. 8.4%, $p < 0.001$) and a rising proportion of brushite stones (0.8 vs. 2.2%, $p < 0.001$). Hypercalciuria was associated with 87% of brushite, and 60% of carbapatite stones. Urinary tract infection was associated with presence of minor amounts of struvite and/or with a carbonation rate of carbapatite > 15%. In CaP stones associated with primary hyperparathyroidism, the main component was carbapatite in 66.9% and brushite in 29.1% of cases. Distal renal tubular acidosis was always associated with carbapatite stones exhibiting a peculiar, virtually pathognomonic, morphology. In conclusion, comprehensive analysis of stones involving morphologic examination is of clinical relevance for improved etiologic evaluation of patients with CaP urolithiasis.

Keywords Calcium phosphate · Brushite · Carbapatite · Nephrolithiasis · Urinary tract infection · Renal tubular acidosis · Primary hyperparathyroidism

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Introduction

Phosphate stones were highly prevalent among stone-formers in the past centuries and were often associated with urinary tract infection (UTI) and poor nutritional conditions both in adults and in children [1–4]. Following the end of World War II, the prevalence of calcium phosphate (CaP) stones, which are described as mainly composed of apatite, gradually declined in parallel with improved management of UTI, whereas the prevalence of calcium oxalate (CaOx) stones relentlessly rose in parallel with marked changes in dietary habits in industrialized countries [5–7].

However, some authors recently reported a tendency for an increasing proportion of CaP in urinary stones over the past decades in the US [8, 9], whereas a slight decrease in the prevalence of stones having CaP as main component was observed in Canada [10]. Of note, all recent studies mentioned a marked parallel increase in the proportion of CaP stones made of infrequent forms such as brushite [8, 9, 11], as well as whitlockite and octacalcium phosphate (OCP) [8]. In fact, the apparently conflicting conclusions of these epidemiologic studies reflect the different definitions

of CaP stones used, whether they referred to the percentage of CaP in stones, to the percentage of stones containing any proportion of CaP, or to the percentage of stones having CaP as main component. In addition, these studies excluded from analysis all CaP stones containing any amount, even minor, of struvite (magnesium–ammonium phosphate, indicative of a past or current UTI by urea-splitting micro-organisms), or did not provide information as to its presence.

Nevertheless, all authors agree to estimate the current proportion of CaP stones (defined as stones containing ≥ 50% of CaP) at about 15% of the total number of urinary stones, with a marked female preponderance, carbapatite (often termed hydroxyapatite) being the predominant CaP phase in both genders [9, 10, 12–15].

CaP stones have in common as a main lithogenic factor a weakly acidic urine, with a significantly higher urinary pH than CaOx stones [9, 16–19]. In addition, hypercalciuria and hyperphosphaturia are often found in CaP stone-formers [9, 17]. Clinically, CaP stones come from diverse etiologies, either genetic, such as idiopathic hypercalciuria and hereditary diseases with urinary phosphate leak [20, 21] or congenital distal renal tubular acidosis [22], or endocrine diseases such as primary hyperparathyroidism [23], or UTI [24]. However, in most cases, CaP stones present as idiopathic and are predominantly found in women, thus raising the question of gender differences in urinary pH and citrate content [9, 25], or in the frequency of UTI episodes [26, 27]. However, despite such diverse etiologic conditions, CaP stones are usually defined only as hydroxyapatite or brushite stones on the basis of their composition identified by Fourier-transform infrared (FTIR) spectroscopy or X-ray diffraction, whereas little attention is paid to morphologic examination and precise distribution of components.

Based on the analysis of more than 50,000 calculi over the past three decades according to a homogeneous protocol combining FTIR compositional analysis and detailed morphologic examination (morpho-constitutional analysis) [28], the aim of the present study was (1) to analyze the epidemiology of CaP urinary stones and its possible variation with time and (2) to analyze the relationships between the crystalline composition and morphology of CaP stones and etiopathogenic factors.

