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ORIGINAL ARTICLE

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Cystine crystal volume determination: a useful tool in the management of cystinuric patients

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Abstract We prospectively determined cystine crystal volume (Vcys) in urine specimens from all consecutive patients with cystine urolithiasis followed at our institution over the past decade, in order to assess its predictive value as to the risk of recurrent cystine stone formation. A total of 57 patients (29 males, 28 females) with homozygous cystinuria entered in the study between January 1990 and December 2000, including 15 children aged less than 15 years and 42 patients aged 15 years or more. The clinical and radiological course was followed until December 2001, for a total of 243 patient-years of follow-up. From study entry until the end of follow-up, we serially examined first voided morning urine specimens in all patients, with determination of the number of cystine crystals per mm^3, and the average size of crystals, thus allowing us to calculate Vcys using a simple formula based on crystal geometry. Recurrence was diagnosed on the basis of serial radiographic examinations using X-rays and echography. Overall, cystine crystals were present in 179 (39%) of the 460 examined urine specimens. Cystine crystalluria was significantly more frequent among the 27 patients who developed new cystine stones (SF) than in the other 30 who remained stone-free (63.3 vs 25.5% of samples, $P < 0.001$). The presence of crystals in ≥50% of serially examined urine samples was more frequently found in patients with recurrent stone formation than in non-recurrent patients (24/27 vs 2/30, $P < 0.001$). The average Vcys value was significantly higher in recurrent SF than in stone-free patients ($8,173 \pm 1,544$ vs 233 ± 150 μ^3/mm^3, $P < 0.001$) and there was no overlap in the individual values of recurrent vs stone-free patients. A Vcys value ≥3,000 μ^3/mm^3 was observed at least once prior to each of the 63 stone recurrences observed in 27 patients (2.3 per patient on the average). In addition, Vcys reflected the efficacy of treatment, with Vcys mean values of $12,097 \pm 3,214$ μ^3/mm^3 at baseline, falling to $2,648 \pm 658$ μ^3/mm^3 on basic therapy (hyperdiuresis plus alkalinization) alone, $1,141 \pm 522$ μ^3/mm^3 on tiopronin therapy (median dose 1,000 mg/day) and 791 ± 390 μ^3/mm^3 on D-penicillamine therapy (median dose 900 mg/day) whereas captopril had no effect ($5,114 \pm 2,128$ μ^3/mm^3). Based on the results of the present study, cystine crystalluria appears to accurately reflect active stone formation in cystinuric patients. Determination of total Vcys provides a simple, cheap and accurate means of predicting the risk of cystine stone recurrence with a Vcys value ≥3000 μ^3/mm^3 as the threshold risk value. We propose that serial Vcys determination be performed simultaneously with the measurement of urine pH and specific gravity to optimally monitor the medical treatment of cystine patients.

Keywords Cystinuria · Cystine crystal volume
Alkalinization · Diuresis · Sulfhydriles · Stone recurrence

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Introduction

Effective chemoprophylaxis of cystine stones is often a difficult task. For years, preventative treatment used hyperdiuresis (≥3 l/day) to reduce urinary cystine

concentration [10], oral alkalinization to improve cystine solubility by means of sodium bicarbonate [10] or potassium citrate [13], and oral administration of sulfhydryl compounds such as D-penicillamine [6, 7] and alpha-mercaptopropionylglycine, or tiopronin [16, 23] to form mixed disulfides. These are much more soluble than cystine itself, thus reducing the amount of free cystine present in the urine. In addition, moderation in animal protein intake is an adjunctive measure aimed at reducing alimentary methionine load [25].

Contemporary medical treatment is based upon a stepwise strategy, using hydration and alkalinization as basic measures, with the addition of thiol derivatives in refractory cases [2, 3, 9, 15, 26]. Because urinary cystine concentration is highest during the night [11, 12, 17], large hydration and alkalinization at bedtime and during the night is recommended [2, 12, 15, 20, 27]. However, despite optimal management, control of stone formation often fails and stone recurrence is common [1, 2, 3, 14, 18, 23].

