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Bertrand Dussol · Jean-Michel Verdier
Jean-Marc Le Goff · Patrice Berthezene · Yvon Berland

Artificial neural networks for assessing the risk of urinary calcium stone among men

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Abstract The pathophysiology of idiopathic calcium oxalate nephrolithiasis involves metabolic abnormalities. Previous studies gave conflicting results about the metabolic factors in stone formers. Artificial neural networks (ANN) are new methods based on computer programming that have outperformed conventional methods in prediction of outcomes in different medical applications. The aim of our study was to compare with ANN the clinical and biochemical parameters implicated in urinary calcium stone between stone formers (SF) and controls (C). We compared 11 clinical and biochemical variables among 119 male idiopathic calcium oxalate SF and 96 C by univariate and multivariate statistical analyses. Univariate analyses included discriminant analysis, logistic regression analysis, and ANN. For multivariate analyses, stepwise discriminant analysis and ANN were performed. Variables included age, body mass index (BMI), family history of nephrolithiasis, supersaturation with respect to calcium oxalate, calcemia, and 24-h urinary calcium, oxalate, uric acid, urea, sodium, and citrate. With univariate and multivariate analyses, ANN were as efficient as classical statistical analyses in discriminating the different parameters. The sensitivity, the specificity, and the percentage of correctly classified patients were similar in all analyses. With ANN, supersaturation (receiver operating characteristic, ROC curves index 0.73) and urea (ROC 0.72) were the most discriminant followed by family history and urinary calcium (ROC 0.67). ROC index was 0.63 for citrate, 0.61 for oxalate and urate, 0.60 for sodium and calcemia, 0.58 for age, and 0.56 for BMI, but these parameters were not statistically different between SF and C. ANN gave additional information since they made it possible to determine the cut-off values of the parameters and their predictive power. Cut-off values for being a stone former were 8.9 for supersaturation and 363 mmol/day for urinary urea with a predictive power of 0.69 and 0.70, respectively. Univariate and multivariate analysis evidenced supersaturation and 24-h urinary urea excretion as the most discriminant parameters between the two populations. In addition to high supersaturation, the negative impact of protein intake was confirmed.

Keywords Calcium oxalate stone · Artificial neural networks · Multivariate analysis · Discriminant analysis · Protein intake · Metabolic factors

B. Dussol (✉) · Y. Berland
Service de Néphrologie, Hôpital de la Conception, 147 Bd Baille, 13385 Marseille Cedex 05, France
E-mail: bdussol@ap-hm.fr
Tel.: +33-4-91383043
Fax: +33-4-91383206

B. Dussol · P. Berthezene · Y. Berland
Centre d'Investigation Clinique, INSERM-APHM, Marseille, France

J.-M. Verdier
Ecole Pratique des Hautes Etudes, Université Montpellier II, Marseille, France

J.-M. Le Goff
AVENTI Co., Marseille, France

Introduction

Nephrolithiasis is a Public Health problem since its prevalence ranges from 1 to 15% in the general population in industrialized countries [1–2], with 50% of relapses every 4 years [3]. In more than 80% of cases, stones are made of either calcium oxalate or a mixture of calcium phosphate and oxalate, and they are usually idiopathic.

Idiopathic calcium stone disease results from genetic, metabolic, and urological factors. Genetic factors are poorly defined but are evident in view of the familial clustering of the disease [4]. Medullary sponge kidney is the main urological factor and is sometimes the only disorder found in stone formers [5]. Metabolic factors are the most prevalent. Results of 24-h urine collection are thus integral to the evaluation and to the selection of the most appropriate intervention to prevent recurrence

[6]. However, data that provide an accurate description of the 24-h urine values in stone formers (SF) and in controls (C) are scarce. In the largest study published to date, the relative risk of stone formation increased with increasing urinary calcium levels but not in a linear fashion. Furthermore, a large proportion of C had also high values of urinary calcium, oxalate, and uric acid [7].

Since biochemical parameters are close in SF and in C, since they interplay, and since the risk of stone formation is likely continuous rather than dichotomous [7], it is possible that multivariate analysis does not have the power to discriminate the clinical and biochemical parameters between the two populations.

