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Serial crystalluria determination and the risk of recurrence in calcium stone formers

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Serial crystalluria determination and the risk of recurrence in calcium stone formers.

Background. Urinary crystal precipitation is the necessary initial step in kidney stone formation. However, clinical relevance of crystalluria in the evaluation of stone formers is disputed.

Methods. We serially determined crystalluria in first-voided morning urine samples, together with full 24-hour urine biochemistry, in 181 patients with idiopathic calcium nephrolithiasis who had formed at least one calcium-oxalate stone and were followed for at least 3 years under our care. All stone events which occurred prior to referral, then after entry in the study were recorded.

Results. As compared with 109 patients who had no evidence of stone recurrence during follow-up, the 72 patients who experienced ≥ one recurrent stone event had a lower daily urine volume (1.74 ± 0.06 vs. 2.26 ± 0.05 L/day (mean ± SEM) ($P < 0.0001$), higher urine calcium and oxalate concentrations, and daily calcium excretion, and they had more frequent crystalluria (68% vs. 23% of urine samples) ($P < 0.0001$). By multivariate Cox regression analysis, the hazard ratio for stone recurrence was 0.32 (95% CI 0.16-0.62) for 1 L increase in daily urine volume, 1.12 (1.09-1.24) for 1 mmol/L increase in urine calcium concentration, 1.24 (1.02-1.50) for 0.1 mmol/L increase in urine oxalate concentration and 27.8 (10.2-75.6) for crystalluria index.

Conclusion. These data provide evidence that crystalluria, when repeatedly found in early morning urine samples, is highly predictive of the risk of stone recurrence in calcium stone formers. Serial search for crystalluria, a simple and cheap method, may be proposed as a useful tool for the monitoring of calcium stone formers, in addition to urine biochemistry.

Formation of crystals in urine is the necessary initial step of lithogenesis in every type of urinary stone disease. Accordingly, crystal formation is widely used in experimental studies aimed at assessing the physicochemical conditions that lead from urine supersaturation to crystal nucleation, growth, and aggregation [1–6]. However, if the formation of stones is always preceded by crystalluria, the reverse is not true and crystalluria may occur without resulting in stone formation. Therefore, the clinical relevance of crystalluria in kidney stone formers remains largely debated. Although some authors proposed crystalluria as an index of stone disease activity in the early seventies [7, 8], routine search for crystalluria has not gained widespread popularity in clinical practice and is not currently recommended in the evaluation of stone formers. Indeed, because crystalluria is occasionally found in 15% to 20% of healthy, nonlithiasic subjects, presence of crystals in a single urine specimen was regarded as not discriminating between stone formers and nonstone formers [9, 10]. However, stone formers have been shown to exhibit more frequent crystalluria, with larger crystals and more numerous and larger crystal aggregates than healthy subjects [7, 11–17]. Therefore, one could hypothesize that presence of persistent crystalluria reflects a propensity for stone formation and may constitute a marker of stone disease activity of potential clinical relevance.

Since 1984, we performed routinely a search for crystalluria, simultaneously with full blood and 24-hour urine biochemistry, at each visit in all stone former patients referred to our stone clinic. We subsequently recorded on line all laboratory data, together with stone episodes, in our lithiasic patients over the past two decades. We were thus able to analyze the relationships between serially determined crystalluria and laboratory parameters and recurrence of stone episodes in the cohort of consecutive patients who were referred to us after they had formed one or several stones. The present study focuses on idiopathic calcium oxalate (CaOx) stone formers, who constitute the largest group of our nephrolithiasis patients.

METHODS

Study population

Between January 1984 and December 2000, 651 patients with idiopathic CaOx urolithiasis were referred to

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our stone clinic. All underwent full blood and 24-hour urine chemistry evaluation, together with search for crystalluria at baseline and at each visit thereafter. Because the average interval for recurrence was about 3 years in idiopathic calcium stone formers in previous studies [18, 19], we defined patients as nonrecurrent only if they had no evidence of formation of new stones for at least 3 years after referral, whereas patients who suffered recurrent stone episode(s) where considered whichever the time duration elapsed from entry in the study until the first recurrent stone episode. The observation period lasted until December 2003, in order that each patient had a follow-up of at least 3 years.

Included in analysis were patients who fulfilled all of the following criteria: idiopathic CaOx nephrolithiasis (CaOx as the main component of stones, with less than 50% of calcium phosphate in mixed stones); age >15 years at first stone episode; at least three follow-up visits with determination of crystalluria; and absence of any clinical condition or drug therapy that may interfere with calcium metabolism. In particular thiazide therapy, if any, was withdrawn for at least 15 days prior to baseline laboratory evaluation. Excluded from analysis were patients with CaOx nephrolithiasis secondary to defined causes such as primary hyperparathyroidism, primary hyperoxaluria, enteric hyperoxaluria, renal tubular acidosis, or systemic diseases, who require specific management, and patients receiving calcium vitamin supplements or biphosphonates for the prevention or treatment of postmenopausal osteoporosis. A total of 181 patients (127 males and 54 females) fulfilled all of these criteria and constitute the study material. Of them, 141 (78%) had formed more than one stone or had multiple calculi before being referred to us, whereas the other 40 (22%) were first stone formers. Patients with medullary sponge kidney were considered for analysis. Medullary sponge kidney was identified on the basis of roentgenologic findings as described elsewhere [20].