The management of medical treatment would be greatly helped if the solubility of cystine could be predicted in individual cases, in order to provide a rationale for the adaptation of treatment dosing. However, as pointed out by Coe et al. [4], if the measurement of urine pH and cystine concentration is easy, solubility of cystine in urine cannot be accurately predicted on the sole basis of free cystine concentration and pH. These authors proposed an assay for directly assessing cystine supersaturation in urine even in the presence of thiol derivatives [4], but the relationship between this promising index and clinical outcomes has not been established. In a previous study, based on a large series of cystinuric patients, we analyzed factors associated with the success or failure of stopping cystine stone formation [2]. We observed no clear difference with respect to daily cystine excretion, achieved urinary pH or thiol-derivative administration. On the contrary a urine volume of less than 3 l/24 h and the presence of cystine crystals and/or specific gravity ≥1,010 on repeat determinations made on morning urine samples often predicted stone formation or growth. However, in order to better quantify the amount of cystine crystals present in urine, we have developed, over the past decade, a method allowing the determination of cystine crystal volume (Vcys) in urine. Such a determination was prospectively performed at each visit for all cystinuric patients under our care. We present here an analysis of the correlations between total Vcys and clinical outcomes, and supply evidence that this parameter closely reflects the efficiency of medical treatment, and provides an accurate index of the risk of recurrence of stone formation.

Patients and methods

We prospectively determined pH, presence of cystine crystalluria and Vcys in 460 first morning urine specimens from 57 patients with homozygous cystinuria followed at our institution from January 1990 until December 2000. The patients comprised 29 males and 28 females. The mean age at start of the study was 24.5 ± 14.6 years for males and 27.0 ± 12.9 years for females. Fifteen patients were aged less than 15 years.

At referral, 31 patients were stone-free, whereas the other 26 had unilateral or bilateral calculi. Baseline cystine excretion ranged from 302 to 1,120 mg/day (mean 824 ± 272 mg). The clinical course of the patients was updated until December 2001, so that all patients had a follow-up of at least 1 year under our care, and at least three urine examinations during follow-up.

An homogeneous medical regimen, as reported elsewhere, was applied to all patients [2]. This involved hyperdiuresis aimed at achieving a total urine volume of at least 3 l/day, alkalinization aimed at maintaining the urine pH at about 7.5 by means of sodium bicarbonate in 46 patients (8–18 g/day according to body weight) or potassium citrate (60–80 meq/day) in six patients. A thiol derivative, either D-penicillamine (600–1,200 mg/day) or tiopronin (500–1,500 mg/day), was added when the standard therapy failed to prevent stone recurrence or growth, at least half of the dose being administered at bedtime. Captopril (100–150 mg/day) was used transiently in six patients.

Clinical and radiological surveillance was scheduled every 3–4 months during the initial 2–3 years, and every 6 months thereafter, or at a shorter interval when needed. Remission (or complete therapeutic success) was defined as arrested new stone formation and sustained stone-free status. Recurrence of stones was defined as radiographic evidence of newly formed stone(s) more than 3 mm in diameter when identified only by echography, or more than a 2 mm increase in diameter of pre-existing stone(s). Therapeutic measures were reinforced after every recurrence was diagnosed. The presence of cystine crystals and Vcys were determined in urine specimens collected during the periods preceding stone recurrence, as well as in periods free of stones.

Samples used for the determination of Vcys were first voided morning urine specimens, because urine cystine concentration has been shown to be highest during the night and therefore first morning urine is the most likely to contain crystals, thus reflecting the risk of stone formation. Well-homogeneized urine samples set in a Malassez cell were examined by light microscopy. Crystal volume was determined using the formula: $Vcys = 0.65 \times N \times L^2 \times T$, where N = number of cystine crystals per mm^3, L = average length of the crystals measured between two opposite angles and T = average thickness of the crystals. Vcys was expressed as μ^3/mm^3 of urine, including both isolated crystals and aggregates. A median number of seven urine specimens per patient was available (range: 3–57). The results are expressed as mean ± SEM unless otherwise specified. We performed statistical analysis using χ-square tests as well as analysis of variance with $P < 0.05$ considered statistically significant.

Results

Incidence of recurrences during follow-up

During follow-up at our institution, 30 patients remained stone-free for a total of 104 patient-years, whereas at least one stone recurrence occurred per patient among the other 27 patients (from one to three recurrences, mean(±SD) 2.3 ± 1.4 episodes per patient) during a total of 139 patient-years. Thus, the incidence of stone recurrence was 0.27/100 patient-years.

