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N. Laube · B. Hoppe · A. Hesse

Problems in the investigation of urine from patients suffering from primary hyperoxaluria type 1

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Abstract Regular calculation of urinary crystallization risk indices in patients suffering from urolithiasis is a recommended measure for treatment adjustment. The more the patient experiences either extensive stone formation or an enhanced recurrence rate, the more important risk index calculations. In patients suffering from primary hyperoxaluria type 1 (PH1), both criteria are met. Different methods of risk determination are known. All strategies for measuring the calcium oxalate (CaOx) crystallization risk of a given urine principally determine this parameter from voided urine. This ''bladder urine'', however, has possibly passed stone material located in the urinary tract and thus may be depleted in lithogenic components. This is commonly the case for patients with PH1, who mostly experience a massive stone burden or severe nephrocalcinosis. Hence, the question arises as to whether we can adequately determine the crystallization risk in the urine of stone-bearing PH1-patients or not. Based on model calculations, we show that the determination of CaOx formation risk in PH1-patients requires knowledge of the restrictions in risk index interpretation: risk indices calculated from urinalysis (e.g. EQUIL) still indicate, even after strong in vivo stone formation, an enhanced but in fact strongly underestimated risk value. However, the outcome ''enhanced'' masks the patient's true risk situation. The BONN Risk Index (BRI), in contrast, discloses the process of extreme in vivo crystal formation. As determined, inter alia, from the urinary concentration of free ionized calcium ($[Ca^{2+}]$), BRI approaches abnormally low values, as, in consequence of CaOx - formation, $[Ca^{2+}]$ tends to values close to zero. Thus, calculations of urinalysis-based risk indices alone are insufficient strategies for the quantification of a PH1 patient's CaOx crystallization risk.

Keywords Primary hyperoxaluria · Crystal formation risk · Depletion effect · Urinalysis interpretation

N. Laube (✉) · A. Hesse
Division of Experimental Urology, Department of Urology,
University of Bonn, Sigmund Freud Strasse 25,
53105 Bonn, Germany
E-mail: norbert.laube@ukb.uni-bonn.de
Tel.: +49-228-2879106
Fax: +49-228-2876344

B. Hoppe
Division of Pediatric Nephrology,
University Children's Hospital, Cologne, Germany

Introduction

Regular calculation of urinary crystallization risk indices in patients suffering from urolithiasis is a recommended measure for treatment adjustment. The more the patient experiences either extensive stone formation (increase in stone mass/time) or an enhanced recurrence rate (colics/time), the more important risk index calculations become. In patients suffering from primary hyperoxaluria type I (PH1), both criteria are met.

Different methods of risk determination are known. All strategies measuring the calcium oxalate (CaOx) crystallization risk of a given urine principally determine this parameter, e.g. the BONN Risk Index (BRI) [1], AP(CaOx) [2], RS(CaOx) [3], from a voided urine. This ''bladder urine'', however, has possibly passed stone material located in the urinary tract and thus may be depleted for lithogenic components. This is commonly the case in patients with PH1, who usually experience a massive stone burden or severe nephrocalcinosis [4].

Hence, the question arises as to whether we can adequately determine the crystallization risk in urines of stone-bearing PH1 patients.

Only a certain amount of the excreted urinary calcium is available for calcium oxalate formation as a variably large fraction is bound, e.g. to proteins. The unbound, i.e. free, ionized urinary calcium contributes to stone formation (risk). The concentration ratio of the free ionized urinary calcium, $[Ca^{2+}]$, and the total urinary calcium, $[Ca_{tot}]$, is highly variable at an individual level.

Investigation of 350 urine samples of recurrent CaOx stone-formers ($n=250$) and healthy volunteers ($n=100$) revealed $[Ca^{2+}]/[Ca_{tot}]$ ratios of between

0.052 and 0.752, and 0.009 and 0.408, respectively. The mean values and related standard deviations were 0.321 (0.121), and 0.208 (0.007), respectively. $[Ca^{2+}]$ was only moderately correlated to $[Ca_{tot}]$. We derived the following relationship between $[Ca^{2+}]$ and $[Ca_{tot}]$: $[Ca^{2+}] = 0.2383 \times [Ca_{tot}]$; $r = 0.703$ (unpublished data).