Study protocol

After baseline evaluation, all patients were managed according to a homogeneous protocol aimed at preventing recurrence of calcium stones. The common basic regimen included high fluid intake to achieve a daily urine output of at least 2 L, well distributed over day and night; dietary calcium intake of 800 to 1000 mg/day; moderate consumption of animal proteins and salt; avoidance of oxalate-rich foods, especially chocolate. When despite these measures hypercalciuria persisted, a thiazide diuretic was added, most often in the form of hydrochlorothiazide (25 mg/day). Allopurinol (200 to 300 mg/day) was prescribed to patients with urinary urate excretion ≥5 mmol/day. Potassium citrate was used only in patients with sustained, marked hypocitraturia (<1 mmol/L).

Clinical surveillance was scheduled at 1 and 3 months after entry in the study to test the effects of the proposed regimen and readjust medical treatment when needed, then every 6 months during the first 2 years, and at yearly interval thereafter or more frequently if clinically needed.

Radiologic surveillance used serial abdominal plain x-ray film and echography; in the recent years, spiral computerized tomography was used as indicated. Intravenous pyelography with low osmolality contrast medium was obtained at least once in every patient in order to detect medullary sponge kidney.

Stone episodes ending in spontaneous passage, and urologic procedures that took place during the prereferral period were recorded with the date of occurrence and type of urologic procedure used. In addition, we included in the prereferral stone count asymptomatic stones present in the urinary tract on baseline radiographic examination. After referral, we prospectively recorded all symptomatic stone episodes whether they ended in spontaneous passage or required any urologic procedure. In addition, we included as recurrence roentgenologic evidence of new stones (i.e., not present on baseline abdominal x-ray film). We determined the time elapsed between onset of stone disease (defined as the date of the first stone episode) and referral, and between entry in the study and first recurrent stone episode, or end of the follow-up period (December 31, 2003). We thus could determine the average annual number of stones formed by each patient during the prereferral period and during the follow-up period.

Laboratory procedures

Twenty-four hour urine collections were obtained at baseline and at each visit thereafter. A single 24-hour collection was taken at each visit because a previous prospective study on 183 calcium stone formers comparing biochemistry parameters in two successive 24-hour urine collections had shown that the highest number of abnormalities was found in the first sample in 85% of cases (unpublished personal data).

Laboratory determinations included 24-hour urine calcium (Ca), phosphate, oxalate (Ox), citrate, magnesium, urea, creatinine, uric acid, sodium, and potassium together with specific gravity. Plasma concentrations of the same parameters (except oxalate and citrate) were simultaneously determined.

Based on the common observation that urine produced during the night is usually the most concentrated, and therefore carries the highest risk of supersaturation and crystal formation, we performed all crystalluria studies in fresh first-voided morning urine samples, according to a uniform protocol described elsewhere [21]. In short,

urine samples brought to the laboratory within 2 hours of voiding were kept at room temperature and were rapidly processed. Urine-specific gravity and pH were measured. Undiluted urine was then homogeneized by gentle shaking and turning over (neither centrifuged nor filtered) and immediately placed in a Malassez cell (CML, Nemours, France) containing 10 mm^3, then examined by light microscopy using a polarizing microscope (Optiphot-2) (Nikon, Champigny-sur-Marne, France). The entire cell was examined at ×200 magnification to localize crystals and aggregates, then with ×400 magnification (high power field). All crystals and aggregates were counted on the entire cell and their size determined using the included micrometric scale. The results were expressed as number of crystals by mm^3. Only one Malassez cell was examined in each instance. Crystalluria examinations were performed in a "blind" manner (i.e., without knowledge of the clinical status of the patients and of the results of laboratory determinations). We did not consider small calcium phosphate grains less than 2 μm in diameter. The mean number of crystalluria studies beyond the baseline one was 6.8 ± 5.3 per patient, with a median of five determinations (range 3 to 33). We defined as "crystalluria index" the ratio of the number of urine samples with crystalluria to the total number of examined urine samples. The average value of all 24-hour urine biochemistry parameters was used in the follow-up period.

Statistical analysis

Data are presented as means ± SEM unless otherwise specified. Intergroup comparisons used Student *t* test, Mann-Whitney test, or analysis of variance (ANOVA) for continuous variables, and the χ^2 and Fisher's exact tests for categoric variables. Differences in medians for not normally distributed parameters were compared using Mann-Whitney U or Wilcoxon rank sum test.

The primary outcome measure was the time from entry in the study until first recurrence of a renal stone, or study end in non-recurrent patients. Secondary outcomes were the total number of recurrent stones formed and the total number of urologic procedures required during the follow-up period. Cox proportional hazards regression analysis used the time duration until first recurrence as end point. Univariate study examined the influence of previous stone disease activity (number of stones/year in the prereferral period), age at start of stone disease, daily urine volume, 24-hour urinary parameters at baseline and during follow-up, and proportion of serial first-voided urine specimens with presence of crystalluria as continuous variables during follow-up. Gender and presence of medullary sponge kidney were entered as categoric variables. Only parameters with a $P < 0.10$ significance were entered in the final multivariate analysis.