Study materials and methods

From 1980 to 2009, we analyzed 50,235 urinary calculi from adult patients. In order to assess possible changes in the relative prevalence of CaP stones and in the distribution of CaP phases, we compared data for the first decade (1980–1989, $N = 4{,}669$ calculi, period 1) and the last one (2000–2009, $N = 27{,}817$ calculi, period 2) of the study period. In order to correlate CaP stone composition and morphology with etiopathogenic factors, we considered only CaP stones for which relevant clinical and laboratory data were available. Thus, hypercalciuria was defined as a urinary calcium excretion > 0.1 mmol/kg/day in both sexes. Primary hyperparathyroidism was diagnosed on the basis of either a high level of serum parathormone despite hypercalcaemia or an inadequate response of parathormone after calcium load. Patients with phosphate leak were defined as having a low serum phosphorus (less than 0.85 mmol/l) and a phosphate threshold normalized for GFR calculated according to the Walton and Bijvoet nomogram [29] and found under the threshold of 0.63 mmol/l with normal serum PTH according to Prié and co-workers criteria [30]. The diagnosis of distal tubular acidosis was established on the basis of plasma bicarbonates and urinary citrate excretion in basal state or after NH_4Cl load in patients presenting with recurrent bilateral stones and/or nephrocalcinosis. Medullary sponge kidney was diagnosed on excretory urograms or by computerized tomography [31, 32].

Our study material consisted of 7,753 CaP stones, including 5,520 without any struvite and 2,233 with some amount of struvite as secondary component. In addition, we determined pH and concentration of lithogenic solutes, in correlation with the nature of crystalluria, in 2,684 first morning urine samples from all comers containing crystals of CaP (or CaOx as controls) examined at our laboratory.

All stones were analyzed according to the same protocol, as described elsewhere [28]. In short, analysis consisted first in morphologic examination by means of a stereomicroscope of the surface and inner structure of the stone, followed by FTIR analysis of the different parts of the stone (core, mid-section and surface) and of a powder of the whole stone for quantitative determination of the proportions of the various components identified in the stone. Special attention was given to the characterization of the second and third components of stones having carbapatite as main component.

CaP stones were defined as stones containing at least 50% of CaP in any form. The carbonation rate (CO_3/PO_4 peaks ratio) of carbapatite stones was determined by FTIR spectroscopy as the ratio between the intensity of the $\nu 3$ band of carbonate ions at 1,420 cm^{-1} and that of the $\nu 3$ band of phosphate ions at 1,035 cm^{-1}.

Data are presented as mean ± SD. The χ^2 test and ANOVA were used for statistical comparisons.

Results

Epidemiological data

Table 1 reports the distribution of the main components of stones analyzed in period 1 and in period 2. The global proportion of CaP stones, including those devoid of any

Table 1 Comparison of stone composition between two periods

Main component	Males		Females		All	
	Period 1 (1980–1989)	Period 2 (2000–2009)	Period 1 (1980–1989)	Period 2 (2000–2009)	Period 1 (1980–1989)	Period 2 (2000–2009)
Calcium oxalates	72.3	75.3[a]	47.3	58.5[c]	64.0	70.1[c]
Uric acids	11.9	11.3	5.4	7.4	9.8	10.1
Calcium phosphates	10.4	9.7	35.8	27.4[c]	18.9	15.1[c]
Without struvite	7.3	7.8	19.9	18.5	11.6	11.1
Carbapatite	9.4	6.9[c]	34.6	24.3[c]	17.8	12.3[c]
Without struvite	6.5	5.0[b]	18.8	15.9[a]	10.6	8.4[c]
Brushite	0.8	2.4[c]	0.7	1.9[b]	0.8	2.2[c]
OCP	0	0.2	0	0.3	0	0.2[a]
ACCP*	0.1	0.1	0.1	0.5	0.1	0.2
Whitlockite	0.1	0.1	0.4	0.4	0.2	0.2
Struvite	1.7	1.0[a]	4.3	2.4[c]	2.6	1.5[c]
Presence of struvite	5.5	3.5[c]	22.3	12.1[c]	11.1	6.1[c]
Others	3.7	2.7	7.2	4.3	4.7	3.2

* ACCP amorphous carbonated calcium phosphate, [a]$p < 0.01$, [b]$p < 0.001$, [c]$p < 0.0001$ versus period 1 for the same group

struvite and those containing some struvite as secondary component, significantly declined with time, from 18.9% in period 1 to 15.1% in period 2 ($p < 0.001$). The prevalence of CaP stones was nearly threefold higher in females than in males in both periods. Carbapatite was consistently the predominant form of CaP, accounting for 94.3% of all CaP stones in period 1 and 81.1% in period 2.

