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Urolithiasis

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Corresponding Author:	Andrea Ticinesi, M.D. Universita degli Studi di Parma Parma, ITALY
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Universita degli Studi di Parma
Corresponding Author's Secondary Institution:	
First Author:	Angela Guerra
First Author Secondary Information:	
Order of Authors:	Angela Guerra
	Andrea Ticinesi, M.D.
	Franca Allegri
	Silvana Pinelli
	Rosalia Aloe
	Tiziana Meschi
Order of Authors Secondary Information:	
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Abstract:	Pure calcium oxalate is the most frequent type of idiopathic kidney stone composition. Fourier transform infrared spectroscopy (FT-IR) allows to detect the ratio of calcium oxalate dihydrate (COD) and monohydrate (COM) crystals in stones, but the clinical significance of this parameter remains uncertain. The objective of this observational study was to verify the association of clinical and laboratory parameters of kidney stone disease with COD/COM ratio in a group of 465 (322 M, age 46 ± 14) patients suffering from idiopathic calcium nephrolithiasis with pure calcium oxalate stones (≥97%). Each participant underwent a complete clinical examination, serum chemistry, 24-hour urine collection for the determination of the profile of lithogenic risk, and had stones analyzed by FT-IR. Most (62%) of the stones has a COD/COM ratio ≤0.25, and the urine chemistry of the corresponding patients showed a low prevalence of urinary metabolic abnormalities. With increasing COD/COM ratio intervals (0-0.25, 0.26-0.50, 0.51-0.75, 0.76-1), a significant association was observed for the number of urological procedures, serum calcium, 24-h urinary calcium excretion, prevalence of hypercalciuria and relative calcium oxalate supersaturation, and a negative trend was detected for the age of the first stone episode (all p-values <0.05). A linear regression model showed that the only parameters significantly associated with COD/COM ratio were 24-h urinary calcium excretion (standardized β=0.464, p<0.001) and urine pH (standardized β=0.103, p=0.013). In pure calcium oxalate idiopathic stones, COD/COM ratio may reflect the presence of urinary metabolic risk factors, and represent a guide for the prescription of urinary analyses.
Response to Reviewers:	RESPONSES TO REVIEWER #1

	“The authors of this submission have performed an investigation in order to detect a possible association between calcium oxalate stone (COM & COD) and clinical characteristics, including 24-hour urinary parameters of lithogenic risk, of a large group of patients with idiopathic calcium nephrolithiasis. Chemical composition of kidney stones and thus the COD/COM ratios were determined by FTIR spectroscopy. The data were well analyzed and thus the conclusion is clear. Here are some suggested revisions: - In the introduction : it is worth to underline that the morphology of crystallites indicates quite precisely the pathology. For example, five morphological subtypes Ia, Ib, Ic, Id and Ie exists for COM kidney stones (Daudon, M., Bader, C. A. & Jungers, P. (1993). Scan. Microsc. 7, 1081-1106 as well as M. Daudon, D. Bazin, G. André, P. Jungers, A. Cousson, P. Chevallier, E. Véron, G. Matzen,Examination of whewellite kidney stones by scanning electron microscopy and powder neutron diffraction techniques, J. Appl. Cryst. (2009). 42, 109-115).” R: We thank the reviewer for the positive comment and for the important suggestions. In the novel version of the manuscript, we have included a better focus on the relationship between calcium oxalate crystal morphology and stone etiopathogenesis, highlighting the mentioned classification of COM kidney stones (see page 2 lines 7-12). The two mentioned papers are now referenced in the manuscript (number 3 and 9). “Also there is a study performed on urine which has to be included in the references : M. Daudon, E. Letavernier, V. Frochot, J.-Ph. Haymann, D. Bazin, P. Jungers, Respective influence of calcium and oxalate urine concentration on the formation of calcium oxalate monohydrate or dihydrate crystals, C. R. Chimie 19 (2016) 1504-1513.” R: We apologize for having missed this important study on the clinical correlates of COD and COM stone composition. In the novel version of the manuscript, this study is referenced (number 24) and discussed (see page 8 lines 12-15 and page 9 lines 8-11). RESPONSES TO REVIEWER #2 “The paper of Guerra et al is an interesting work analysing clinical characteristics of patients forming calcium oxalate monohydrate stones or calcium oxalate dihydrate stones. Although relevant, I seem that the paper needs to be reconsidered in some methodological points.” R: We thank the reviewer for the positive comments and constructive criticism. All the methodological concerns have been carefully considered and the manuscript has been modified accordingly. “1. Control group characteristics and criteria for their enrolment could be more extensively detailed at page 3, line 29-33.” R: In the novel version of the manuscript, we have introduced more information on the characteristics of controls and criteria for their inclusion in the study (see page 3 lines 15-22). The mandatory criteria for control selection were the absence of a personal history of kidney stones/renal colic and the absence of retained stones at abdominal ultrasound performed immediately before urine collection. “2. Please specify conditions for 24-hour urine storage in order to measure their pH.” R: In the novel version of the manuscript, more details on the methods of urine collection, preservation and analysis have been added (see page 4 lines 4-11). This methodology is well standardized at our Stone Clinic, and has remained substantially the same over the last 30 years. “3. References 9-10, reported at page 4, to support limits of hypercalciuria and hyperoxaluria do not appear appropriate to me.” R: The references have been deleted from the novel version of the manuscript. Unfortunately, there are no widely accepted definitions of hypercalciuria and hyperoxaluria, and “convenience” definitions were adopted in this investigations.