Table 1. Characteristics of the 181 patients in the prereferral and follow-up periods

	Prereferral period	Follow-up period
Duration *years*	8.7 ± 0.7	6.8 ± 0.3
Number of stone episodes		
Mean number/patient	4.83 ± 0.45	1.07 ± 0.14[a]
Number/patient-year	0.67 ± 0.07	0.21 ± 0.03[a]
Number of spontaneous passages	3.39 ± 0.43	0.82 ± 0.12[a]
Number of urologic procedures (total)	1.42 ± 0.13	0.18 ± 0.04[a]
Total number of urologic procedures	256	34
Open surgery (%)	79 (30.8)	0
Percutaneous nephrolithotomy	12 (4.7)	4 (11.8)
Extracorporeal shock wave lithotripsy	114 (44.5)	22 (64.7)
Ureteroscopy	51 (19.9)	8 (23.5)

Data are given as mean ± SEM. Comparison follow-up period vs. prereferral period (Mann-Whitney test)
[a] $P < 0.0001$.

Receiver operating characteristics (ROC) curve analysis was used to determine the cut-off values of significant variables with respect to the risk of stone recurrence. All *P* values are two-sided. A *P* value < 0.05 was considered significant. Statistical procedures used the NCSS 2000 software (Jerry L. Hintze, Kaysville, UT, USA).

RESULTS

The studied cohort comprised 181 patients, all Caucasian (127 males and 54 females). There was a marked male preponderance with a gender ratio of male to female of 2.35. A family history of urolithiasis was found in 75 patients (41.4%), and medullary sponge kidney was present in 76 patients (42%).

At baseline, 38.6% of patients (56.6% of those with medullary sponge kidney and 33.4% of those without medullary sponge kidney) had one or more renal stones at radiographic evaluation; 66.3% were hypercalciuric at referral (calcium excretion ≥0.1 mmol/kg/day on free diet in both genders) and 49.7% had a daily urine volume ≤1.5 L.

As shown on Table 1, the overall number of stone episodes significantly decreased in the follow-up period as compared to the prereferral period. The number of stone episodes ending in spontaneous passage declined from 3.39 ± 0.43 to 0.82 ± 0.12 per patient ($P < 0.0001$), a decrease of 76%, and the number of urologic procedures from 1.42 ± 0.13 to 0.18 ± 0.04 per patient ($P < 0.0001$), a decrease of 86.5%. As a result, the total number of formed stones, (including three asymptomatic new stones formed during follow-up) decreased from 4.83 ± 0.45 to 1.07 ± 0.14 stone/patient (a reduction by 78%) between the prereferral and the follow-up period. All urologic procedures were fewer than in the prereferral period, and in particular no open surgery was performed after referral.

Table 2. Characteristics of patients with (R) and without (NR) stone recurrence during the follow-up period

	NR ($N = 109$)	R ($N = 72$)	P value
Gender *Male/female*	74/35	53/19	0.41
Mean age at first stone episode *years*	32 (15–68)	28 (15–64)	0.027
Number with medullary sponge kidney (%)	34 (31.2)	42 (58.3)	0.001
Number with family history (%)	41 (37.6)	34 (47.2)	0.20
Prereferral period			
Duration *years*	7.5 (0.1–33)	5.0 (0.1–25)	0.36
Number of spontaneously passed stones/patient	2.80 ± 0.44	4.28 ± 0.85	0.09
Number of urologic procedures/patient	1.40 ± 0.17	1.43 ± 0.22	0.92
Number of stone episodes per patient-year	0.53 ± 0.09	0.89 ± 0.13	0.02
At referral			
Mean age at referral *years*	44 (20–72)	36 (15–71)	0.0003
Body mass index kg/m^2	23.8 ± 0.33	23.3 ± 0.40	0.33
Serum creatinine at referral *μmol/L*	89.7 ± 1.38	90.2 ± 2.1	0.85
Number of single stone formers (%)	25 (22.9)	15 (20.8)	0.74
Number of stone-free patients at referral (%)	61 (55.9)	47 (65.3)	0.21
Mean urine volume *L/day*	1.6 (0.8–3.3)	1.4 (0.6–2.9)	0.0001
Patients with urine volume ≤*1.5 L/day* (%)	44 (40.4)	46 (63.9)	0.003
Urine calcium concentration *mmol/L*	3.9 (0.9–12)	6.0 (2.2–12)	<0.0001
Urine calcium excretion *mmol/day*	6.4 (1.8–16.4)	7.9 (3.4–15.6)	0.0019
Urine calcium excretion *mmol/kg/day*	0.11 (0.02–0.23)	0.13 (0.02–0.21)	0.017
Hypercalciuria ≥*0.1 mmol/kg/day* (%)	63 (57.8)	57 (79.2)	0.003
Urine oxalate concentration *mmol/L*	0.24 (0.09–0.62)	0.27 (0.10–0.47)	0.07
Urine oxalate excretion *mmol/day*	0.40 (0.13–0.88)	0.36 (0.11–0.67)	0.18
Urine citrate concentration *mmol/L*	1.8 (0.2–4.8)	1.9 (0.1–5.8)	0.24
Urine citrate excretion *mmol/day*	2.7 (0.3–7.4)	2.8 (0.1–6.1)	0.64
Urine urea excretion *mmol/day*	388 (160–757)	397 (192–674)	0.42
Urine urea excretion *mmol/kg/day*	5.9 (2.3–8.7)	5.9 (2.3–10.2)	0.78
Follow-up period			
Duration *years*	6.88 ± 0.41	6.79 ± 0.53	0.90
Number treated with thiazide (%)	42 (38.5)	26 (36.1)	0.74
Time to first recurrence *years*	—	2.41 ± 0.14	
Number of spontaneous passage/patient	0	2.07 ± 0.25	
Number of urologic procedures/patient	0	0.46 ± 0.09	
Number of recurrences/patient	0	2.67 ± 0.25	
Number of recurrences/patient-year	0	0.37 ± 0.05	
Average urine volume *L/day*	2.1 (1.2–4.0)	1.7 (0.7–2.8)	<0.0001
Patients with urine volume ≥*2 L/day* (%)	73 (66.9)	16 (21.1)	<0.0001
Urine calcium concentration *mmol/L*	2.4 (0.5–6.7)	4.2 (1.7–9.1)	<0.0001
Urine calcium excretion *mmol/day*	5.3 (1.8–15.1)	7.1 (2.6–13.4)	<0.0001
Urine oxalate concentration *mmol/L*	0.20 (0.06–0.37)	0.27 (0.10–0.49)	<0.0001
Urine oxalate excretion *mmol/day*	0.44 (0.16–0.98)	0.43 (0.19–0.93)	0.79
Urine citrate concentration *mmol/L*	1.3 (0.3–3.5)	1.6 (0.1–4.5)	<0.007
Urine citrate excretion *mmol/day*	2.9 (0.4–6.5)	2.8 (0.1–5.5)	0.48
Urine urea excretion *mmol/day*	403 (180–656)	382 (175–643)	0.59
Urine urea excretion *mmol/kg/day*	5.7 (2.3–10.6)	5.8 (2.2–10.1)	0.89
Number of crystalluria determinations	4 (3–28)	5 (3–33)	0.53
Mean crystalluria index	0.23 ± 0.03	0.68 ± 0.03	<0.0001
Crystalluria in ≥50% of samples (%)	17 (15.6)	63 (87.5)	<0.0001
Serum creatinine at end of follow-up *μmo/L*	92 ± 2	93 ± 2	0.60