This change essentially reflected a significant decline in the overall proportion of struvite-containing CaP stones, from 7.3 to 4.0% ($p < 0.001$), the decline being more marked in females than in males. In contrast, the overall proportion of CaP stones free of struvite remained unchanged, at 11.6% in period 1 and 11.1% in period 2, a non-significant difference.

However, among the latter group, the relative proportion of stones made of carbapatite as main component declined from 10.6 to 8.4% between periods 1 and 2, whereas the prevalence of brushite markedly increased (from 0.8 to 2.2%) as well as that of OCP. In parallel, the presence of brushite in stones was constantly increasing (Fig. 1) to reach 4.3% in the recent years and the proportion of carbapatite stones with a carbonation rate > 15% also increased from 15.5% in period 1 to 19.2% in period 2 (data not shown).

Of note, CaP in the form of carbapatite was a very common crystalline phase in urinary stones since it was identified by selective infrared analysis in 80% of all stones in both sexes (data not shown). Indeed, in most cases, it was a minor component of the stone, which accounted for less than 5% of the stone weight. However, despite its low proportion in the stone, CaP was often clinically relevant either as an initiating phase of the lithogenic process as observed

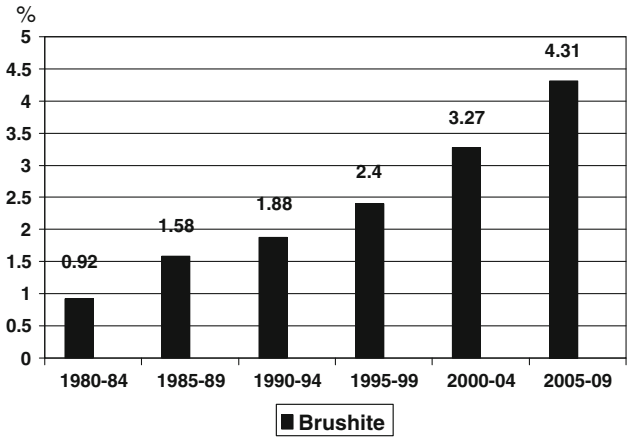

Fig. 1 Increasing occurrence of brushite-containing stones over the three past decades

in stones developed from a Randall's plaque [33, 34] or as a crystalline phase associated to calcium oxalate in various pathological conditions.

Relationships between urine pH and biochemistry, and CaP crystalline phases

Table 2 reports pH values and concentrations of calcium, phosphate and citrate in 722 first morning urine samples, with respect to the type of CaP crystals, as compared with samples containing CaOx dihydrate (COD) crystals ($n = 1{,}656$) and samples without any crystal ($n = 7{,}479$).

The mean urine pH was significantly higher in the presence of CaP crystals of any type than of COD crystals. Mean pH values were higher for amorphous carbonated

Table 2 Calcium salts crystalluria (mean ± SEM) in first morning urine samples

Crystalline phase	Number	pH	Calcium[a]	Phosphate[a]	Citrate[a]	Creatinine[a]	Ca/Cr
Weddellite (pure)	1,656	5.88 ± 0.02*	6.85 ± 0.08	29.85 ± 0.37	2.51 ± 0.05[b]	13.39 ± 0.15[x]	0.65 ± 0.01
Octahedrons	1,082	5.88 ± 0.02*	5.78 ± 0.13	29.33 ± 0.81	2.43 ± 0.09[b]	13.15 ± 0.16[x]	0.54 ± 0.02
Dodecahedrons	483	5.89 ± 0.03*	8.96 ± 0.19*	31.76 ± 1.50	2.63 ± 0.21[b]	14.14 ± 0.24[x]	0.92 ± 0.06
Brushite (pure)	20	6.42 ± 0.08	5.08 ± 0.49	26.12 ± 3.06	1.12 ± 0.20	8.25 ± 0.79	0.70 ± 0.9
Carbapatite (pure)	55	6.68 ± 0.06[c]	2.76 ± 0.25[e]	16.78 ± 1.72[e]	1.76 ± 0.21[d]	8.12 ± 0.71	0.52 ± 0.07
ACCP (pure)	647	7.19 ± 0.02	4.25 ± 0.76[e]	18.69 ± 0.37[c,e]	2.42 ± 0.06[c]	8.13 ± 0.17	0.71 ± 0.04
No crystalluria	7,479	6.13 ± 0.01	2.59 ± 0.02[e]	17.71 ± 0.15[e]	1.84 ± 0.02[e]	8.66 ± 0.06[e]	0.36 ± 0.005