Almost any urine, even from non-stone formers, is indicated by, inter alia, a certain relative supersaturation with respect to CaOx [RS(CaOx)]. Thus, urinary composition reflects a metastable solution and a certain positive driving force exists to form crystals.

In the case of precipitation of calcium oxalate, both urinary $[Ca_{tot}]$ and $[Ca^{2+}]$ decrease equal to salt formation (law of mass conservation). As a result of continued depletion, $[Ca_{tot}]$ and $[Ca^{2+}]$ tend towards a minimum level which is defined by the extent and duration of crystal formation. Theoretically, at the end of crystal formation, RS(CaOx) = 0, i.e., supersaturation equals 1.

What are the consequences of crystallization taking place in vivo? In particular, in patients suffering from PH1, the formation of mineral deposits, not only in the urinary tract, but also in the entire body, is a common implication of the disease [4]. Thus, it is reasonable to assume that crystal formation is a permanent process in PH1 patients altering, at least, their urinary composition.

Precipitation systematically reduces the concentrations of the lithogenic substances in the solution, e.g. urine. Thus, the determined in vitro crystallization risk results in a diminished estimate of the true situation. In fact, crystallization risk indices estimated from PH1 patients relatively often reflect low values which clearly do not correlate with the individual severity of stone disease [5].

In a first approach, the extent of the depletion effect can be determined for each patient's individual situation [6, 7, 8].

Model calculations

Some simple model calculations can illustrate the problem (Table 1).

Assuming a "typical" normal subject's 24-h-urine composed of, inter alia, 5.0 mmol/l $[Ca_{tot}]$ and 0.5 mmol/l $[Ox_{tot}]$; the volume is 1.5 l. From these data urinary Ca and oxalate excretions of 7.5 mmol/day and 0.75 mmol/day, respectively, can be obtained; this urine reflects a normocalciuric and "mild hyperoxaluric" (limit 0.56 mmol/day [9, 10]) state. As for any urine, it depicts a certain relative supersaturation with respect to CaOx, RS(CaOx), i.e., the urinary composition reflects a metastable solution and a certain positive driving force to form crystals.

Based on the mean $[Ca^{2+}]/[Ca_{tot}]$ ratio of 0.208 in healthy subjects, and the assumed urinary composition, the respective $[Ca^{2+}]$ value of the healthy subject amounts to 1.04 mmol/l. This concentration is still in excess of the corresponding $[Ox_{tot}]$ of 0.5 mmol/l, and the (unknown) concentration of free, unbound ionized oxalate, $[Ox^{2-}]$, with $[Ox^{2-}] \leq [Ox_{tot}]$.

Thus, in the theoretical case of quantitative CaOx-formation, a maximum of 49.37 mm^3 of salt can be formed out of 1.5 l of urine ($M = 65.82$ cm^3/mol, [6]), if the total amount of oxalate is available for calcium complexation, i.e. $[Ox_{tot}] = [Ox^{2-}]$, and $[Ca^{2+}] = 0$ mmol/l at the end of crystal formation. This hypothetical volume corresponds to a sphere of approximately 4.55 mm in diameter. It is obvious that the *real* $[Ox^{2-}]$ value limits the amount of CaOx potentially formed.

For further simplification, let us assume that $[Ox^{2-}] = 0.5$ $[Ox_{tot}]$. Now, only 0.25 mmol/l urinary oxalate can be used for CaOx formation. From the precipitated volume of 24.68 mm^3, a sphere of approximately 3.61 mm in diameter can be obtained from 1.5 l of the model urine per day.