Hypercalciuria is in fact generally defined as urinary calcium excretion >200 mg/24 h (see Pak CY et al. Kidney Int 2011), but some investigations demonstrated that the risk of stone formation increases continuously with increasing urinary calcium excretion values, so that no threshold for hypercalciuria can be certainly identified (Curhan GC et al. Kidney Int 2001). Moreover, different “convenience” definitions of hypercalciuria were used in nephrolithiasis studies (Escribano J et al. Cochrane Database Syst Rev 2014), and the definition establishing 4 mg/kg/day of urinary calcium excretion as threshold for hypercalciuria is commonplace in clinical practice (see Leslie SW et al. StatPearls 2019). We underline that a rigorous definition of hypercalciura and hyperoxaluria was beyond the aims of the present investigation.
“4. The Authors declared to analyse characteristics of patients grouped in quartiles of COD/COM ratio: according to usual statistical criteria, each quartile has to include 25% of patients (n=116-117 in the present study); on the contrary the present study divided patients in four groups defined according to the value of the COD/COM ratio (0.0.25, 0.26-0.5, 0.51-0.75, 0.76-1), a method which does not identify quartiles. Description of these analyses has to be revised in the abstract, discussion, statistical method and result sections and tables.”

R: We agree with the reviewer. In the previous version of the manuscript, the definition of COD/COM ratio quartiles was not appropriated. Each COD/COM ratio quartile should in fact have included 25% of the study population, and thus interquartile limits should have been different. However, we believe that a categorization of COD/COM ratio in four ranges (0-0.25; 0.26-0.50; 0.51-0.75; 0.76-1) is much more practical for clinical interpretation of the parameter. For this reason, we preferred to maintain this method of categorization, changing the incorrect definition of “quartiles”. The manuscript has been revised in accordance with this choice. Each category has been labeled as “COD/COM interval”.

“5. In the statistical analysis section it is reported that data were expressed as mean ± standard deviation or mean and 95% confidence intervals or median and interquartile range. However, mean±SD was used to express the large part of the variables in the manuscript, whereas median and IQR was used to describe COD/COM ratio (at page 6) and duration of the disease (in table 1) as non-parametric variables. Mean and 95% confidence intervals was used only at page 9 in the manuscript discussion and not in result section. Therefore, methods to express variables need to be revised at page 5.”

R: We agree with the reviewer and apologize for the lack of clarity of the previous version of the “statistical analysis” paragraph. In the novel version of the manuscript, the section on how data were expressed and handled has been modified as follows: “Continuous variables were expressed as mean ± standard deviation or, for non-normally distributed variables, median and interquartile range (IQR). Dichotomous variables were expressed as percentages” (page 5 lines 10-11).

“6. In table 1, ESWL number and stone rate could be considered as non-parametric variables and reported as median and interquartile range.”

