The Abnormal Red-Cell Oxalate Transport Is a Risk Factor for Idiopathic Calcium Nephrolithiasis: A Prospective Study

Giovanni Gambaro, Francesco Marchini, Antonio Piccoli, Maria Angela Nassuato, Franca Bilora, and Bruno Baggio

ABSTRACT

An abnormal erythrocyte transmembrane oxalate flux was described in recurrent idiopathic calcium nephrolithiasis. To verify whether it might represent a risk marker of renal stone disease, two prospective studies were carried out. One hundred ninety patients with idiopathic calcium nephrolithiasis who were enrolled at their first episode of lithiasis during the period 1984 to 1986, form the basis of the first prospective study. The impact of erythrocyte oxalate transport anomaly, gender, familial occurrence of nephrolithiasis, hypercalciuria, hyperoxaluria, and hyperuricosuria on stone recurrence by both bivariate and multivariate analysis of frequencies was assessed. The predictive value of the erythrocyte anomaly for a patient's becoming a stone former was also assessed in five nephrolithiasic families. Recurrence occurred in 57.9% of patients; this was significantly associated with the erythrocyte anomaly, hyperoxaluria, and male gender. However, when using multivariate analysis, only gender and the erythrocyte anomaly were statistically significant and were independent predictors of recurrence. The probability of stone recurrence predicted by the logistic model ranged from 30.1% for women with normal erythrocyte oxalate transport, to 73.4% for men with the erythrocyte anomaly. The family follow-up showed that only subjects with the erythrocyte abnormality become renal stone-formers in the 8-yr survey. By showing the predictive value of the erythrocyte oxalate anomaly for recurrent calcium nephrolithiasis, our findings support the notion that this anomaly is a risk factor in renal stone disease.

Key Words: Renal stone, recurrence, hypercalciuria, hyperoxaluria, predictivity

In 1984, we described an anomalous erythrocyte transmembrane oxalate flux (Kox) in a group of patients with recurrent idiopathic calcium nephrolithiasis (ICN) (1). This defect is observed in primary calcium lithiasis, is not present in secondary forms, and is determined genetically as an autosomal monogenic trait with complete penetration and variable expressivity (2). Moreover, more rapid intestinal absorption of oxalate is associated with increased erythrocyte oxalate flux (2). The Kox anomaly seems to depend on abnormal phosphorylation levels of some membrane proteins, including the band 3 anion carrier (3). The anomaly seems to be worldwide and highly represented in renal stone-formers because it has been reported in the United States by Motola et al. (4) and, in abstract form, in India by Narula et al. (5), in the United States by Jenkins et al. (6), and in Japan by Takahiro et al. (7).

The role of this anomaly in nephrolithiasis is still unknown. Although a number of physiopathological mechanisms have been suggested to link the abnormal erythrocyte oxalate flux with the pathogenesis of calcium nephrolithiasis (8), there is no definite demonstration of this. Certainly the demonstration that this erythrocyte anomaly is a risk factor of nephrolithiasis should support its pathogenetic role.

We addressed this hypothesis by a prospective analysis of the tendency of renal stone disease (1) to recur in a cohort of single-stone-forming patients, in relation to the presence or lack of presence of the Kox anomaly, and previously proposed stone risk markers; and (2) to manifest in five nephrolithiasic families in which the Mendelian inheritance of the anomaly had been previously reported (2).

METHODS

Study Protocol

The patients in this study were subsequently selected from over 650 stone-formers who were admitted to our outpatient clinic during the period 1984 to 1986. To avoid any bias by previous behavioural and/or pharmacological therapies on the natural history of the disease, and confusion in the evaluation of relapses at follow-up, only patients with ICN at their first episode of lithiasis and who were stone-free at the beginning of the study were enrolled. The calcic nature of stones was deduced from chemical analysis and/or by radiological examination. Secondary forms of renal lithiasis were excluded on the basis of biochemical parameters (blood levels of total calcium, phosphate, urate, parathyroid hor-
mone whole molecule, ionized calcium or urine cAMP when necessary, urine cystine, anamnestic records (enteric diseases, gout, prolonged immobilization, urinary tract infections), and radiological findings (intravenous urography and echography). The first-stone ICN patients were given only generic dietary (balanced, normocaloric diet) and behavioral suggestions (water intake to maintain diuresis at approximately 2 L/day), and no pharmacological treatment was administered for stone prevention. Thus, 231 subjects fulfilled the above criteria, and were enrolled; however, 41 were excluded from our analysis because they had taken some drugs that are known to possibly interact with renal lithiasis during the observation period, or were lost to follow-up. Therefore, 190 patients form the basis of the present investigation: 65 women (mean age, 34 yr; range, 18 to 56 yr) and 125 men (mean age, 32 yr; range, 17 to 51 yr). In 80%, the chemical composition of the passed stone was available and disclosed that 64% of the stones were pure calcium-oxalate and 36% were mixed (oxalate, phosphate) calcium stones. In the remaining 20%, the radiological examination revealed a radioopaque gravel.