Crystalluria

Overall cystine crystalluria was present in 179 (39%) of the 460 analyzed specimens. The presence of crystals was

significantly more frequent in patients with persisting stone formation than in those with arrested formation (63.3% vs 25.5% of samples, $P < 0.001$). The presence of crystalluria in more than half of the examined samples was observed in a significantly higher proportion of recurrent stone formers than of patients with arrested formation: 24/27 (88.8%) vs 2/30 (6.7%) ($P < 0.001$).

Relationship between crystal number and Vcys

Vcys was dependent on both crystal number and size (Table 1). When Vcys was very low, $< 500\ \mu^3/mm^3$, the average number of cystine crystals was $< 1/mm^3$ and their average dimension was about 11 μm. When the crystal mass was 500–$< 3{,}000\ \mu^3/mm^3$, the average density of crystals was seven times greater, and their average diameter was larger. In patients with the highest Vcys values, $\geq 3{,}000\ \mu^3/mm^3$, both the average diameter and density of crystals rose strikingly, the latter being nine times higher than in the group 500–$< 3{,}000\ \mu^3/mm^3$.

Cystine crystal volume and recurrence

A total of 63 stone recurrence episodes were observed in 27 patients (2.3 episodes per patient). The average value of Vcys was significantly higher in patients with recurrent stone formation than in those with arrested formation ($8{,}173 \pm 1{,}544$ vs $233 \pm 150\ \mu^3/mm^3$, $P < 0.001$). A Vcys value $\geq 3{,}000\ \mu^3/mm^3$ was observed at least once in all patients who subsequently developed evidence of stone recurrence (mean = 2.3 urine specimens per recurrent stone patient), but never in non-recurrent patients.

As shown in Fig. 1, there was no overlap between the lowest Vcys value observed in recurrent stone formers ($4{,}284\ \mu^3/mm^3$) and the highest value observed in patients who remained free of recurrence ($2{,}857\ \mu^3/mm^3$).

Influence of therapy

The average value of Vcys according to the current therapy is shown on Table 2.

Hyperdiuresis with alkalinization reduced the average value of Vcys from $12{,}097 \pm 3{,}214\ \mu^3/mm^3$ at baseline to $2{,}648 \pm 658\ \mu^3/mm^3$ ($P < 0.05$). Addition of captopril to the basal treatment did not result in a significant beneficial effect. In contrast, tiopronin (at the median dose of 1,000 mg/day) and D-penicillamine (at the median dose of 900 mg/day) significantly reduced Vcys when compared to hydration and alkalinization alone.

Irrespective of drug therapy, urinary pH had a significant influence on Vcys (Table 3). Urine pH between 7.5 and 8.0 was associated with effective cystine solubilization, as reflected by a Vcys $< 500\ \mu^3/mm^3$.

Table 1 Relationships between Vcys, number and size of cystine crystals

Vcys (μ^3/mm^3)	< 500	500–$< 3{,}000$	$\geq 3{,}000$
No. of samples	73	40	66
Mean length of crystals (μm)	11.2 ± 0.7	15.7 ± 1.4[a]	18.3 ± 1.2[b]
Crystals (no./mm^3)	0.7 ± 0.1	4.8 ± 0.9[b]	46.9 ± 11.3[c]

[a] $P < 0.01$ vs < 500; [b] $P < 0.001$ vs < 500; [c] $P < 0.01$ vs 500–3,000

Discussion

Patients with cystine urolithiasis are exposed to a high risk of recurrent stone formation. Poorly controlled cystinuria often leads to progressive impairment of renal function, and even to end-stage renal failure [1, 14, 18].

Cystine excretion in homozygous subjects is as high as 600–1,400 mg/day [2], whereas the limit of solubility of cystine in urine is about 250 mg/l at pH values < 7.5 [10].