R: We agree with the reviewer. Table 1 has been modified accordingly. The comparison of ESWL number and stone rate across different groups of stone formers, stratified by COD/COM ratio intervals, was then performed using Kruskal-Wallis test. For this reason, p-values changed.

“7. Significance of findings was reported with 3 p values in tables 2 and 3 but 2 p values in tables 1 and 4. I seem that the Authors considered unadjusted p values raising from ANOVA (the so-called p for trend), p values adjusted for confounding variables (p for trend from ANCOVA) and p values obtained with multiple comparisons between groups (Bonferroni test). It is not immediately clear why 3 p values were not reported in tables 1. The use of these p values could be more extensively explained in statistical methods.
8. In table 4 the Authors compared values of variables estimated using ANCOVA. If this is correct, this method has to be detailed in statistical analysis section at page 5 and specified in the text at page 6 and table 4.”

R: The whole statistical analysis paragraph has been revised in order to improve comprehension by readers (see page 5 lines 14-26). In Tables 1, 2 and 3, normally

	distributed clinical and laboratory parameters were compared among groups of patients, stratified by intervals of COD/COM ratio, using one-way analysis of variance (ANOVA) for crude comparisons, and analysis of covariance (ANCOVA) for comparisons adjusted for covariates (age, sex, duration of disease, BMI). The Bonferroni test for multiple comparisons was applied if adjusted p values were <0.05. Non-normally distributed variables were compared among groups of patients by Kruskal-Wallis test. In Table 4, a comparison between stone formers, stratified by COD/COM ratio intervals, and stone-free controls was made. Unfortunately, stone formers and controls showed mild, but statistically significant, differences in age, sex distribution, body mass index, and urinary volume. For this reason, in Table 4 urinary parameters were handled as mean ± standard deviation adjusted for age, sex, BMI, and, for urinary supersaturation indexes, also for urinary volume. Comparisons were made using ANCOVA. Bonferroni test was again applied if adjusted p values were <0.05. Tables 1, 2, 3 and 4 have been carefully revised to improve comprehension on what kind of statistical analyses were applied in each case. In Table 1, crude p values obtained with ANOVA (dichotomous variables or continuous variables with normal distribution) or Kruskal-Wallis test (continuous variables with non-normal distribution) were reported for all lines. Adjusted p values, obtained with ANCOVA, were reported only for those variables where adjustment for covariates is meaningful in clinical terms. Finally, p for trend values obtained with ANOVA (linear trends) were reported only if <0.05 (statistically significant). RESPONSE TO EDITOR-IN-CHIEF'S ADDITIONAL COMMENT “Please note that this journal's policy is only to allow a maximum of five to six authors unless the article is the product of a multi-centre research study. Your article currently has 7 authors, which is not permitted. If you decide to submit a revised version of your manuscript, please reduce the number of authors to six or fewer. Also, if you wish to submit any articles to Urolithiasis in future, I would be grateful if you could please adhere to this policy (see Instructions for Authors on journal website).” R: We apologize that our original submission was not adherent to this editorial policy. After discussion among all authors, we decided to remove from the author team Dr Antonio Nouvenne, who gave advice for study conception and assistance in manuscript drafting, but was not directly involved in data collection, analysis and interpretation. The manuscript has now six authors. Dr Nouvenne has been mentioned in the acknowledgement section.

Idiopathic calcium nephrolithiasis with pure calcium oxalate composition: clinical correlates of the calcium oxalate dihydrate/monohydrate (COD/COM) stone ratio

Angela Guerra[1,2], Andrea Ticinesi[1,2]*, Franca Allegri[1,2], Silvana Pinelli[2], Rosalia Aloe[3], Tiziana Meschi[1,2]

[1]Geriatric Rehabilitation Department, University-Hospital of Parma, Via Antonio Gramsci 14, 43126 Parma, Italy

[2]Department of Medicine and Surgery, University of Parma, Via Antonio Gramsci 14, 43126 Parma, Italy

[3]Diagnostic Department, U.O. Diagnostica Ematochimica e S.S.D. Biochimica ad Elevata Automazione, University-Hospital of Parma, Via Antonio Gramsci 14, 43126 Parma, Italy

Corresponding author:

*Andrea Ticinesi, MD, PhD, Geriatric-Rehabilitation Department, Parma University-Hospital (Azienda Ospedaliero-Universitaria di Parma), via Antonio Gramsci 14, 43126 Parma, Italy.