Stone growth rates of 25 mm^3/day in high-risk stone-formers have been observed [6, 7]. This growth rate, when taking place over a period of 1 year, corresponds, for example, to a diameter increase of a single spherical stone of from initially 5 mm to ≈26 mm.

In the model urine calculations above, the hypothetical stone growth rate also amounts to approximately 25 mm^3/day.

When analyzing the remaining urine, a $[Ca_{tot}]$ and a $[Ox_{tot}]$ of 4.75 mmol/l and 0.25 mmol/l can be obtained. The corresponding Ca and Ox excretions are 7.125 mmol/day and 0.375 mmol/day, respectively. It is obvious that, as a side effect of the stone growth related

Table 1 Parameters used and model calculations. Ca* calcium excretion, Ox* oxalic acid excretion, N healthy volunteers, PH1 patients suffering from primary hyperoxaluria type 1, Max. CaOx maximum volume of calcium oxalate monohydrate ($M = 65.82$ cm^3/mol) which can be theoretically formed from urine if either Ca or Ox is completely used up. Data obtained from recurrent CaOx stone-formers

Parameter		Unit	N	PH1	Max CaOx [mm^3/day]	
a	$[Ca_{tot}]$	mm/l	5.0	5.0	N	PH1
b	$[Ox_{tot}]$	mm/l	0.5	5.0		
c	V	l/day	1.5	1.5	[a,b,c]49.37	[a,b,c]493.65
d	Ca*	mm/l	[a,c]7.5	[a,c]7.5	↓	↓
e	Ox*	mm/l	[a,b]0.75	[b,c]7.5		
f	$[Ca^{2+}]/[Ca_{tot}]$	-	0.208	0.321[1]		
g	$[Ca^{2+}]$	mm/l	[a,f]1.04	[a,f]1.61	[b,c,g]49.37	[b,c,g]158.96
h	$[Ca_{bound}]$	mm/l	[b,g]3.96	[b,g]3.39	↓	↓
i	$[Ox^2] = 0.5[Ox_{tot}]$	mm/l	0.25	2.5	[c,g,i]24.68	[c,g,i]158.96

urinary depletion, the urinary composition apparently turned into a ''normooxaluric'' state.

Similar rough computations can be made for a ''model PH1-patient'': this person typically has a total urinary oxalate concentration up to ten times above normal, amounting to 5.0 mmol/l ($[Ca_{tot}] = [Ox_{tot}]$), and a higher $[Ca^{2+}]/[Ca_{tot}]$ ratio of 0.321. Table 1 shows that the CaOx volume of approximately 160 mm^3, which can be obtained from this urine, is, as expected, much higher than the one computed from a healthy person's urine. Even after this dramatic crystallization process, urinalysis of the bladder urine will result in $[Ca_{tot}] = 2.5$ mmol and $[Ox_{tot}] = 2.5$ mmol/l. The corresponding excretion values are both 3.75 mmol/day.

Even from this strongly depleted urine, RS(CaOx) and AP(CaOx) values can be computed as $[Ca_{tot}]$ and $[Ox_{tot}]$ remain positive because the initially strongly protein-bound Ca cannot contribute to crystal formation, but appears in urinalysis. The BRI, which focuses on the free, unbound urinary $[Ca^{2+}]$, however, will drop to values close to zero as $[Ca^{2+}]$ approaches 0 mmol/l, due to the presumably extremely high corresponding $[Ox^{2-}]$. This is in contrast to the situation in ''normal stone formers'' where $[Ca^{2+}] > [Ox^{2-}]$.

Discussion

In the case of PH1-patients, $[Ox_{tot}]$ of the initially formed urine normally exceeds 0.5 mmol/l; these patients often present with strongly elevated urinary $[Ox_{tot}]$ of the (potentially) depleted bladder urine exceeding 1.0 mmol/l. While the $[Ca_{tot}]/[Ox_{tot}]$ ratio of healthy subjects amounts to around 10, that of PH1-patients often reaches values close to 1. Due to the extremely high crystallization pressure, the above outlined scenario of a stone formation related urinary depletion will be more quantitative: $[Ca_{tot}]$ and $[Ox_{tot}] \rightarrow$ minimum, $[Ca^{2+}] \rightarrow 0$ mmol/l. In fact, due to the law of mass action, a certain steady state concentration of free lithogenic ions still remains in solution at end of crystal formation. In supersaturated CaOx solutions, approximately 99% of the ions precipitate as CaOx. Thus, $[Ca^{2+}]$ will not reach 0 mmol/l.