Data are given as mean (± SEM) for normally distributed parameters and as medians (with range) for not normally distributed parameters.

During follow-up, 72 patients experienced at least one recurrent stone episode (R group) whereas the other 109 remained free of any recurrence for at least 3 years after entry in the study (NR group). Characteristics of patients in the R and NR groups are given in Table 2. The two groups did not differ with respect to gender distribution, family history of stones, proportion of single stone formers, presence of stones at baseline, body mass index (BMI), and proportion of patients treated with thiazide, but age of patients at clinical onset of urolithiasis was significantly lower in the R than in the NR group. Patients in the R group had a higher stone disease activity in the prereferral period (0.89 ± 0.13 stone episodes/patient-year) than did NR patients (0.53 ± 0.09) ($P = 0.02$), whereas the number of required urologic procedures was similar.

During the follow-up period, R patients experienced a significantly lesser incidence of stone episodes as compared with their corresponding prereferral period (0.37 ± 0.05 vs. 0.89 ± 0.13 episodes/patient-year) ($P < 0.001$), and the number of required urologic procedures also decreased significantly.

At baseline, daily urine output was significantly lower and daily calcium excretion was significantly higher in the R than in the NR group, whereas daily oxalate and citrate

excretion did not differ, nor urinary urea excretion. The difference was even more marked when considering urine calcium concentration.

During follow-up, the average daily urine output rose in both R and NR groups, but to a lesser extent in the former than in the latter. The mean increase in daily urine volume was close to 0.5 L/day in the NR group, as compared with only about 0.3 L/day in the R group. The mean average daily urine output during follow-up was thus markedly higher in NR than in R patients (2.26 $\pm$ 0.05 vs. 1.74 $\pm$ 0.06 L/24 hours) ($P < 0.0001$).

Mean daily urine calcium excretion decreased in both groups, but more markedly so in the NR group. As a result of both higher diuresis and lower calcium excretion, urine calcium concentration was strikingly lower in NR than in R patients (2.47 $\pm$ 0.10 vs. 4.36 $\pm$ 0.19 mmol/L) ($P < 0.0001$). In contrast, average daily oxalate excretion did not significantly differ from the prereferral period, and was similar in the R and NR groups. However, oxalate concentration decreased in the NR group, but not in the R group, and thus was significantly lower in the former than in the latter during follow-up. Average daily citrate excretion remained unchanged with respect to the prereferral period in both groups, and did not differ between R and NR groups, but urinary citrate concentration was higher in the R group, thus reflecting a lower diuresis. Urea excretion remained essentially unaltered in both groups, and did not differ between R and NR groups during follow-up.

Crystalluria was found in a significantly higher proportion of urine samples in R than in NR patients (68 $\pm$ 3 vs. 23 $\pm$ 3%) ($P < 0.0001$). Of note, presence of crystalluria at baseline or occurrence of crystalluria during follow-up did not differ whether or not patients had preexisting stones. Crystalluria was found in 45% of urine samples of stone-free patients and in 38% of those with renal stone(s) present at baseline or during follow-up (a not significant difference). Overall, crystalluria was found in $\geq$50% of individual urine samples in 87.5% of R patients, as compared to 15.6% of NR patients, a highly significant difference ($P < 0.0001$).