Artificial neural networks (ANN) are alternatives to linear, parametric statistical models [8–9]. Neuronal networks are computer-based pattern recognition methods with loose similarities with the nervous system. Individual variables of the network, called ''neurones'' can receive inputs from other neurones and define nonlinear relationships among them to improve accuracy. In nonlinear models, inputs that are not evidenced by conventional statistical analysis can gain weight and thereby become discriminating factors. Neural networks can recognize patterns in highly complex datasets [10]. They have outperformed classical statistical analysis and are more accurate in multivariate analysis of clinical data [11–12]. Neuronal networks are increasingly used in medical decision support [13–16]. They are used in image analysis, signal processing, and laboratory medicine [10]. We already used ANN to define the best-risk factors in breast cancer and found that plasminogen activator and tumor size gave the best discrimination between patients with recurrence or not at 5 years [17].

The aim of our study was to compare the clinical and biochemical parameters implicated in urinary calcium stone in order to add knowledges in the pathophysiology of the disease.

Materials and methods

Study population

Stone formers

Stone formers were recruited among male patients attending our nephrology outpatient clinic between January 1997 and January 2000. They were included regardless of the number of stone episodes. Forty percent of the subjects were first-time stone formers, 60% being recurrent. The diagnosis of calcium oxalate stones was based on chemical analysis of spontaneous or surgically eliminated stones. All patients were evaluated in the Center of Clinical Investigations to exclude systemic disease, including primary hyperparathyroidism, sarcoidosis, vitamin D excess, bowel disease of any kind, renal tubular acidosis, primary hyperoxaluria, or urinary tract infections. The metabolic evaluation was performed at least 6 weeks after their last stone episode. Besides, none had hereditary or acquired anatomical disorders of the kidney or the urinary drainage system except medullary sponge kidney that was present in seven patients. Hence 119 idiopathic calcium SF, aged between 18 and 60 years, were included in the study. They all gave written informed consent to participate in the study, and the local ethics committee approved the trial.

For patients on medications that could affect biochemical parameters, medications were discontinued 2 weeks before their 24-h urines were collected. SF were told to pursue their usual diet. None of them had been placed on a low-calcium diet.

Controls

The control group was composed of 96 healthy males aged between 18 and 60 years without a personal history of stones who attended the Detection Center of the Primary Medical Insurance Fund of Bouches du Rhône (South East of France). In this Center, all members of the National Insurance scheme are called every 5 years for a medical checkup. These subjects were recruited during three 2-month periods: May–September 1998, January–April 1999, and October–December 1999. As seasonal fluctuations in stone frequency have been reported, recruitment was done over a year. Controls never had systemic or renal diseases, and were taking no medications that could influence mineral metabolism. They were analyzed in terms of personal characteristics and correctly effected the 24-h urine collections.

Characteristics of SF and C are presented in Table 1. The mean age, weight, and body mass index (BMI) were similar for SF and C (note that the two groups were not matched at all). A family history of stone disease was more frequent in SF (49%) than in C (18%). Urinary volumes were higher in SF but this was expected since 60% of them were recurrent SF and thus had already been advised to increase their fluid intake.

Methods

Personal characteristics

For SF and C, the following medical parameters were recorded: age, BMI, and family history of stone disease, defined as the elimination of a stone or the presence of a stone in one member of the family (parents, brothers, sisters, and children).

Data collection

Plasma calcium was measured on a Hitachi 747 Analyzer (Roche-Boehringer, Mannheim, Germany). We collected 24-h urines on thymol, and measurements were carried out by two trained technicians. Samples were all measured by a DAX autoanalyzer (Bayer Diagnosis,

Table 1 Characteristics of the controls and idiopathic calcium stone formers

Variables (mean ± SD)	Stone formers ($n=119$)	Controls ($n=96$)	t Tests P value
Age (year)	42.7 ± 10.0	42.7 ± 10.8	NS
Weight (kg)	75 ± 11.0	73 ± 9.4	NS
Body mass index (BMI)	25.0 ± 3.2	24.2 ± 2.9	NS
Family history (%)	49%	18%	< 0.01
Urinary volume (l/day)	1.99 ± 0.8	1.37 ± 0.4	< 0.001
Calcemia (mmol/l)	2.42 ± 0.10	2.36 ± 0.09	< 0.001
CaOx supersaturation	13.3 ± 8.7	7.0 ± 4.1	< 0.001
Urinary calcium (mmol/day)	6.7 ± 4.2	4.4 ± 2.0	< 0.001
Urinary calcium/creatinine (mmol/mmol)	0.53 ± 0.33	0.40 ± 0.19	0.05
Urinary urea (mmol/day)	355 ± 120	270.6 ± 79	< 0.001
Urinary urea/creatinine (mmol/mmol)	28.0 ± 9.5	24.5 ± 7.1	0.05
Urinary oxalate (mmol/day)	0.29 ± 0.18	0.21 ± 0.11	NS
Urinary oxalate/creatinine (mmol/mmol)	0.023 ± 0.014	0.019 ± 0.01	NS
Urinary uric acid (mmol/day)	3.3 ± 1.3	2.8 ± 0.9	NS
Urinary uric acid/creatinine (mmol/mmol)	0.26 ± 0.10	0.25 ± 0.08	NS
Urinary citrate (mmol/day)	2.39 ± 1.25	2.27 ± 0.7	NS
Urinary citrate/creatinine (mmol/mmol)	0.19 ± 0.10	0.19 ± 0.06	NS
Urinary sodium (mmol/day)	164 ± 72	136 ± 48	< 0.001
Urinary sodium/creatinine (mmol/mmol)	12.9 ± 5.7	12.3 ± 4.4	< 0.01