The generally accepted medical treatment aimed at preventing the formation of cystine stones is based on hyperdiuresis and alkalinization to increase cystine solubilization, with the addition of a thiol derivative to decrease urinary free cystine excretion [2, 3, 9, 15, 26]. However, clinical outcomes do not consistently correlate with free cystine daily excretion, because the solubility of cystine, which depends on supersaturation in urine,

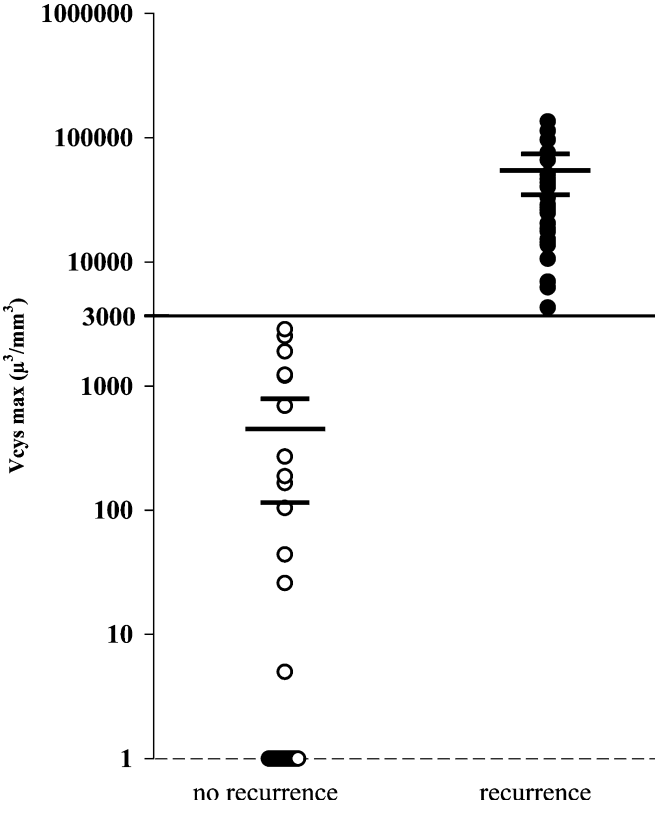

Fig. 1 Relationship between cystine crystal volume (Vcys, log scale) and stone recurrence

Table 2 Vcys (μ^3/mm^3) according to therapy

	Baseline	Basal (alkalinisation + hydration)	Basal + captopril	Basal + tiopronin	Basal + D-penicillamine
No. of samples	45	232	46	72	65
Crystalluria (presence %)	61.8	33.7	34.4	35.4	20.0
Vcys (μ^3/mm^3)	12,097 ± 3,214	2,648 ± 658[a]	5,114 ± 2,128	1,141 ± 522[b,c]	791 ± 390[b,c]
Vcys range (μ^3/mm^3)	0–168,214	0–50,285[a]	0–93,474	0–26,111	0–21,407

[a] $P < 0.05$ vs baseline; [b] $P < 0.01$ vs baseline, [c] $P < 0.01$ vs basal

Table 3 Effect of urine pH on Vcys ($\mu^3/mm^3 \pm SE$)

pH	< 6.0	6.0–6.4	6.5–6.9	7.0–7.4	7.5–8.0	≥8.0
No. of samples	29	33	45	67	61	42
Vcys (μ^3/mm^3)	16,965 ± 6,897	9,196 ± 3,143	4,498 ± 1,412	1,333 ± 466[a]	707 ± 287[b]	94 ± 61[c]

[a] $P < 0.05$, [b] $P < 0.02$, [c] $P < 0.01$ vs pH < 7.0

cannot be accurately estimated on the basis of urinary pH and cystine concentration [4, 21, 22] even using a pH nomogram [10]. Therefore, there is a need for laboratory criteria providing a direct assessment of the efficacy of medical treatment, in order to guide changes in therapeutic strategy and drug dosing.

Sakhaee [26] proposed the adaptation of the daily dose of thiols to baseline total cystine excretion, in order to reduce the crystallization risk. However, the molar quantity of cystine that D-penicillamine or tiopronin is able to complex is low and variable [4, 16] and there is no direct relationship between cystine excretion and solubilization. Lindell et al. [16] proposed the adjustment of the tiopronin dosage on the measurement of urinary free cystine. Again, however, there is no direct relationship between free cystine concentration and solubility.