[x] $p < 0.01$, *$p < 0.001$ versus CaP crystals of any type, [a] expressed as mmol/l, [b]$p < 0.01$, [c]$p < 0.001$ versus brushite, [d]$p < 0.01$, [e]$p < 0.001$ versus weddellite

Table 3 Lithogenic factors related to the main component of the stone

Stone component	Occurrence of UTI (%)	Occurrence of hypercalciuria (%)	Occurrence of HPT (%)
Calcium phosphate stones			
Carbapatite ≥ 50%	33.4[a,b,c]	63.1[a]	5.7[a]
Brushite ≥ 50%	21.5[b,c]	87.6	16.6
Whitlockite ≥ 50%	65.1	44.4	NA
ACCP ≥ 50%	69.8	60.0	NA
OCP ≥ 50%	37.5[d]	55.6	NA
Calcium oxalate stones			
Whewellite ≥ 50% (without weddellite)	10.0[a,b,c,e]	11.8[a,e,f]	0.1[a,e,f]
Weddellite ≥ 50%	8.2[a,b,c,e]	83.6[e]	4.4[a,g]

NA not assessed

[a] $p < 0.0001$ versus brushite

[b] $p < 0.0001$ versus whitlockite

[c] $p < 0.0001$ versus amorphous carbonated calcium phosphate (ACCP)

[d] $p < 0.01$ versus brushite, whitlockite or ACCP stones

[e] $p < 0.0001$ versus carbapatite

[f] $p < 0.0001$ versus weddellite

[g] $p < 0.05$ versus carbapatite

calcium phosphate (ACCP) (7.19) and carbapatite (6.68) than for brushite (6.42), itself higher than for COD (5.88).

Urine calcium and phosphate concentrations were significantly higher in the presence of brushite than of carbapatite or COD, whereas the opposite was found with respect to citrate concentration. Of note, the mean value for creatinine concentration was significantly higher in the presence of COD crystals by comparison to other urine samples, which is highly suggestive of a low diuresis in the former.

Lithogenic factors associated with the various CaP phases

Hypercalciuria was found in 87.6% of patients with brushite stones, and in 60% of those with carbapatite or ACCP stones. Of note, primary hyperparathyroidism was found in 16.6% of patients with brushite stones, as compared with only 5.7% of those with carbapatite stones and less than 5% of patients producing calcium oxalate stones (Table 3). Tubular acidosis, complete or incomplete, was found in 15% of patients with carbapatite stones (data not shown). History of UTI was reported in a very high proportion of patients with ACCP (69.8%) or whitlockite (65.1%) stones. Moreover, UTI was also frequently found in patients whose CaP stones contained a significant amount (>20%) of whitlockite or ACCP as secondary component, or a carbonation rate > 15%, whereas history of UTI was less frequent in the case of brushite stones (21.5%) or in the case of carbapatite or OCP stones (33 and 37.5%, respectively).

Table 4 Occurrence of CaP crystalline phases as main component in other physiological or pathological conditions

Main component	HPT	dRTA	Phosphate leak	MSK and other kidney malformative uropathies	Treatment with CAI	Pregnancy
No. of CaP stones	127	70	46	288	39	161
% among stones related to the condition	48.0	100	19.6	30.1	76.5	66.0
Carbapatite	66.9	95.7	76.1	85.4	94.8	85.7
Brushite	29.1	1.4	23.9	12.9	0	6.8
Octacalcium phosphate	2.4 (2.4)[a]	0 (2.8)[a]	0 (2.2)[a]	1.4 (0.7)[a]	2.6 (2.6)[a]	5.0 (23.2)[a,1]
Whitlockite	0	0	0	0.3	0	0.6
PACC	1.6	2.9	0	0	2.6	1.9

HPT primary hyperparathyroidism, *dRTA* distal renal tubular acidosis, *MSK* medullary sponge kidney, *CAI* carbonic anhydrase inhibitors (acetazolamide, topiramate, etc.)