NS not significant

Germany) for calcium, urea, creatinine, and urate. Oxalate was measured by an enzymatic method (Biorea) and citrate by spectrophotometer (Boehringer-Mannheim kit). Urinary pH was not measured since it does not influence calcium oxalate supersaturation.

The accuracy of the individual daily urine collections was evaluated on the basis of their creatinine content in relation to body weight. Urinary solutes were expressed in mmol/day (24-h excretion). The relative calcium oxalate supersaturation ratio was calculated with EQUIL93 software [18].

Statistical analyses

Univariate analyses

Linear discriminant analysis A linear discriminant analysis with a Mahalanobis distance was performed on each variable, using the classify function of the Matlab Statistics Toolbox. For each variable, the sensitivity, specificity, and percentage of correctly classified patients were calculated.

Logistic regression analysis using arbitrary categories for individual variables Categorical variables were compared using the chi-square test. Logistic regression was used to estimate odds ratio for being a kidney stone case after simultaneous adjustment for multiple factors. To estimate odds ratio, clinically reasonable increments of change were selected for the different parameters. The P values for trend in the multivariate models were calculated after the median values were assigned to the categories. We calculated 95% confidence intervals for all relative risks. All P values are two-tailed.

Artificial neural networks for univariate analyses and cut-off determinations For each variable x, class-conditional probability densities $f_0(x)$ and $f_1(x)$ were estimated in the control (C_0) and case (C_1) groups by probabilistic neural networks (PNNs). PNNs implement kernel estimates of probability density functions [19]. PNN are nonlinear models that are adjusted on the data by a single parameter. To prevent overfitting of the data, a leave-one out cross-validation method was used. We already used this approach to perform nonlinear discriminant analysis and to predict outcome in node-negative primary breast cancer [17]. A calibration method was used to better smooth the probability densities.

Cumulative distributions were deduced from trapezoidal integration of the probability density functions, and the Cramer–Von Mises fitting statistical test was used to evaluate whether the models were well fitted to the data. If they were not, fitting was refined by decreasing the network parameter.

For each variable, cut-off values were estimated for which $f_1(x) \geq f_0(x)$. Specificity and sensitivity were calculated using an affectation criterion such that if $f_1(x) \geq f_0(x)$ x is affected to C_1; otherwise x is affected to C_0.

For this affectation criterion:

$$\text{Specificity} = \int_{f_1(x)<f_0(x)} f_0(x)\mathrm{d}x = 1 - \int_{f_1(x)\geq f_0(x)} f_0(x)\mathrm{d}x$$

$$\text{Sensitivity} = \int_{f_1(x)\geq f_0(x)} f_1(x)\mathrm{d}x$$

We also calculate the receiver operating characteristic (ROC) indicator representing the area under the ROC curve by using procedures described in [9]. The ROC indicator gives an estimate of the discriminant power of the variable.

The nonparametric test of Wilcoxon for unpaired data was used to compare each variable between the control and the case group.

Multivariate analyses

Multivariate stepwise discriminant analysis This analysis was performed by selecting variables that were entered stepwise to progressively discriminate the two populations by multivariate analysis. The threshold of selection to enter or to remove (F of Fischer) was 0.15. We excluded variables that did not contribute with F levels higher than 0.15 after the inclusion of the other significant variables. The highest probability to classify each case in a defined group was obtained by the discriminant equation computed with the contribution of the significant variables.

Artificial neural networks for multivariate analyses Posterior probabilities of belonging to the stone formers group was deduced as in [17] from estimations of the probability densities $f_0(x_1, x_2)$ and $f_1(x_1, x_2)$. A leave-one out cross validation method was used, and specificity and sensitivity were calculated using an affectation criterion such that if $f_1(x_1, x_2) \geq f_0(x_1, x_2)(x_1, x_2)$ is affected to C_1; otherwise (x_1, x_2) is affected to C_0.