While in PH1 patients, urinalysis-based risk indices reflect a certain amount of ''residual risk'', BRI, in contrast, reflects only low values which are clearly below the limit value of BRI = 1/l as $[Ca^{2+}]$ drops close to zero (Fig. 1).

No crystallization risk value determined from highly depleted bladder urine can sufficiently reflect the patient's real risk state.

Normal CaOx stone-formers are indicated by a high RS(CaOx) and a high BRI [1]. In contrast, PH1 patients tend to reflect high but not, as one may expect, extremely high RS(CaOx) (Fig. 2), in combination with a low to very low BRI.

The question is, why RS(CaOx) in PH1 patients is not higher than in ''normal CaOx stone-formers''. This is explained by the fact that RS(CaOx) is com-

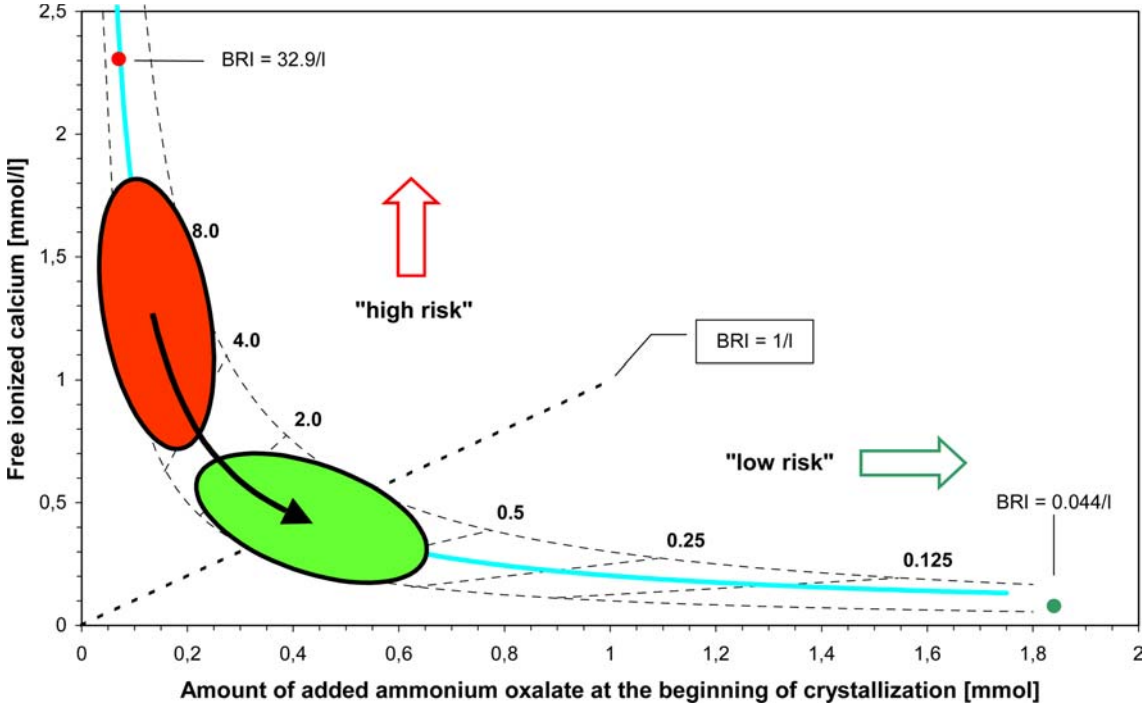

Fig. 1 Hypothetical situation to illustrate the effect of in vivo stone formation on the BONN Risk Index. Due to high urinary oxalate concentrations in the initially formed urine of PH1 patients, ''mass'' precipitation of CaOx takes place in the urinary tract. The initial urinary concentration of free ionized calcium consecutively drops to moderate to low concentrations; the results of crystal formation risk investigations in the voided urine, thus, reflect a misleading, only virtually low, risk situation for the patient. In extreme cases of in vivo crystal formation we observed BRI values below 0.25 /l