[a] As second component in carbapatite stones

[1] $p < 0.0001$ versus other conditions

CaP stone composition and morphology in specific etiopathogenic conditions

Table 4 shows the composition of CaP stones in well-defined etiopathogenic conditions. Carbapatite was the main crystalline phase in a majority of cases, irrespective of the pathology. Primary hyperparathyroidism was associated with stones mainly made of CaP in 48% of cases. Among them, carbapatite represented 66.9% and brushite 29.1% of cases. These stones often exhibited a peculiar morphology as a consequence of the presence of either brushite (type IVd) or a mixture of carbapatite and weddellite (type IV + II) (Fig. 2).

Distal renal tubular acidosis was associated in virtually all cases with carbapatite stones of peculiar morphology (type IVa2 in our classification), characterized by an embossed and smooth surface with glazed appearance and small cracks (Fig. 2). Carbapatite was the main crystalline phase in 95% of these stones (Table 4). Acetazolamide- or topiramate-induced stones were also made of carbapatite in 95% of cases, but their morphological type was IVa1 (Fig. 2).

CaP stones associated with urinary phosphate leak were made of carbapatite or brushite.

CaP stones produced by patients with medullary sponge kidneys or other malformative anomalies such as horseshoe kidneys were mainly made of carbapatite (85.4% of cases) or brushite (12.9%).

Stones revealed during pregnancy were mainly made of CaP in 66% of cases. Among carbapatite stones, as many as 23.2% contained OCP as the second component, a tenfold higher proportion than observed in all other conditions. Of interest, OCP-containing stones were found mainly in the second and third trimesters of gestation [35].

Discussion

Epidemiology of CaP renal stones

Our data, based on a large series of kidney stones analyzed in the past three decades, indicate a significant decline in the global prevalence of CaP stones over time, considering together CaP stones devoid of struvite and those containing some amount of struvite. This distinction was possible thanks to use of FTIR which is able to identify even very small amounts of struvite. This decline essentially reflected a marked decline in the prevalence of struvite-containing CaP stones, whereas the prevalence of CaP stones totally devoid of struvite remained essentially unchanged over time. The rarefaction of CaP stones containing some proportion of struvite paralleled the simultaneous decline in the prevalence of infection stones, i.e. stones predominantly made of struvite, an undisputable marker of lithogenesis induced by urea-splitting micro-organisms [36]. In contrast, within the group of struvite-free CaP stones, the proportion of stones having carbapatite as the main component slightly decreased (from 10.6 to 8.4%), whereas the proportion of those mainly made of brushite markedly increased (from 0.8 to 2.2%), thus compensating the decline of the former.

Our findings are in general agreement with those reported in other recent epidemiological studies. Mandel et al. [8] found no significant variation in the proportion of stones containing hydroxyapatite (without associated struvite) among 10,163 stones analyzed before 1989 [37] and 33,198 stones analyzed between 1990 and 2002 (26.9 vs. 27.9%, NS), whereas they observed a significant increase in the prevalence of brushite-containing stones (from 1.7 to 4.1%). In Canada, Gault and Chafe [10] observed a decline in the percentage of stones containing ≥50% of CaP without struvite from 18% (in 1980–1983) to 14%