Results

Univariate analyses

Univariate discriminant analysis

The sensitivity, specificity, and percentage of correctly classified patients are shown for each variable in Table 2, using a classification derived from a classical linear discriminant analysis. The most predictive variables to correctly classify the patients were by decreasing order supersaturation (67%), urinary urea (64.7%), and urinary calcium (61.9%). Supersaturation by far was the most specific (83.3%), whereas urea the most sensitive (67.2%).

Logistic regression analysis using arbitrary categories for individual variables

The odds ratio for calcium stone disease according to categories of the different variables are shown in Table 3. The main result was the sharp increase in stone formation observed with increased values of supersaturation and of urinary urea. For the highest categories, the odds ratio was 9.89 for the supersaturation and 14.74 for urinary urea. The ratio increased in a linear fashion with increasing values for these two parameters.

The odds ratio of stone formation was significantly increased for the highest categories of urinary calcium (> 7.2 mmol/day), oxalate (> 0.325 mmol/day), and uric acid (> 3.8 mmol/day). The odds ratio for these three parameters were lower than that for supersaturation and urinary urea but they increased in a linear fashion with increasing values.

Highest categories of calcemia (> 2.44 mmol/l) and of urinary sodium (> 190 mmol/day) were associated with a small risk of stone formation but the risk did not increase in a linear fashion. Urinary citrate was associated with a lower risk but not in all the categories. Age and BMI were not associated with risk of stone formation.

Univariate analysis using artificial neural networks

The results of the analysis by ANN are shown in Table 4. Supersaturation (ROC 0.73), urinary urea (ROC 0.72), family history, and urinary calcium (ROC 0.67) were the most discriminant and predictive parameters. Urinary citrate, oxalate, and uric acid, calcemia, age, and BMI had lower ROC indices and were not significantly different between the two groups.

Artificial neural networks to define cut-off values

The cut-off values with their predictive and discriminant power, calculated with ANN, are shown in Table 5. No significant cut-off values were found for age, BMI, and urinary citrate.

Cut-off values associated with calcium stone formation were > 2.36 mmol/l for calcemia, > 8.9 for supersaturation, > 363 mmol/day for urinary urea nitrogen, > 5.7 mmol/day for urinary calcium, > 0.21 mmol/day for urinary oxalate, > 3.65 mmol/day for urinary uric acid, and > 170 mmol/day for urinary sodium.

Distribution curves of supersaturation and of 24-h urinary urea are shown in Figs. 1 and 2. Cut-off values were assessed by the intersection point of the two curves.

Table 2 Sensitivity, specificity, and percentage of patients correctly classified according to the linear discriminant analysis

Variables	Sensitivity	Specificity	Percentage of patients correctly classified
CaOx supersaturation	53.8	83.3	67.0
Urinary urea (mmol/day)	67.2	61.5	64.7
Urinary calcium (mmol/day)	57.1	67.7	61.9
Calcemia (mmol/l)	60.5	57.3	59.1
Urinary sodium (mmol/day)	56.3	56.3	56.3
BMI	52.9	59.4	55.8
Age	59.7	50.0	55.3
Urinary oxalate (mmol/day)	43.7	64.6	53.0
Urinary uric acid (mmol/day)	52.9	51.0	52.1
Urinary citrate (mmol/day)	45.4	50.0	47.4

The urinary variables were expressed by 24-h excretion (mmol/day)

Table 3 Odds ratio for kidney stones according to categories of variables by logistic regression analysis