Work Telephone number: 0039-0521703871; Mobile: 00393471845191; Fax: 0039-0521702383

E-mail address: aticinesi@ao.pr.it

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Abstract

Pure calcium oxalate is the most frequent type of idiopathic kidney stone composition. Fourier transform infrared spectroscopy (FT-IR) allows to detect the ratio of calcium oxalate dihydrate (COD) and monohydrate (COM) crystals in stones, but the clinical significance of this parameter remains uncertain. The objective of this observational study was to verify the association of clinical and laboratory parameters of kidney stone disease with COD/COM ratio in a group of 465 (322 M, age 46 ± 14) patients suffering from idiopathic calcium nephrolithiasis with pure calcium oxalate stones (≥97%). Each participant underwent a complete clinical examination, serum chemistry, 24-hour urine collection for the determination of the profile of lithogenic risk, and had stones analyzed by FT-IR. Most (62%) of the stones has a COD/COM ratio ≤0.25, and the urine chemistry of the corresponding patients showed a low prevalence of urinary metabolic abnormalities. With increasing COD/COM ratio intervals (0-0.25, 0.26-0.50, 0.51-0.75, 0.76-1), a significant association was observed for the number of urological procedures, serum calcium, 24-h urinary calcium excretion, prevalence of hypercalciuria and relative calcium oxalate supersaturation, and a negative trend was detected for the age of the first stone episode (all p-values <0.05). A linear regression model showed that the only parameters significantly associated with COD/COM ratio were 24-h urinary calcium excretion (standardized β=0.464, p<0.001) and urine pH (standardized β=0.103, p=0.013). In pure calcium oxalate idiopathic stones, COD/COM ratio may reflect the presence of urinary metabolic risk factors, and represent a guide for the prescription of urinary analyses.

Key words: urolithiasis; hypercalciuria; hyperoxaluria; kidney stones; calcium oxalate.

Introduction

Under infrared spectroscopy, calcium oxalate crystals of calcium stones may appear in two distinct molecular forms: whewellite, that is, calcium oxalate monohydrate (COM), and weddellite, that is, calcium oxalate dihydrate (COD). These forms are associated with different etiology of stones [1, 2] and are also associated with different surface morphology of calculi [3].

COM depends on urinary excretion of oxalate and is typical of conditions of hyperoxaluria, such as primary hyperoxaluria, intestinal diseases or dietary regimens with a high oxalate load [4-8]. According to the surface morphology and crystallite appearance at environmental scanning electron microscopy, five different types of COM stones can be identified [3, 9]. Each of them corresponds to different pathophysiological mechanisms: low diuresis or slight intermittent hyperoxaluria (type Ia), low diuresis and slight intermittent hyperoxaluria and hypercalciuria (type Ib), primary hyperoxaluria (type Ic), hyperoxaluria with anatomical alterations (type Id) and enteric hyperoxaluria (type Ie) [9].

COD is instead typically found in stones of patients who have a high urinary calcium excretion, with or without hyperoxaluria, due to primary hyperparathyroidism, Paget bone disease, prolonged immobilization, sarcoidosis, myeloma, bone metastasis, acromegaly, hyperthyroidism, renal or enteric hypercalciuria [3, 10].

In this context, the detection of prevalent COM or COD composition in stones passed by patients with calcium lithiasis may serve as a guide for detecting stone etiology and prescribing appropriated second-level diagnostic tests [11, 12].

However, the most common etiology of calcium stones is idiopathic, accounting for around 80-85% of patients visited in stone clinics [1, 11, 12]. In these patients, the presence of COM, COD, or a combination of the two in passed stones examined by infrared spectroscopy has uncertain significance.

The objective of this observational study was to detect the possible associations between calcium oxalate stone composition, in terms of COM, COD and their ratio, and the clinical characteristics, including 24-hour urinary parameters of lithogenic risk, of a large group of patients with idiopathic calcium nephrolithiasis (ICN).