Several authors proposed monitoring urinary pH and specific gravity over whole day and night cycles, with the adaptation of the dose of alkali and/or thiols to achieve a pH of about 7.5 and a specific gravity < 1010 over the entire nyctohemera [9, 11, 16]. Maintaining urinary pH and specific gravity within these limits during the night is especially important [12, 20], but monitoring urine at night is not practicable. Moreover, there is again no close relationship between urinary pH, cystine concentration and cystine solubility.

Because urine supersaturation is the driving force for crystallization in cystinuria, the direct measurement of supersaturation should provide a more relevant assessment of the potential solubility of cystine. Pak and Fuller [22] proposed a method for such assessment, but this was only valid in the absence of cystine binding drugs. More recently, Coe and co-workers [4, 21] proposed a solid phase assay of urine cystine supersaturation which is valid both in the presence and absence of cystine binding drugs, but its predictive value on the risk of stone formation remains to be clinically validated.

We propose here a simple, easy and cheap means of assessing the effect of any treatment schedule, based on the quantitative evaluation of cystine crystalluria. We elected to use first voided morning urine specimens, because the highest risk of cystine crystal formation occurs during the night [10, 12, 17], and the first voided morning sample reflects the composition of urine formed during the preceding nocturnal period. Recently, Fjellstedt et al., prospectively using separate day and night urine collections in 26 cystinuric patients followed over 3.5 years, detected 56 episodes of high urinary cystine concentration, half of which occurred during the night, and would have been missed on the basis of only 24 h urine analysis [12]. The predictive value of Vcys was established on the basis of a large cohort of patients followed for a long duration. In our experience, the absence of any cystine crystal, or a Vcys < 3,000 μ^3/mm^3 was associated with the absence of cystine stone formation as assessed by clinical imaging criteria, whereas the presence of multiple crystals (> 20/mm^3), together with Vcys > 3,000 μ^3/mm^3, was predictive of stone recurrence, even if observed in only one urine sample. This index was highly discriminating, as there was no overlap between the lowest value of Vcys in recurrent patients (4,284 μ^3/mm^3) and the highest value in non-recurrent patients (2,857 μ^3/mm^3). Therefore, we propose to adopt a rounded threshold value of 3,000 μ^3/mm^3.

As emphasized in Table 2, the critical point in the management of cystinuric patients is the day to day attention to medical advice for permanently maintaining high diuresis and suitable alkalinization of urine. Recently, Pietrow and co-workers underlined the difficulty of cystinuric patients achieving therapeutic success due to poor compliance with medical recommendations [24]. In our experience, several patients developed new stones following short periods with very high Vcys while on basal therapy due to low urine pH and/or insufficient diuresis. This was the result of poor compliance. As illustrated by the higher mean Vcys in their first morning urine sample, patients who are only treated with alka-

linization and hydration measures are more at risk of stone recurrence than patients on thiol derivative therapy. These results underline the crucial importance for each patient of simultaneously maintaining high values for both diuresis and urine pH day after day. Some patients may develop new stones when treated with a thiol derivative if they do not comply with the daily recommended drug dose. Another point of clinical relevance is that the immediate correction of therapeutic measures (reinforced alkalinization and high diuresis) following the observation of a Vcys above 3,000 μ^3/mm^3 may prevent stone recurrence as we observed in two new patients recently managed in our institution.

Our study:

1. confirms the effectiveness of basal therapy with hyperdiuresis and alkalinization to reduce or suppress the precipitation of cystine crystals, as Vcys was significantly lower in patients receiving this treatment at baseline. However, the optimal reduction of Vcys was observed only if the urine pH value was above 7.0 and urine volume >3l/day, as previously observed [2].
2. argues against the efficacy of captopril to reduce cystine crystalluria, in contrast with the findings of some authors [28] but in agreement with others [5, 8, 19].
3. confirms the effectiveness of D-penicillamin and tiopronin in reducing the risk of cystine crystallization in addition to alkalinization and hyperdiuresis.

In conclusion, Vcys is a direct reflection of the activity of crystal formation in cystinuric patients. Therefore, Vcys determination provides a simple and easy method of assessing the solubility of cystine, and predicting the risk of cystine stone formation, thus allowing an adjustment of therapy when needed. We propose that the serial determination of Vcys be added to serial measurements of urine pH and specific gravity to optimally monitor the medical treatment of cystinuric patients.

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