Variables	Stone formers	Controls	Odds ratio	95% CI
Age (years)				
< 35	28	27	1.00	
35–43	32	27	1.16	0.56–2.42
43–51	34	17	1.86	0.86–4.03
> 51	25	25	0.94	0.44–2.04
BMI				
< 22.60	24	29	1.00	
22.60–24.45	29	26	1.37	0.64–2.93
24.45–26.54	34	19	2.15	0.99–4.66
> 26.54	32	22	1.77	0.82–3.80
Calcemia (mmol/l)				
< 2.33	22	33	1.00	
2.33–2.39	32	22	2.20	1.02–4.75
2.39–2.44	31	22	2.12	0.98–4.60
> 2.44	34	19	2.67	1.22–5.82
CaOx supersaturation				
< 5.55	18	36	1.00	
5.55–8.66	20	34	1.20	0.52–2.74
8.66–13.01	36	18	4.10	1.83–9.19
> 13.01	45	8	9.89	4.07–24.04
Urinary calcium (mmol/day)				
< 3.4	21	33	1.00	
3.4–5.3	24	30	1.27	0.58–2.80
5.3–7.2	31	23	2.16	0.99–4.70
> 7.2	43	10	6.14	2.65–14.22
Urinary urea (mmol/day)				
< 227	19	35	1.00	
227–325	25	29	1.65	0.74–3.70
325–387	26	28	1.78	0.80–3.98
> 387	49	4	14.74	5.73–37.95
Urinary oxalate (mmol/day)				
< 0.149	24	30	1.00	
0.149–0.215	24	30	1.00	0.46–2.17
0.215–0.325	34	20	2.12	0.98–4.57
> 0.325	37	16	2.81	1.28–6.16
Urinary uric acid (mmol/day)				
< 2.1	26	29	1.00	
2.1–3.1	25	28	0.99	0.46–2.12
3.1–3.8	28	26	1.20	0.56–2.56
> 3.8	40	13	3.21	1.46–7.10
Urinary citrate (mmol/day)				
< 1.7	36	18	1.00	
1.7–2.2	25	25	0.50	0.23–1.09
2.2–2.9	20	38	0.26	0.12–0.58
> 2.9	38	15	1.24	0.56–2.72
Urinary sodium (mmol/day)				
< 100	27	28	1.00	
100–151	26	27	0.99	0.46–2.12
151–190	25	29	0.89	0.41–1.90
> 190	41	12	3.27	1.47–7.25

Multivariate analyses

Stepwise discriminant analysis

The stepwise discriminant analysis is shown in Table 6. Supersaturation, calcemia, urinary urea excretion, BMI, and urinary oxalate excretion were the variables useful to discriminate the two populations. Supersaturation was by far the most discriminant variable with an F value (42.58) more than two times higher than the second one, that is calcemia with an F value of 17.32 and urinary urea with an *F* value of 14.79. The *F* values of the other significant variables were even lower.

With this analysis, sensitivity was 66.4% and specificity 87.5%, and 75.8% of the patients were correctly classified (Table 7).

Multivariate analysis using artificial neural networks

A two-variable ANN model was built using supersaturation and urinary urea, which were the most predictive variables in univariate ANN analysis. The performance of this two-variable nonlinear model was very close to that of the linear model using the stepwise discriminant analysis with five variables. Sensitivity was 62.2% and specificity was 89.6%, and the percentage of correctly classified patients was 74.4%.

As this nonlinear model was based on only two variables, it allowed visualization of a map that could be used in clinical practice (Fig. 3).

Discussion

The main result of the study was that ANN were not superior to classical statistical analyses in discriminating clinical and biochemical parameters between SF and C. This is true for univariate analyses since the sensitivity, the specificity, and the percentage of correctly classified patients were similar using linear discriminant analysis (Table 2) or ANN (Table 4). This result was somewhat expected since ANN are an adaptive nonlinear regression tool best suited for multiple covariates.

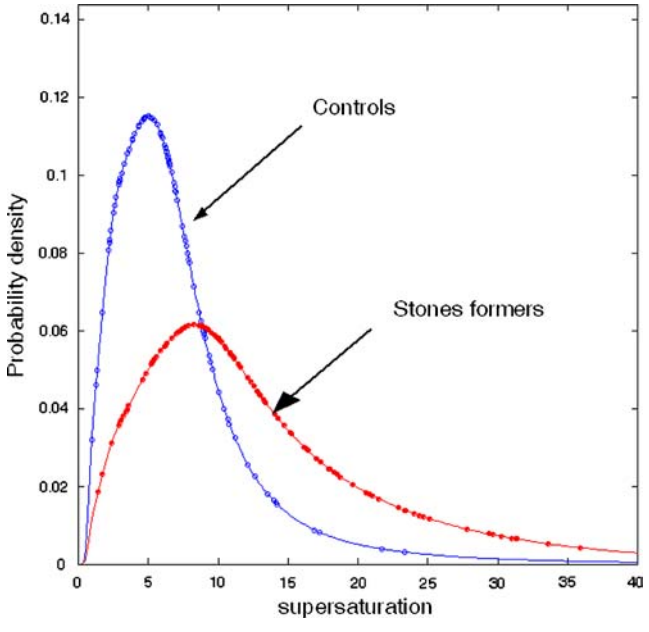

Fig. 1 Distribution curves of CaO*x* supersaturation in stone formers and in controls. The ROC value was 0.73. The cut-off value is 8.9 (intersection point of the curves)