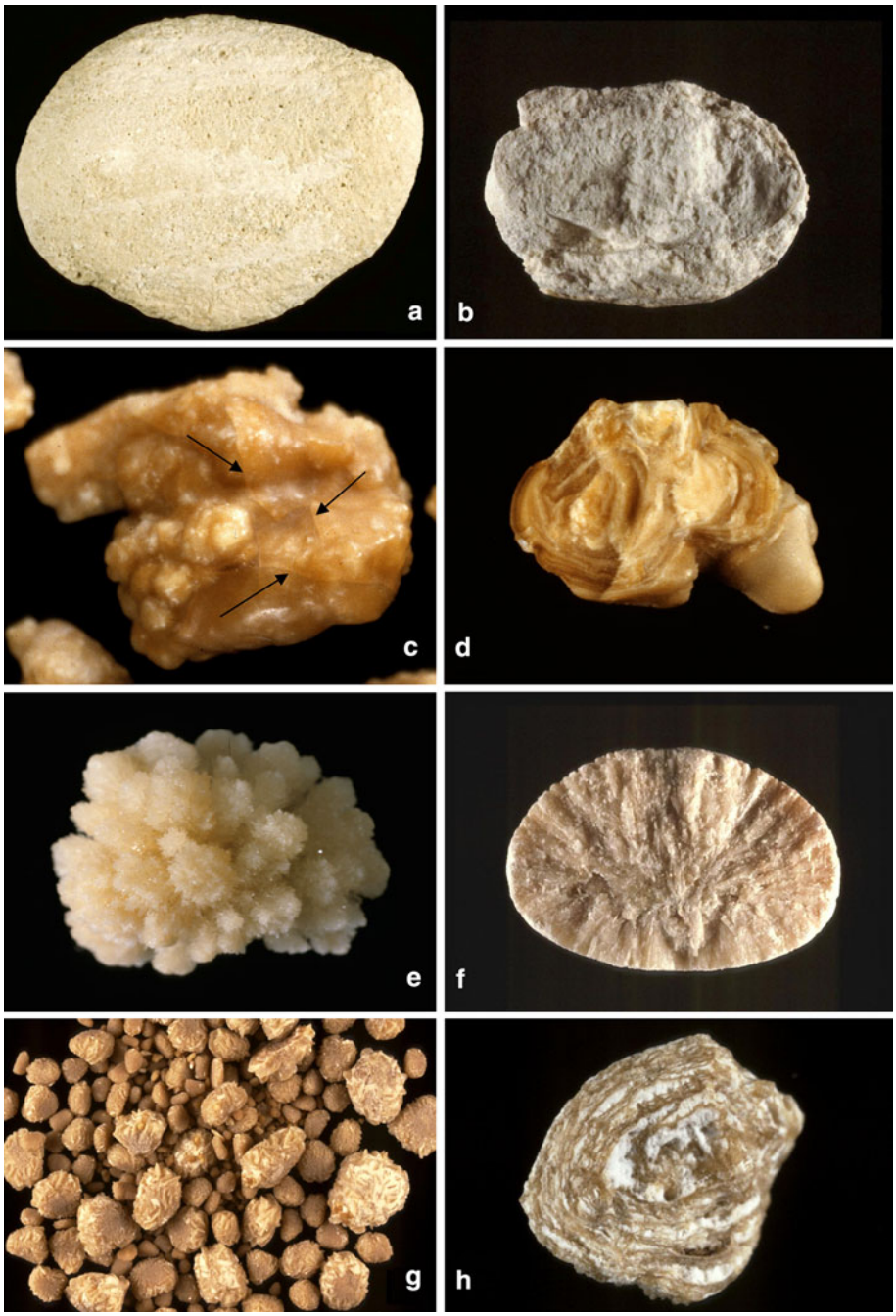

Fig. 2 Typical morphological aspects of CaP stones. **a** Type IVa1 stone (carbapatite), rough and homogenous surface, *whitish color*. This type of stone is observed mainly in cases of UTI with or without some amount of struvite, in patients with idiopathic hypercalciuria and relatively high urine pH, and in stones induced by carbonic anhydrase inhibitors. **b** Cross-section of type IVa1 stone showing poorly organized structure, *whitish color*. **c** Type IVa2 stone (carbapatite). Embossed and smooth surface with glazed appearance and small cracks (*arrows*), *brown-yellow color*. This peculiar morphology is almost exclusively found in stones associated with distal renal tubular acidosis (congenital acidosis, Sjögren's syndrome, medullary sponge kidney, etc.). **d** Cross-section of type IVa2 stone showing a concentric structure with alternately *thick brownish layers* and *thin whitish layers*. **e** Type IVd stone (brushite). Surface made of aggregated rod crystals of brushite with a radiating organization, *cream to pale yellow-brown color*. Such type of stones is frequently found in primary hyperparathyroidism. **f** Cross-section of type IVd stone made of compact concentric layers with a radiating organization of the crystals. **g** Stones of mixed type IV + II. The surface is made of bipyramidal crystals of weddellite mixed with a rough structure of carbapatite, *pale yellow-brown color*. Such mixed type is commonly found in resorptive hypercalciuria, and especially in primary hyperparathyroidism. **h** Cross-section of a mixed type IV + II stone. Typical aspect of alternate layers of weddellite (*yellow-brown*) and of carbapatite (*whitish*)