The mean time from entry in the study to first recurrence was 2.4 $\pm$ 1.2 years (median 2.3 and range 0.5 to 5 years) in the R group, whereas no recurrence was observed in the NR group over a mean follow-up duration of 6.9 $\pm$ 4.2 years (range 3 to 15 years). By ROC curve analysis, the cut-off values for the risk of developing stone recurrence during follow-up were as follows: average follow-up urine output 1.94 L/day [sensitivity 0.69, specificity 0.76, and positive predictive value (PPV) 0.82; negative predictive value (NPV) 0.62; and area under the curve (AUC) 0.78]; average follow-up urine calcium concentration 3.78 mmol/L (sensitivity 0.71, specificity 0.88, PPV 0.80, NPV 0.82, and AUC 0.88), and crystalluria index 0.50 (sensitivity 0.88, specificity 0.84, PPV 0.79, NPV 0.91, and AUC 0.88). The ROC curves for crystalluria and urine volume versus stone recurrence are shown in Figures 1 and 2.

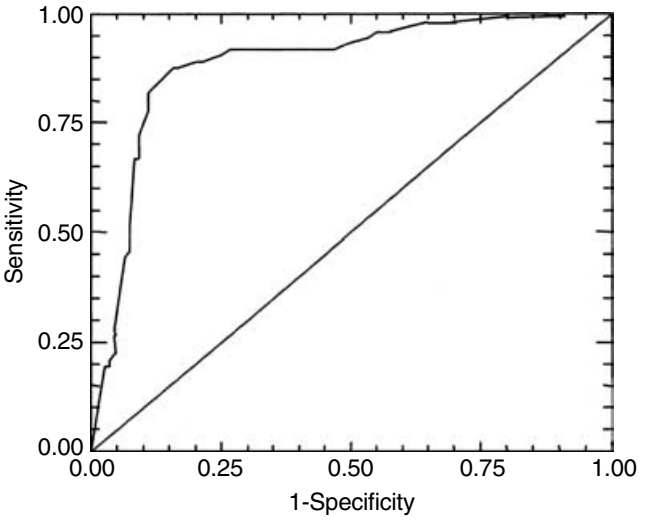

Fig. 1. Receiver operating characteristic (ROC) curve for the risk of stone recurrence with respect to crystalluria index.

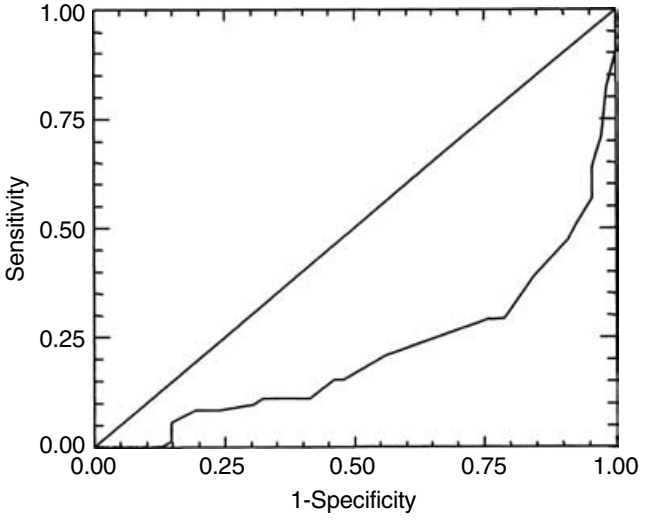

Fig. 2. Receiver operating characteristic (ROC) curve for the risk of stone recurrence with respect to average daily urine volume during follow-up (protective effect).

As compared with the 105 CaOx stone formers without medullary sponge kidney, the 76 patients with medullary sponge kidney had a more severe stone disease (Table 3). Their mean number of stone episodes was nearly three times higher both before and after referral, and the proportion of medullary sponge kidney patients with recurrence of stone formation was nearly two times higher. Nevertheless, a striking reduction in the number of symptomatic stone episodes and even more in the number of required urologic procedures was observed after entry in the medical protocol by comparison with the prereferral

Table 3. Data on calcium oxalate (CaOx) stone formers with or without medullary sponge kidney

	Without medullary sponge kidney ($N = 105$)	With medullary sponge kidney ($N = 76$)	P value
Prereferral			
Number of stone episodes/patient	2.99 ± 0.28	7.38 ± 0.94	<0.0001
Number of urologic procedures/patient	1.31 ± 0.15	1.55 ± 0.24	0.38
At referral			
Baseline urine calcium excretion *mmol/day*	7.1 (1.9–16.4)	6.9 (1.8–15.6)	0.59
Baseline daily urine volume *L/day*	1.6 (0.6–3.3)	1.45 (0.8–3.4)	0.03
Follow-up			
Number with stone recurrence (%)	30 (28.5)	42 (55.3)	0.0003
Number of stone episodes/patient	0.55 ± 0.10	1.76 ± 0.28	<0.0001
Number of urologic procedures/patient	0.12 ± 0.05	0.26 ± 0.07	0.10
Average urine volume *L/day*	2.1 (0.7–3.9)	1.9 (0.9–3.5)	0.002
Urine calcium excretion *mmol/day*	6.4 (1.8–15.1)	5.6 (1.8–12.7)	0.25
Urine oxalate excretion *mmol/day*	0.44 (0.16–0.99)	0.42 (0.18–0.93)	0.39
Urine citrate excretion *mmol/day*	2.9 (0.4–9.5)	2.8 (0.1–6.3)	0.78
Crystalluria index	0.38 ± 0.03	0.45 ± 0.04	0.15

Results are expressed as in Table 2.