Table 4 ROC indices, sensitivity, specificity, and percentage of patients correctly classified according to the univariate analysis using ANN

Variables	ROC	Sensitivity (%)	Specificity (%)	Percentage of patients correctly classified
CaO*x* supersaturation	0.73	63.9	74.0	68.4
Urinary urea (mmol/day)	0.72	52.1	87.5	67.9
Family history	NA	48.7	82.3	63.7
Urinary calcium (mmol/day)	0.67	57.1	68.8	62.3
Urinary citrate (mmol/day)	0.63	35.3	85.4	57.7
Urinary oxalate (mmol/day)	0.61	60.5	61.5	60.9
Urinary uric acid (mmol/day)	0.61	37.8	82.3	57.7
Urinary sodium (mmol/day)	0.60	45.4	72.9	57.7
Calcemia (mmol/l)	0.60	68.9	47.9	59.5
Age	0.58	58.0	55.2	56.7
BMI	0.56	59.7	50.0	55.3

The urinary variables were expressed by 24-h excretion (mmol/day)
NA nonavailable

Table 5 Cut-off values (in mmol or in mg or g) with their predictive (Se + Sp/2) and discriminant power (ROC indices) using ANN

Variables	Cut-off value (mmol)	Cut-off value (mg or g)	(Se + Sp)/2	ROC indices
Age^{NS}	–		0.57	0.58
BMI^{NS}	–		0.55	0.56
Calcemia (mmol/l)*	> 2.36	94.4 mg	0.58	0.60
CaOx supersaturation*	> 8.9	–	0.69	0.73
Urinary urea (mmol/day)*	> 363	22.0 g	0.70	0.72
Urinary calcium (mmol/day)*	> 5.7	228 mg	0.63	0.67
Urinary oxalate (mmol/day)*	> 0.21	19.1 mg	0.61	0.61
Urinary uric acid (mmol/day)*	> 3.65	610 mg	0.60	0.61
Urinary citrate (mmol/day)^{NS}	–	–	0.60	0.63
Urinary sodium (mmol/day)*	> 170	3.91 g	0.59	0.60

NS nonsignificant
*$P < 0.01$ (Wilcoxon test)

Multivariate ANN were as efficient as the classical stepwise discriminant analysis (Table 7). The efficiency of ANN was expected since they are particularly useful in complex problems; they can analyze a large number of linear and nonlinear variables without the operator knowing the variables or making assumptions between them [10]. The pathophysiology of idiopathic calcium stone disease is very complex, and stone formation results from multiple genetic and environmental factors. These factors often interplay. For example, hypercalciuria is dependent on genetic background, on nutritional factors, such as calcium intake, but also on sodium and protein consumption [20]. Hyperoxaluria is mediated not only by an increase in lean body mass, but also by oxalate intake and the amount of calcium in the gut [21].

However, ANN were more informative than classical analyses on two points. In multivariate analyses, ANN, based on only two variables, made it possible to build a map to determine the risk of stone formation (Fig. 3). Knowing the supersaturation and the 24-h urinary urea, one can easily assess the patient's risk of stone formation. This is an advantage over the stepwise discriminant analysis since one needs to know five variables to calculate the risk with the same sensitivity and specificity. Second, ANN made it possible to determine the cut-off value of the parameters and their respective predictive power (Table 5) with the exception of citraturia. Above these values, the risk of stone formation is increased. The cut-off values for urinary calcium was 5.7 mmol/day, for urinary uric acid 3.65 mmol/day, and for urinary oxalate 0.21 mmol/day. Even with ANN, we were unable to find a cut-off value under which hypocitraturia may be defined (note that the definition is not clear in the literature, [22]). The cut-off value for urea was 22 g/day, meaning that a diet containing more than 68 g/day or 0.9 g/kg/day of protein (according to the median body weight of the SF in this study) confers a risk of stone formation. Most of these values were slightly below the usual definition [23–25]. These lower values may be explained by the rather active disease in our patients since most of them were recurrent SF.

The second result of the study was the evidence that, whatever the analysis performed, supersaturation was the most discriminant variables. This finding agrees with the physicochemical approach of urolithiasis, since the driving force for stone formation is supersaturation of the urine with respect to a calcium salt [26]. This result indicates that supersaturation should be calculated in clinical practice. It should also prompt nephrologists to tailor the treatment on supersaturation by aiming to decreasing it below 8.9, since this was the threshold value above which the risk of calcium stone formation increased in this study.