Before admission to the study, erythrocyte oxalate self-exchange was evaluated in all patients as previously described (2), and at least three basal analyses of 24-h urinary calcium (atomic absorption), oxalate (oxalate oxidase method), and urate (urate method) excretion rates were performed. By setting the normal upper limits for 24-h urine excretion rates of calcium, oxalate, and urate at 7.56, 0.50, and 4.96 mmol, respectively (9), 21% of the patients were hypercalciuric, 28% hyperoxaluric, and 11% hyperuricosuric. Furthermore, 3.5% of them presented contemporaneously all of the above urinary abnormalities, 5.8% of patients were hyperoxaluric and hyperuricosuric, 2.3% was hypercalciuric and hyperuricosuric, and 4.6% were hyperoxaluric and hyperuricosuric. During the observation period, the patients were examined at least once each year in our clinic; follow-up was completed in December 1993.

Patients were considered to be recurrent stone-formers if they had experienced at least one more stone episode after the first one that led them to our observation. Recurrences were deduced from spontaneous expulsion, radiographic demonstration of opaque stones, or the need for open or closed urological interventions. In these patients, the observation period stopped at relapse, and they were then treated according to the metabolic activity of the disease: some continued with dietary/behavioral treatment, whereas others received pharmacological therapy (thiazides, allopurinol, citrate), and some patients were lost to follow-up. The clinical features of these patients after relapse were not analyzed.

In addition to Kox, the impact on stone recurrence of the following risk factors was determined: gender, familial occurrence of nephrolithiasis, hypercalciuria, hyperoxaluria, and hyperuricosuria. Continuous parameters were transformed into binary (yes, no) variables, with the following cut-off values and inequalities for the abnormal category: Kox higher than 0.58 x 10^-2 min^-1; urine excretion rates of oxalate, calcium, and urate over 0.50, 7.56, and 4.96 mmol/24 h, respectively. The cut-off value 0.58 x 10^-2 min^-1 for Kox was determined in 1986 as the 99% upper normal value of the Kox distribution in control subjects (2). Furthermore, we recently reevaluated 40 of the 42 members (two had died) of the five families in the original study, which described the hereditary transmission of the oxalate self-exchange anomaly (2). By 1986, 32 of these subjects had never formed stones, although 19 subjects presented with the cell abnormality.

### Statistical Methods

Statistical analysis was performed by the Receiver Operating Characteristic (ROC) curve (10), and with the BMDP statistical package (11). The ROC curve was obtained by plotting both the sensitivity (on the vertical axis) and the false-positive rate (on the horizontal axis) in predicting recurrence for each defined Kox. The association between stone recurrence and risk factors was evaluated by both bivariate (chi-squared test, BMDP program 4F) and multivariate analysis of frequencies (multiple logistic regression, BMDP program LR). Family data were analyzed by the McNemar's test (BMDP program 4F).

### RESULTS

Among the 190 ICN patients, 110 (57.9%) had experienced recurrence at some time point of the observation period (Table 1). The median follow-up in recurrent and non-recurrent subjects was 3.4 yr (range, 0.6 to 5.1 yr), and 8.1 yr (range, 6.9 to 9.7 yr), respectively. Recurrent versus non-recurrent ICN patients did not differ in age at admission to the study (first-stone episode), prevalence of familial occurrence, hyperuricosuria, and hypercalciuria (Table 1). On the contrary, the renal stone recurrent disease was significantly associated with hyperoxaluria, male gender, and abnormal Kox (Table 1). Since 1986, we have used the value 0.58 x 10^-2 min^-1 as the cut-off value for Kox, which was the 99% upper normal value of control subjects (2). In the study presented here, the same cut-off value was associated with 74% sensitivity and 53% specificity in identifying recurrence during the follow-up (Figure 1). Moreover, the same cut-off value

### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non-Recurrent Stone-Formers</th>
<th>Recurrent Stone-Formers</th>
<th>Analysis Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (%)</td>
<td>42.1</td>
<td>57.9</td>
<td></td>
</tr>
<tr>
<td>Age (yr, median (range))</td>
<td>33 (21 to 56)</td>
<td>34 (17 to 52)</td>
<td></td>
</tr>
<tr>
<td>Gender (Female)</td>
<td>42.5</td>
<td>28.2</td>
<td></td>
</tr>
<tr>
<td>Familial History of Renal Stones (%)</td>
<td>38.7</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td>Hypercalciuria (%)</td>
<td>17.9</td>
<td>23.8</td>
<td>\chi^2 = 4.22, P = .044</td>
</tr>
<tr>
<td>Hyperoxaluria (%)</td>
<td>20.0</td>
<td>34.9</td>
<td>\chi^2 = 2.66, NS</td>
</tr>
<tr>
<td>Hyperuricuria (%)</td>
<td>11.4</td>
<td>10.3</td>
<td>\chi^2 = 0.94, NS</td>
</tr>
<tr>
<td>Abnormal Kox (%)</td>
<td>47.5</td>
<td>73.6</td>
<td>\chi^2 = 4.04, P = .044</td>
</tr>
</tbody>
</table>

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could be selected on the ROC curve as the closest point to the upper-left corner (Figure 1). At this point, the discriminative ability of a test is maximized, and the number of erroneous diagnosis is minimized. Furthermore, the area under the ROC curve (0.645; 95% confidence interval [CI], 0.56 to 0.73), which represents the overall accuracy of a test, was significantly different from the area value 0.5 of the 45-degree ROC curve (i.e., the locus of points for test results in which the sensitivity equals the false-positive rate).

We then evaluated the impact of risk factors by the multivariate analysis after accounting for their mutual relationships. Considering stone recurrency as the dependent variable, and the other factors (familial occurrence, uricosuria, calcuria, oxaluria, and Kox) as predictors, multiple logistic regression analysis (stepwise procedure) was performed. Only gender and Kox were selected as statistically significant ($P < 0.0001$ and $P = 0.03$, respectively) and independent predictors of recurrency, with a standardized partial regression coefficient of 2.14 for gender, and 3.67 for Kox in the regression equation, and a risk odds ratio of 2.0 (95% CI, 1.1 to 3.8) and 3.2 (95% CI, 1.7 to 6.0) respectively with a not-statistically-significant difference. The probability of stone recurrency predicted by this model ranged from 30.1% for women with normal Kox to 73.4% for men with abnormal Kox (Table 2). Attempts to improve these results by modifying the cut-off value of Kox was ineffective.

The family follow-up study showed that five new subjects became renal stone-formers; all had an abnormal Kox. Among the 13 subjects without the Kox anomaly, none developed the stone disease in the 8-yr survey (Figure 2). The statistical significance of these results (chi-squared test, 9.6; $P = 0.002$) was essentially a result of the high specificity of the test.

Figure 1. The Receiver-Operating Characteristic (ROC) curve of Kox as separator variable between recurrent and non-recurrent patients. The graph of the sensitivity versus the false-positive rate (i.e., 1-specificity) was drawn moving from the highest Kox value (origin) to the lowest value. The optimal cut-off value was identified as that closest to the upper-left corner. Oxalate-ROC, straight line; area, 0.6451; SE, 0.0410.

**DISCUSSION**

This study was carried out in a stone population that seems to have a trend for recurrency that is very similar to that previously reported by others. Indeed, almost 60% of stone patients experienced a first relapse within 5 to 6 yr after the first renal stone, and most of them were men (12–18). Our follow-up was sufficiently long to allow an observation period of at least 6.9 yr in non-relapsing patients; this time interval can be matched with the median time to recurrence that has been recorded in many surveys (12–18).

The salient point of the investigation presented here is that subjects with the Kox anomaly have a high probability for renal stone formation. In fact, in ICN patients at their first stone episode, it is possible to predict which subjects will experience disease recurrency through a combination of Kox with gender.
Indeed, the overall probability of stone recurrency may be as high as 73% in the worst conditions (men with Kox higher than $0.58 \times 10^{-2} \text{ min}^{-1}$), and as low as 30% in the best conditions, that is, women with normal Kox. The performance of the logistic model in predicting recurrency could not be enhanced by the inclusion of hyperoxaluria, hypercalciuria, hyperuricosuria, or familial occurrence; in other words, none of these variables increased the detection of patients prone to recurrency.