Table 4. Univariate Cox regression analysis for the risk of recurrence

Risk factors	Z value	Hazard ratio (95% CI)	P value
Age at onset (per 1 *year*)	−2.38	0.98 (0.96–0.99)	0.017
Medullary sponge kidney (present)	3.73	2.45 (1.53–3.92)	0.0002
Family history (present)	1.73	1.15 (0.98–1.34)	0.08
Baseline urine volume (per 1 *L/day*)	−3.52	0.36 (0.20–0.63)	0.0004
Baseline calcium excretion (per 1 *mmol/day*)	2.18	1.07 (1.01–1.15)	0.029
Baseline calcium concentration (per 1 *mmol/day*)	4.81	1.24 (1.14–1.36)	<0.0001
Crystalluria index	8.64	35.8 (15.9–80.7)	<0.0001
Crystalluria in ≥50% of samples	7.69	15.8 (7.8–31.8)	<0.0001
Follow-up urine volume (per 1 *L*)	−6.66	0.17 (0.10–0.29)	<0.0001
Follow-up calcium excretion (per 1 *mmol/day*)	4.28	1.18 (1.09–1.28)	<0.0001
Follow-up calcium concentration (per 1 *mmol/L*)	8.29	1.62 (1.45–1.82)	<0.0001
Follow-up oxalate excretion (per 100 *pmol/day*)	0.32	1.28 (0.29–5.76)	0.75
Follow-up oxalate concentration (per 100 *μmol/L*)	6.02	2.32 (1.76–3.05)	<0.0001
Follow-up citrate excretion (per 1 *mmol/day*)	−0.40	0.97 (0.81–1.15)	0.69
Follow-up citrate concentration (per 1 *mmol/L*)	0.97	1.15 (0.87–1.54)	0.33

period. Urine lithogenic parameters did not differ between patients with or without medullary sponge kidney; crystalluria was found more frequently in the former, but the difference did not reach statistical significance.

Results of univariate Cox regression analysis are given in Table 4. Age at onset of stone disease, presence of medullary sponge kidney, baseline urine volume, baseline urine calcium excretion and concentration, as well as average follow-up urine volume, daily calcium excretion, urine Ca and Ox concentrations, and crystalluria were significantly associated with the risk of stone recurrence.

Results of multivariate Cox regression analysis for the whole series are given in Table 5. We entered in multivariate Cox regression analysis only presence of medullary sponge kidney, and average follow-up values of daily urinary volume, Ca and Ox concentrations, and crystalluria index, because there were strong correlations between baseline and follow-up daily urine volume and between daily excretion and concentration of Ca and Ox, as well as between crystalluria index and presence of crystals in ≥50% of samples. Follow-up average daily urine parameters were selected as reflecting the effects of preventive measures implemented after referral.

In this model, five factors were significantly and independently associated with the risk of recurrence: presence of medullary sponge kidney, follow-up Ca and Ox concentrations, follow-up daily urine volume and crystalluria index. The latter revealed the most powerful predictor of stone recurrence, with a hazard ratio of nearly 28 whereas presence of crystalluria in at least half of urine samples was associated with a hazard ratio of 16.8. Urine volume was the second most powerful factor, with the risk of recurrence decreasing by 68% for each 1 L/day increase in daily urine output.

When limited to the subgroup of 76 patients with medullary sponge kidney, multivariate Cox regression analysis identified as independent risk factors for recurrence only three parameters: average daily urine output [hazard ratio (HR) 0.40 per 1 L/day (95%CI 0.15-0.99)] ($P = 0.041$), average calcium concentration [HR 1.30 per 1 mmol/L (95% CI 1.08-1.56)] ($P < 0.005$), and crystalluria [HR 26.2 (95% CI 11.1-86.2)] ($P < 0.0001$).

Table 5. Multivariate Cox regression analysis based on average follow-up values

Risk factors	Z value	Hazard ratio (95% CI)	*P* value
Presence of medullary sponge kidney	2.97	2.15 (1.30–3.56)	0.003
Follow-up calcium (per 1 *mmol/L*)	2.12	1.12 (1.09–1.24)	0.03
Follow-up urine volume (per 1 *L/day*)	−3.35	0.32 (0.16–0.62)	0.0008
Crystalluria index	6.51	27.8 (10.2–75.6)	<0.0001
Follow-up oxalate (per 0.1 *mmol/L*)	2.20	1.24 (1.02–1.50)	0.028

DISCUSSION

The present study is the first to analyze in parallel the association of serially determined crystalluria and urine biochemistry parameters with CaOx stone recurrence in a long-term, prospective cohort study. Our data provide strong evidence that frequent crystalluria (i.e., found in at least half of first morning urine samples) is the most reliable indicator of the risk of recurrence in CaOx stone formers.

Our working hypothesis was that repeated finding of crystalluria in a patient should reflect the risk of sustained crystallization, leading to stone formation. To gain clinical relevance, this hypothesis had to be verified by means of a prospective study based on the record of all newly formed stones in a cohort of stone formers followed over a long time duration. We elected to study first-voided, morning urine samples because they have been shown to exhibit the highest concentration of solutes and therefore are at highest risk of containing crystals [22, 23].