(in 1995–1998) of the sum of CaOx and CaP stones which itself accounted for 80% of the total number of stones analyzed during the study period. In contrast, Parks and co-workers [9] reported an increase in the average content of CaP in calcium stones, in parallel with a marked increase in the prevalence of brushite stones, particularly in men, between 1970 and 2003.

Lithogenic factors specific for CaP stones

A higher urinary pH is the most characteristic difference between CaP and CaOx stone-formers, with values measured on 24-h urine samples in the order of 6.3 in the former as compared to 5.6 in the latter [17]. Indeed, a high urinary pH increases the urinary supersaturation of CaP [12, 16, 38, 39]. Of note, brushite stones form in urines with a lesser pH value than carbapatite stones, and a higher calcium concentration [13]. In first morning urine samples, which express the maximal stone risk due to maximal water conservation during the overnight period [40], we observed an amplification of the lithogenic factors, with even higher pH levels and calcium concentrations in all types of CaP. The highest pH value was observed for ACCP, the highest phosphate and calcium concentrations and the lowest pH value and citrate concentration in the brushite group. Intermediate values were found for carbapatite (Table 2).

Clinical relevance of morpho-constitutional analysis of CaP stones

Comprehensive analysis of CaP stones, combining morphologic examination and compositional analysis by means of FTIR, with identification of stone components, quantification of their respective proportions, and determination of the carbonation rate of apatite, contributes to improved etiologic diagnosis.

In the great majority of cases, CaP stones present as idiopathic, i.e. without a defined causal disease, and most often CaP is admixed with variable amounts of CaOx. However, stones predominantly made of carbapatite in the absence of UTI often are associated with some degree of hypophosphatemia and urinary phosphate leak, the mechanism of which is still incompletely understood [30].

Some metabolic diseases lead specifically to the formation of stones predominantly made of CaP such as primary hyperparathyroidism or distal renal tubular acidosis [23].

Stones mainly made of carbapatite are also found (often in association with nephrocalcinosis) in hereditary diseases such as Dent's disease or mutations in genes coding for renal phosphate-transporter regulatory proteins [20, 41, 42]. It may be anticipated that in the near future molecular genetic studies will improve our knowledge of monogenic or polygenic disorders of renal tubular handling of calcium, phosphate and acid excretion, thus leading to better targeted therapeutic strategies.

Brushite stones, although much less frequent than carbapatite ones, pose an intriguing problem. Because brushite is thermodynamically unstable, it is rapidly converted into the more thermodynamically stable carbapatite [43]. The reasons for which brushite persists as the major component of CaP stones in some patients, mostly males, and shows an increasing prevalence over the recent years, are presently not understood and require further studies. One explanation could be a shortened delay between stone formation and the stone removal by urological procedures. Indeed, brushite stones are especially recurrent, resistant to ESWL fragmentation and poorly responsive to medical metaphylaxis [44]. As recently reported by Evan et al. [45], brushite stones are often associated with some signs of nephropathy with cortical fibrosis, tubule atrophy and plugging of collecting ducts, suggesting that such stones correspond to a more severe disease than other lithogenic conditions. Relationships between brushite and etiopathogenic conditions remain to be determined. However, in our experience, the high occurrence of primary hyperparathyroidism associated to brushite stones by comparison to other types of calcium stones should especially prompt us to look for this metabolic disorder.

Indeed, in clinical practice, the distinction between the crystalline phases of calcium salts, and especially CaP, is usually not considered relevant for diagnosis and medical management. Thus, the full benefits that might be gained from comprehensive morphologic and FTIR examination of stones remain largely ignored or underappreciated. Our experience provides evidence that morpho-constitutional stone analysis is much more informative than simple FTIR or XRD analysis, and is of clinical relevance.