It is not surprising that hypercalciuria and familial occurrence are not related to recurrency in ICN, as other workers have already reported similar findings (14,18). Robertson and Peacock (19) previously found an association between hyperoxaluria and recurrency, which we also found by bivariate frequency analysis. However, in the study presented here, when hyperoxaluria was forced to enter a logistic model with Kox and gender in the multivariate analysis of frequencies, its statistical weight was not significant, probably because of the higher prevalence of hyperoxaluria in men. Oxaluria values are known to be higher in male stone-formers (20,21), and in view of the strong association between male gender and recurrency of stones, oxaluria mistakenly appears related to recurrency. The prognostic value of gender in nephrolithiasis is long known. Indeed, disease prevalence and its tendency to recur are higher in men than in women (14,22,23). In our analysis, Kox was associated to a higher risk odds ratio for recurrency than gender (3.2 versus 2.0). However, the difference was not statistically significant, as the 95% CI values were overlapping.

We described an abnormal Kox in ICN patients in 1984 (1). In the following years, we reported a number of observations in these patients that supported a physiopathological relationship between abnormal anion cell physiology (as mirrored by the Kox in the erythrocyte) and nephrolithiasis. In particular, in ICN patients with the erythrocyte oxalate anomaly, we observed a higher intestinal absorption (2), and increased renal clearance of oxalate (24), an abnormal tubular handling of urate (25), and a deranged natriuretic response to furosemide (26). Because these functional abnormalities might modify the physicochemical status of urine, they could be pathophysiological related to ICN.

The demonstration of the predictive value of Kox for recurrent ICN, the results of the ROC curve (Figure 1), and the family survey seem to us to be very intriguing fragments of the complex scenario of the relationship between the oxalate cell anomaly and stone pathophysiological that we are trying to draw.

The ROC curve indicates an optimal cut-off point for Kox close to its normal value ($0.58 \times 10^{-2} \text{ min}^{-1}$) (2). The identity of two differently defined cut-off values of Kox sounds to us as a confirmation on a clinical basis (the ROC curve was derived on a clinical phenomenon, i.e., the actual recurrence) of a purely statistic definition of normality (the upper 99th percentile of the normal distribution of the control group).

The family survey was carried out in kindreds, in which parental transmission of the Kox anomaly was addressed and described in 1986 (2); 8-yr follow-up findings showed that some subjects eventually became new renal stone-formers. Interestingly, this occurred only in individuals who had the Kox anomaly, whereas other family members without the abnormal Kox did not form any stones. Therefore, this family survey strengthens the idea that the Kox anomaly is a risk marker of ICN, because, at least in these kindreds, it predicted new cases of the disease with high specificity. Whether this may be extrapolated to the general population requires further studies with a different experimental design.

As a minor point, a byproduct of this study is the possible application in the clinical setting of the test, the erythrocyte oxalate self-exchange, and the derived simple algorithm, because of their capability to identify the ICN patients at high risk of recurrence. The need of similar tests is self-evident because they might enhance the therapeutic attention offered to these subjects, and thus help to prevent stone recurrences.

Until now, stone risk factors have been generally proposed on the bases of cross-sectional investigations of urinary composition; thus, hypercalciuria, hyperoxaluria, hyperuricosuria, and more or less complex indices calculated from the above parameters and others (27–30) were advanced as risk factors of stone formation for a possible clinical application, as inferred from their physicochemical role. Furthermore, on the basis of cross-sectional or retrospective investigations, it was suggested that hypercalciuria and hyperoxaluria were related to the past natural history ofolithiasis (19,21). However, to validate an assay or a test as a predictive or risk marker of nephrolithiasis or recurrency, a prospective survey is mandatory. To the best of our knowledge, no study has challenged the so-called "stone risk factors" prospectively, with the exception of this study and the investigation by Strauss et al. (31) that, however, differs from ours in term of the goal (prediction of stone relapse during treatment), enrolled patients (all were recurrent stone-formers), and evaluated parameters.

In conclusion, by demonstrating the predictive value of Kox for the stone disease and its recurrences, this study supports the notion that the Kox anomaly is a risk factor in calcium nephrolithiasis.

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REFERENCES

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