The population analyzed in this study is not representative of common stone formers populations and does not reflect our own whole cohort of calcium stone formers nor the proportion of recurrent patients. Due to our status of tertiary referral center, our cohort presents an overrepresentation of severe, relapsing forms of idiopathic CaOx urolithiasis and especially of patients with medullary sponge kidney. Indeed, 78% of patients already were recurrent stone formers when referred to us, 66% were hypercalciuric, and 42% had medullary sponge kidney as underlying condition. Medullary sponge kidney has been shown to be associated with an especially high rate of stone production [20, 24, 25] and this was the case in the present series. In the whole population of 651 patients with idiopathic calcium nephrolithiasis evaluated at our stone clinic from 1984 to 2000, medullary sponge kidney accounted for a markedly less proportion (25.1%). In addition, only patients with at least 3 years of follow-up were included in order to reliably assess nonrecurrence as several studies showed that time duration until first recurrence was about 3 years [18, 19]. There was a high rate of drop out of follow-up with time among patients, as already observed by Parks, Asplin, and Coe [19]. A possible explanation is that patients with a less severe form were more prone to neglect medical surveillance than patients with frequent recurrences, such as those with MSK. Although most of our patients had a rather severe form of nephrolithiasis, an overall improvement was observed, with a global reduction in stone formation by 77% during follow-up as compared with the prereferral period, even in medullary sponge kidney patients. Of note, a decreased recurrence frequency was also observed in the R group, with a mean annual incidence of stone episodes reduced by 58%, and of urologic procedures by 67% as compared with the prereferral period.

Our patients were managed according to a homogeneous protocol over the whole study period. In view of the report of Bataille et al in 1983 [26], we adopted in 1984 the recommendation of dietary calcium intake of no less than 600 mg/day, and following the study of Curhan et al in 1993 [27], we prescribed a calcium intake of 800 to 1000 mg/day to all of our calcium stone formers. Based on the data of Robertson et al [28] we recommended since the early 1980s a moderate animal protein and salt intake. Based on the findings of Pak et al [29] and Hosking et al [30] we recommended all stone formers to maintain a high fluid intake, ensuring a daily urine output of at least 2 L, well distributed over night and day.

In this regard, our present data confirm and extend previous observations on the primary importance of urine dilution for preventing stone recurrence. Pak et al [29] were the first to formally demonstrate that urine dilution significantly increases the formation product ratio of CaOx, thus reducing the risk of crystal nucleation. Borghi et al [31] observed that increasing nocturnal urine volume by a 500 mL water load administered at 11:30 p.m. resulted in nearly halving the CaOx relative supersaturation in both normal controls and normocalciuric CaOx stone formers. On a clinical point of view, Hosking et al [30] observed that, among stone formers with a similar initial urine volume, those who exhibited no recurrence during follow-up had a significantly higher daily urine volume than those who had recurrent stone formation (about 2.1 vs. 1.7 L/day). In large epidemiologic studies comparing dietary habits and incidence of stones in cohorts of men or women aged ≥40 years without history of renal colic, the risk of kidney stones was inversely correlated with fluid intake [27, 32]. Subsequently, Curhan et al [33] examined 24-hour urine chemistries in 913 subjects diagnosed with a first kidney stone compared with 309 healthy controls randomly sampled from the same cohorts. Mean daily urine volume was lower in cases than in controls in either gender. In a prospective randomized interventional study

in 199 male CaOx stone formers, Borghi et al [34] showed that patients who sustained a urine volume >2 L/day, instead of <1.1 L/day, had a significantly lower incidence of recurrent stone formation over a 5-year follow-up period.

Our study highlights the importance of a high urine volume to prevent CaOx stone recurrence, and confirms the validity of the universal recommendation to increase urine volume to at least 2 L/day [35, 36]. The mean average urine volume during follow-up in our NR group was 2.26 L/day, compared to 1.74 L/day in the R group, and ROC curves identified a daily output of 1.94 L as the cut-off value.

In agreement with the findings of Curhan et al [33], we found that baseline 24-hour urinary calcium excretion was significantly higher in the R than in the NR group. The difference was even more marked when considering urine calcium concentration, which was nearly 50% higher in the R than in the NR group, an observation in keeping with the lower mean baseline urine volume in the R group. Moreover, the mean increment in urine output was higher (by nearly 500 mL/day) in NR patients, compared to only 300 mL/day in R patients, whose baseline diuresis already was about 250 mL/day lower than in patients who subsequently had no recurrence during follow-up. It has been suggested that part of CaOx stone formers spontaneously have a weak thirst, and therefore a low fluid intake and suboptimal urine volume, thus resulting in a higher risk of recurrence [37]. As a matter of fact, oligodipsia was declared by two thirds of our patients with a spontaneously low urine volume. On a practical point of view, this finding highlights the importance of motivating stone formers to achieve and sustain a urine volume of at least 2 L/day, with fluid intake at bedtime in order to avoid nocturnal urine hyperconcentration.