Table 6 Multivariate stepwise discriminant analysis

Significant variables	F	Prob $>F$	Wilks'λ	Prob $<\lambda$
CaOx supersaturation	42.58	<0.0001	0.83	<0.0001
Calcemia (mmol/l)	17.32	<0.0001	0.77	<0.0001
Urinary urea (mmol/day)	14.79	0.0002	0.72	<0.0001
BMI	10.53	0.0014	0.69	<0.0001
Urinary oxalate (mmol/day)	2.47	0.0994	0.68	<0.0001

The study also reinforced the role of protein intake in the occurrence of idiopathic calcium stone disease. Much experimental and clinical work has evidenced that protein intake has a negative impact in calcium oxalate nephrolithiasis. Wasserstein et al showed a clear relation between dietary protein intake and urinary calcium excretion; the slope of this relationship was much greater for patients with recurrent nephrolithiasis than for the controls [20]. We found the same relation in 108 SF and 210 C [27]. We also demonstrated, in a randomized trial, that in patients who decrease their protein intake for 4 months, urine calcium output decreases significantly [28]. Curhan et al in a prospective study on more than 45,000 males with no history of kidney stones found that animal protein intake was directly associated with the risk of stone formation [29]. Furthermore, a high protein diet may increase urinary uric acid and oxalate [30]. In fact, Giannini et al. demonstrated for hypercalciuric patients that moderate protein restriction not only decreases calcium excretion but also improves the entire lithogenic profile by decreasing urinary uric acid and oxalate and by increasing urinary citrate [31]. Recently Borghi et al. evidenced among hypercalciuric recurrent SF that a low-protein diet combined with a normal calcium intake provides greater protection than the traditional low calcium and normal protein diet [32]. As our study clearly showed that protein intake is deleterious in calcium nephrolithiasis, this is a new argument for patients to decrease their protein intake.

Other parameters seem to play a minor role in the pathophysiology of calcium stone disease. The values of urinary oxalate were lower than in some previous reports [21], but not in all [27, 33–34]. Possible explanations for these differences include differences in analytic methods, dietary oxalate intake, or time trends. Urinary oxalate and uric acid were associated with a significant but low relative risk by multivariate analysis. This result agrees with previous studies showing slightly but consistently higher values of urinary oxalate [7] and of urinary uric acid [27] in SF compared with C by conventional statistical analysis. But by ANN, these two

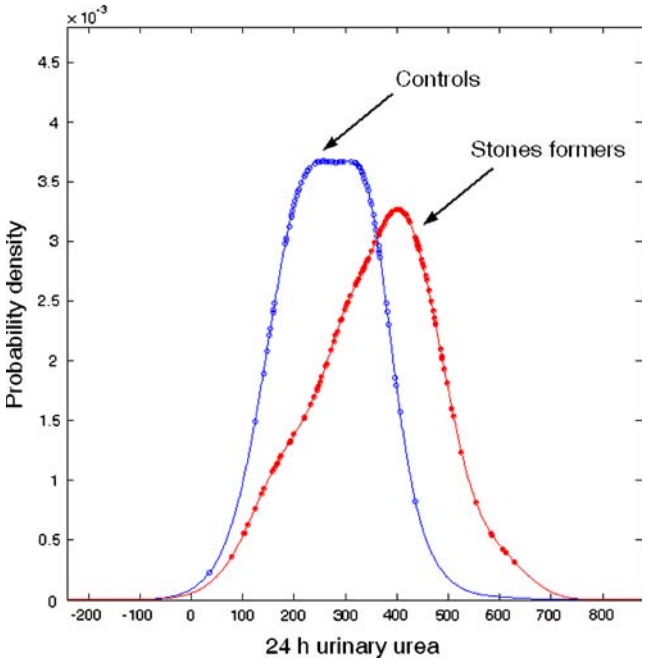

Fig. 2 Distribution curves of 24-h urinary urea in stone formers and in controls. The ROC value was 0.72. The cut-off value is 363 mmol/day (intersection point of the curves)

parameters did not differ significantly between the two groups. This does not mean that oxalate and uric acid play no role in the pathophysiology of calcium stone. In fact, a postprandial spike of oxalate has a greater impact on stone formation than 24-h excretion [35]. In the same way, dissolved uric acid directly promotes calcium oxalate precipitation by the classic salting out effect [36].

All the analyses showed that urinary citrate was not inversely associated with the risk of stone formation, as previously shown [7]. The implication of low urinary citrate in calcium nephrolithiasis seems doubtful, it may not be useful to evaluate this parameter in clinical practice.