Materials and methods

Study participants

All subjects over 18 who completed a medical and urinary metabolic evaluation at our Stone Clinic from 2009 to 2017 were eligible for study enrolment. Inclusion criteria were the presence of ICN, infrared spectroscopy analysis of stones completed at our laboratory within three months from urinary metabolic evaluation, and pure calcium oxalate stone composition (defined as calcium oxalate crystals ≥97%). Subjects with known calcium stone etiology, such as primary hyperoxaluria, enteric hyperoxaluria, primary hyperparathyroidism or other bone diseases associated with hypercalciuria, were excluded from the study. Subjects with chronic kidney disease (creatinine clearance <60 ml/min), renal tubular acidosis, recurrent urinary tract infections, congenital or acquired anomalies of the kidney and the urinary tract, spina bifida, or cystic fibrosis were excluded as well. Subjects with missing clinical or laboratory data were not considered for the final analysis.

Thus, the main study population was composed exclusively of calcium stone formers with documented pure calcium oxalate stone composition and no known etiology. From an epidemiological perspective, this circumstance represents the majority of cases with calcium nephrolithiasis [12, 13].

A database of urinary profiles of lithogenic risk from a group of non-stone forming controls who underwent urinary metabolic evaluation at our Stone Clinic was also considered, to compare the urine composition of patients with different COD/COM ratio in their stone composition with normal standards. These controls (mean age 42±12 years old, male:female ratio 1:2, body mass index [BMI] 24±4 kg/m^2) were selected according to the absence of episodes of renal colic in their personal history and absence of retained stones at abdominal ultrasound at the moment of urine collection. Subjects with congenital or acquires anomalies of the urinary tract, recurrent urinary tract infections, creatinine clearance <60 ml/min and suspected diseases of calcium metabolism were not considered.

Clinical and urinary metabolic evaluation

According to the clinical protocol adopted in our stone clinic [13], a comprehensive medical history, with particular focus on the stone disease course and risk factors, was collected from all participants. Family history and age of onset of the first stone episode were carefully collected [14]. The coexistence of kidney stones with hypertension, that represents an important risk factor for urinary metabolic abnormalities [15], was also particularly assessed.

Height, weight, and arterial pressure were measured. Abdominal ultrasound or X-ray were performed to detect retained stones and their radio-opacity. Blood tests, including serum creatinine, calcium, phosphorus, uric acid, parathormone (PTH), and 25-hydroxyvitamin D (25-OH-D) were performed.

Each participant also collected a 24-hour urine sample for the urinary metabolic profile of lithogenic risk [13]. During the collection, urine was equally distributed in two containers: one containing 2 ml of chlorhexidine gluconate 20% and the other 15 ml of 18% hydrochloric acid. The panel of urinary analyses, performed on the same day the collection was concluded, included pH, sodium, potassium, chloride, creatinine, ammonium, urea, uric acid, citrate (all measured from the chlorhexidine container), calcium, magnesium, oxalate, sulfate and phosphate (measured from the hydrochloric acid container). Urine volume was also assessed considering the content of both urine containers. Urinary relative supersaturations for lithogenic salts, representing an index of the risk of stone recurrence [16], were calculated by using the Equil2 software [17].

Hypercalciuria was defined as a 24-hour urinary calcium excretion ≥4 mg/kg/day, while hyperoxaluria was defined as a 24-hour urinary oxalate excretion >45 mg/day.

Stone analyses

Stones passed by participants or extracted during urologic procedures were examined at our stone clinic laboratory by Fourier transform infrared spectroscopy (FT-IR). This technique allows the detection and quantification of COM and COD crystals in stones.

Mixtures with different percentages of COD and COM, selected from patients' kidney stones, were prepared and used for the calibration. The pure COM and COD infrared spectra used for calibration, corresponding to the reference ones [18], are shown in Supplementary Material (Figure S1). COM has a band with absorption peak at 1315 cm^{-1} and COD at 1325 cm^{-1}, respectively. Among kidney stones with spectra corresponding to COM [18], the one with the lowest value of the 1325/1315 cm^{-1} ratio was chosen as the reference for pure COM. In fact, due to the additivity of the Lambert-Beer law, the presence of minimal traces of COD in the sample increases the absorbance to a greater extent at 1325 cm^{-1} than at 1315 cm^{-1}, increasing COD/COM ratio. Conversely, pure COD was selected from samples with spectra equal to COD [18] and with the highest value at 1325/1315 cm^{-1}.