Hypercalciuria, hyperoxaluria, and hypocitraturia have been shown to act as the main lithogenic factors in idiopathic calcium urolithiasis [38–40]. However, most studies were based on the calculation of urinary CaOx supersaturation, which is a theoretic criterion of the risk of crystallization and stone formation. Only two studies, including one by Curhan et al [33] and the present one, provide a multivariate analysis of the association of urinary parameters with the more relevant criterion of symptomatic stone episodes. The case/control study of Curhan et al [33] concluded to a significant, graded correlation between incidence of stones and both daily urine volume and calcium excretion, but not with citrate, oxalate, or uric acid excretion. The most critical lithogenic determinant appeared as the urine concentration of calcium. Our data, based on the incidence of recurrent stone episodes in a cohort of patients all of whom had a history of stones, are fully concordant. We observed a significant influence on stone recurrence of daily urine volume and of calcium (but not oxalate or citrate) excretion, and of Ca and Ox urinary concentrations, giving clinical confirmation to our previous observation that CaOx crystallization in urine is in close relationship with the CaOx molar product [41]. The positive relationship between stone recurrence and oxalate concentration found in our patients suggests that calcium stone formers may be more sensitive to oxalate concentration to form CaOx crystals than normal subjects, in agreement with the results of crystalluria studies reported by Fan and Chandhoke [42].

Many indices have been proposed to evaluate the risk of being a stone former. They are mostly based on 24-hour urine biochemistries, such as the saturation-inhibition index proposed by Robertson et al [43], the relative CaOx supersaturation by Equil 2 computerized software [44], or the AP(CaOx) index proposed by Tiselius et al [45], and other indices [46]. However, even if such indices are able to discriminate between cohorts of nonstone forming healthy subjects and of stone formers, there is considerable overlapping in the values of these indices at the individual level. In contrast, serial crystalluria determination revealed a simple and reliable method to assess the risk of recurrence in individual stone formers. The strength of our study relies on its prospective design, with long-term follow-up of a cohort of patients managed according to a homogeneous protocol and followed with serial determinations of both urine biochemistry and crystalluria.

Frequency of the finding of crystalluria was strikingly higher in recurrent stone formers than in their nonrecurrent counterparts, and appeared as a stronger predictive index of recurrent stone formation than were biochemistry parameters. However, neither crystal number and size nor presence of aggregates allowed discrimination between recurrent and nonrecurrent stone formers. Of note, crystalluria was not influenced by the presence of kidney stones, as crystalluria was not found more frequently in patients with stones present in the kidneys at baseline or during follow-up than in those who remained stone free. This finding has the important implication that crystalluria may be used as a marker of stone disease activity irrespective of the presence or absence of stone(s) in the kidney.

The positive and negative predictive values of crystalluria index for a cut-off value of 0.5 as determined by ROC curve analysis could be influenced by the prevalence of the exposure. Indeed, not surprisingly, the number of crystalluria determinations was slightly higher in the R group than in the NR group, thus reflecting that patients with recurrences were more frequently seen than those free of recurrence. In any case, it remains that crystalluria was the most powerful predictor of the risk of recurrence.

Assessment of crystalluria appears of clinical interest in the management of idiopathic calcium stone formers, as already shown in patients with cystinuria [47] or primary

hyperoxaluria [48]. Indeed, crystalluria globally reflects the risk of stone formation as it integrates the complex interactions between promoters and inhibitors of lithogenesis, both known and unknown, either measurable or not. In clinical practice, finding of crystals in the morning urine sample should alert the physician that one or several urinary lithogenic factors determined by urine chemistry analysis are not adequately controlled. Such a finding suggests considering daily urine volume and distribution together with excretion of calcium, oxalate, uric acid, citrate, magnesium, urea, and sodium excretion, in order to readjust dietary measures and institute or reinforce targeted drug therapy. In particular, finding of CaOx crystals in the first morning urine sample together with a high specific gravity highly suggests an excessive concentration of urine during the night, thus requiring increased fluid intake at bedtime. In addition, evidence of crystalluria contributes to motivate the patient to comply with recommendations.

In our patients, recurrence of stones was consistently preceded by the persistence or reappearance of crystalluria. Based on this finding, reinforcement of preventive measures, especially increase and/or better distribution of fluid intake, was usually followed by vanishing of crystalluria in parallel with arrest of stone recurrence.

Medullary sponge kidney appeared as an aggravating condition, as manifested by a higher spontaneous incidence of stone episodes than in patients without medullary sponge kidney and a higher proportion of recurrences during follow-up as observed by others [24, 25], despite similar baseline and follow-up values of daily urine volume and calcium excretion. As indicated by multivariate analysis, the protective effect of increased urine volume was less apparent than in the whole series, thus suggesting that a higher daily urine volume is to be achieved in medullary sponge kidney patients to reduce the risk of stone recurrence. This finding highlights the role of mechanic factors superimposed to metabolic ones [20], and confirms the need for hyperdiuresis in medullary sponge kidney patients in order to maximally reduce urine CaOx supersaturation and accelerate urine flow.

CONCLUSION

Our study provides evidence that crystalluria is highly indicative of stone disease activity in CaOx stone formers, and predictive of stone recurrence especially when repeatedly present in urine samples. In our experience, the predictive value of crystalluria was much greater than that of daily urine volume, 24-hour calcium excretion, or even urine calcium or oxalate concentration, the most influent factors in CaOx stone formers, thus indicating that other known or still unidentified factors contribute to the risk of CaOx stone formation. Indeed, crystal precipitation is the resultant of all factors, both promoters and inhibitors, measured and unmeasured, acting in urine to trigger crystal formation, the first step in lithogenesis. Because crystalluria study is a reliable, simple, and cheap method, serial crystalluria determination may be proposed as a useful clinical tool in the monitoring of CaOx stone former patients, in addition to urine biochemistry.

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