Relationships between CaP stones and UTI

Identification of any amount of struvite in a CaP stone is highly suggestive of past or current history of UTI by urea-splitting micro-organisms, even if struvite is not the main component. Indeed, struvite supersaturation requires primarily a very high urine pH, which can be produced only through the splitting of urea resulting in the release of abundant carbonate and ammonium ions, and also a high magnesium and phosphate urine content [36].

However, if the latter condition is not fulfilled, patients with urease-producing micro-organisms may not form struvite in spite of an elevated urine pH, which nevertheless is able to induce CaP crystallization. Moreover, due to the local production of carbonic anhydride, apatite phases are enriched with carbonate ions, thus resulting in a high ratio of carbonate to phosphate ions (carbonation rate). The evidence of the association of a high carbonation rate with UTI

was provided in a previous study, which showed a positive correlation between the carbonation rate (and ACCP content) of struvite-free CaP stones, and the number of bacterial imprints shown by scanning electron microscopy at the surface of CaP stones [46]. Thus, a high carbonation rate in a CaP stone is highly suggestive of UTI even in the absence of detectable struvite.

The proposed threshold of 15% for the carbonation rate was based on correlations with the medical history of patients [13]. Indeed, a carbonation rate $\geq$ 15% was found in only 7% of CaP stones from patients without history of UTI, as compared to 46% in patients with proven UTI and even 88% when struvite was also present in the stone. Of note, the female predominance among CaP stone-formers may be, at least in part, in relation with the higher incidence of UTI episodes in women, an hypothesis supported by the preferential occurrence of CaP stones in women in the third decade [9, 10, 15], i.e. in the period of highest incidence of UTI.

Peculiar characteristics of CaP stones in specific metabolic diseases

Both the composition and morphology of CaP stones are to be considered. In primary hyperparathyroidism, we observed stones that are mostly composed of carbapatite or of a mixture of COD and carbapatite, with about 15% made predominantly of brushite, whereas pure COM was never found in stones from these patients. Therefore, stones made of brushite suggest search for primary hyperparathyroidism, whereas the presence of COM with type I morphology virtually eliminates this diagnosis [47].

Stones from patients with distal renal tubular acidosis, either congenital or acquired (such as in Sjogren's syndrome), are reported to be made of carbapatite [23] without more precision. We observed that stones formed by patients with dRTA indeed have carbapatite as their main component, and more importantly exhibit a very peculiar morphology (type IVa2) quite different from that of usual carbapatite stones (Fig. 2) in 95% of cases. This peculiar morphology is virtually pathognomonic of hereditary or acquired dRTA. A similar morphology of CaP stones is also observed in stones formed in patients with medullary sponge kidneys, likely as a consequence of local defects in distal urine acidification in affected collecting ducts. In contrast, stones developed in patients on long-standing treatment with carbonic anhydrase inhibitors are made of carbapatite with a common morphology (type IVa1 or IVb).

Pregnancy

Stones revealed during the course of pregnancy often are considered to pre-exist to gestation. However, changes in stone composition according to the pregnancy stage highly suggest a lithogenic contribution of the metabolic adaptive changes associated with the gravid state. First, the proportion of carbapatite stones is twofold more frequent in pregnancy (66%) than in age-matched non-pregnant women (31.4%, $p < 0.0001$) [35]. Moreover, among carbapatite stones, we found a parallel increase in OCP as the second component of stones and the stage of pregnancy, the prevalence of OCP being as high as 32.7% in stones found in the last trimester of pregnancy, as compared to 4.4% ($p < 0.0001$) in non-pregnant women. In view of the thermodynamic instability of OCP, which spontaneously converts to carbapatite within weeks, the high occurrence of OCP in pregnant women highly suggests that these stones were formed de novo during gestation, rather than pre-existent.

Conclusion

In conclusion, precise identification of the crystalline phases of urinary stones preferably by means of FTIR, combined with careful morphologic examination, provides wider and more precise etiologic information than simple compositional analysis. Clinical relevance of morpho-constitutional analysis, as proposed here, is of particular interest with respect to CaP stones, which constitute a very heterogeneous group with multiple disparate etiologies, including some infrequent, albeit severe, monogenic diseases. As stone composition and morphology reflect the physico-chemical causes of lithogenesis, comprehensive CaP stone analysis will decisively improve etiologic evaluation of CaP stone-formers.

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