What are our study limitations? We studied only males because among females a preliminary study with ANN showed calcemia to be the main discriminant factor between SF and C (personal communication). This argues for a difference in the pathophysiology of stone disease between males and females, with predominant endocrine factors (infraclinical hyperparathyroidism by example) among females.

Our cohort was 100% Caucasian but stones occur less frequently in other racial groups [37]. We excluded stone formers over 60 years of age because our control

Table 7 Sensitivity, specificity, and percentage of patients correctly classified according to the multivariate analyses: stepwise discriminant analysis (five variables) and ANN (two variables)

	Sensitivity	Specificity	Percentage of patients correctly classified
Stepwise discriminant analysis (five variables)	66.4	87.5	75.8
CaO*x* supersaturation and urinary urea (mmol/day) according to ANN	62.2	89.6	74.4

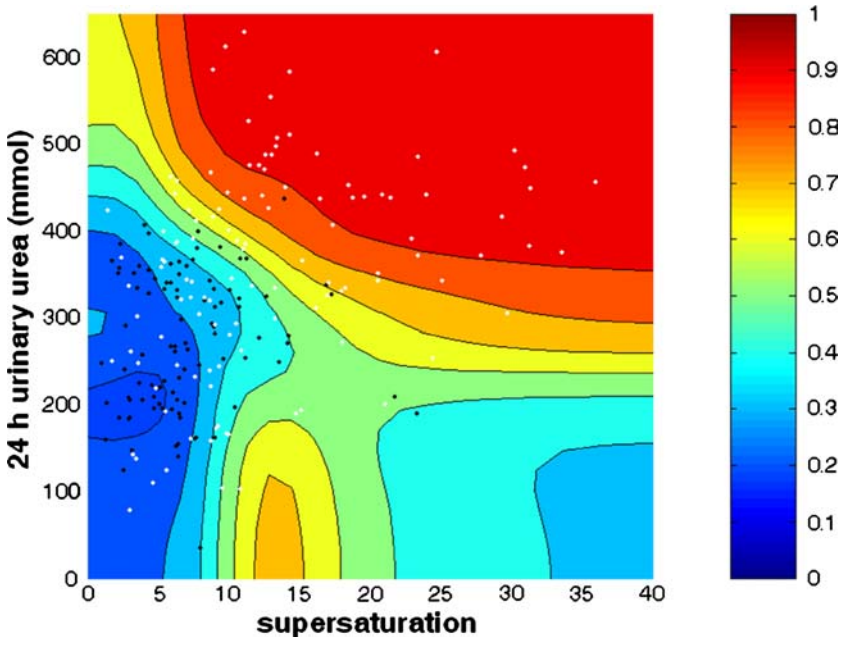

Fig. 3 Posterior probability (risks) of belonging to the stone formers group according to CaO*x* supersaturation and 24-h urinary urea excretion. *White points* stone formers; *black points* controls

group did not include individuals over 60. This point is not likely to limit the generalizability of our results because idiopathic calcium stone disease occurs mainly between 30 and 60. In fact in individuals over 60, the prevalence of nephrolithiasis decreases and stones are often made of uric acid or struvite [38]. In this study, all stone formers had the same kind of stone, that is, pure calcium oxalate or a mixture of calcium oxalate and phosphate with a predominance of oxalate over phosphate. This type of stone is the most prevalent in industrialized countries. None of the SF were taking medications or were prescribed a particular stone diet expect for water intake. Thus our SF group was representative of the population suffering from calcium stone. Controls fitted SF for age and BMI, and were representative of the general French population.

Because only a single 24-h urine collection was performed, the means are likely properly centered, but the standard deviations may be larger than in case of multiple collections. Since stone formers had greater variations in urinary parameters (for example, 24-h urinary calcium), this study may have overestimated the differences between the two populations. Finally even if 24-h urinary creatinine was in the range expected for body weight (0.2 mmol/kg/day, data not shown), some errors in the completeness of the urine collections are still likely, particularly in C because of poorer motivation.

In summary, ANN were not clearly superior to classical statistical analyses but gave additional informations on the pathophysiology and on the treatment of calcium nephrolithiasis (1) by determining the cut-off values of the parameters involved in the disease and their predictive power (2) by making it possible to build a map to determine the risk of stone formation. Finally, ANN and other analyses pointed out supersaturation and urinary urea as the most discriminant variables, confirming the negative impact of protein intake in the disease.

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