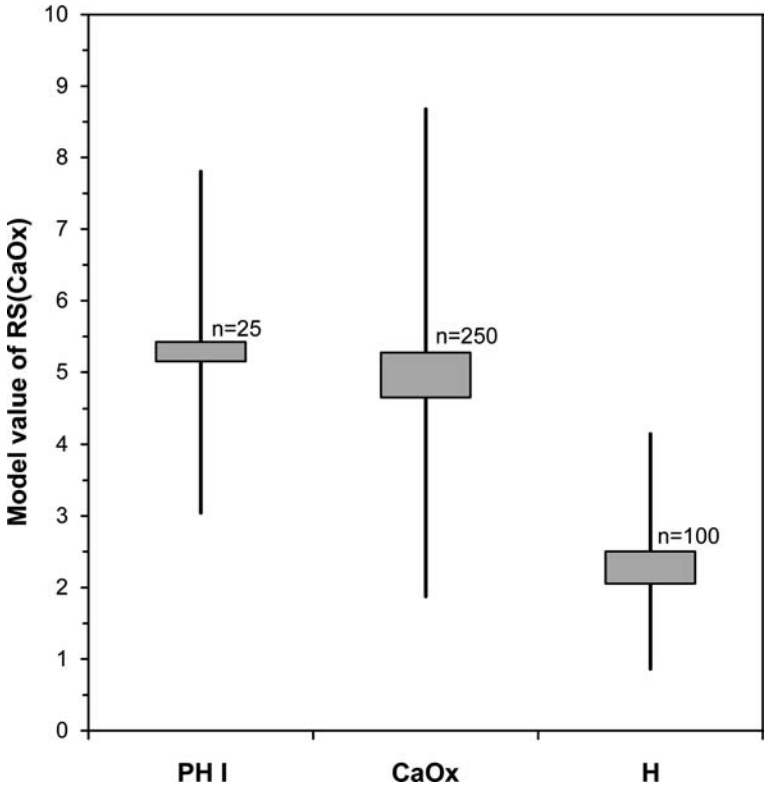

Fig. 2 Calculated model values of the urinary relative supersaturation with respect to calcium oxalate, RS(CaOx) [3]. *Boxes* indicate difference between arithmetic mean (*top*) and median (*bottom*) of observed RS(CaOx). *Vertical lines* depict the range of the standard deviation. Whereas the healthy subjects' urines (*H*) reflect, as expected, low RS(CaOx), the respective values of PH1 and recurrent CaOx stone formers, however, are nearly identical, although one may assume that PH1 patients should be indicated by much higher RS(CaOx)

puted from [Ca_{tot}], i.e., both the bound and unbound calcium are considered in the result. Since in PH1-patients a high probability exists that [Ca^{2+}] is close to zero, [Ca_{tot}] in these patients is only governed by the concentration of bound Ca. Obviously, in normal CaOx stone-formers and PH1 patients, this concentration is similar.

Urinary close-to-zero [Ca^{2+}], determined from a native urine sample, provides a strong indication of in vivo crystal formation.

Thus, RS(CaOx) and AP(CaOx) calculations alone are insufficient strategies for the quantification of a PH1 patient's CaOx crystallization risk. It may be argued that a therapy-related moderate increase in BRI in PH1 patients is a first indication of therapeutic success, as the bladder urine now reflects an enhanced residual crystal formation risk, i.e., quantitative CaOx precipitation has not taken place in vivo.

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