For the FT-IR analyses of stones, pellets were prepared mixing pulverized stone (1%) with potassium bromide (99%). Absorbance spectrum was recorded using a Shimadzu FTIR – 8400S spectrophotometer (Shimadzu

Corporation, Kyoto, Japan), with a measurement range between 400 and 4000 cm^{-1}, resolution 4 cm^{-1}, number of scans 45. The absorbance intensity of recorded spectra ranged between 0.2 and 0.8, to avoid deviations from the Lambert-Beer law.

The relationship between the percentage of COD in the mixture (x) and the absorbance ratio at 1325/1315 cm^{-1} (y) was described by a quadratic equation ($0.078x^2 + 0.352x + 0.734$, $R^2 = 0.9995$), shown in the Supplementary Material (Figure S2). This equation was used to determine the COD/COM ratio in the calcium oxalate kidney stones of patients enrolled in this study.

Statistical analyses

Continuous variables were expressed as mean ± standard deviation or, for non-normally distributed variables, median and interquartile range (IQR). Dichotomous variables were expressed as percentages. Stone formers were stratified by COD/COM ratio intervals of their stone composition, as following: 0-0.25 (first interval), 0.26-0.50 (second interval), 0.51-0.75 (third interval), 0.76-1 (fourth interval). Normally distributed continuous and dichotomous clinical and laboratory parameters were compared among groups of patients, stratified by intervals of COD/COM ratio, using one-way analysis of variance (ANOVA) for crude comparisons, and analysis of covariance (ANCOVA) for comparisons adjusted for covariates (age, sex, duration of disease, BMI). The Bonferroni test for multiple comparisons was applied if adjusted p values were <0.05. Non-normally distributed continuous variables were compared among groups of patients by Kruskal-Wallis test.

For comparisons between patients, stratified by intervals of COD/COM ratio, and controls, urinary parameters were handled as mean ± standard deviation adjusted for age, sex and BMI, since these variables were different between patients and controls. Urinary supersaturations were also adjusted for urinary volume. Comparisons were then made using ANCOVA. Bonferroni test was again applied if adjusted p values were <0.05.

The relationship between urinary parameters and COD/COM ratio in stone composition was also assessed by linear regression models.

All p-values were considered significant for $p<0.05$. Analyses were performed with the SPSS software v.24 (SPSS Inc., Chicago, IL, USA).

Results

From 2009 to 2017, 947 stone samples (from 677 males and 270 females) with pure calcium oxalate composition were analyzed in our Stone Clinic. However, 482 subjects were excluded from the analysis for missing clinical or laboratory data or not meeting inclusion criteria. Thus, the study was conducted on a group of 465 stone formers (322 males, 143 females, age 46 ± 14) and their stones.

In stone formers, the COD/COM ratio median was 0.20 (IQR 0.10-0.40), so that most participants (62%) fell within the first interval of COD/COM ratio (0-0.25). The clinical characteristics of patients, stratified by COD/COM ratio intervals, are reported in Table 1. Patients with the highest COD/COM ratio had a higher number of extra-corporeal shock-wave lithotripsy procedures ($p = 0.017$ with Kruskal-Wallis test) and a lower age of onset of kidney stone disease (p for trend = 0.001 with ANOVA) (Table 1). However, the trend for an earlier onset of the disease was confirmed only in those without a family history of stones (p for trend = 0.003 with ANOVA), and not in those self-reporting a family history of stones (Table 1, Figure 1A).

There was also a trend for an increase in serum calcium with increasing COD/COM ratio (p adjusted with ANCOVA for age, sex, duration of disease and BMI = 0.014), while other serum parameters were not different across COD/COM ratio intervals (Table 2).

The analysis of 24-hour urinary parameters of lithogenic risk across COD/COM intervals is depicted in Table 3. With increasing COD/COM ratio, significantly higher levels of 24-h calcium excretion, calcium oxalate and calcium phosphate supersaturation (all p values adjusted with ANCOVA for age, sex, duration of disease and BMI <0.001) could be demonstrated. Moreover, urinary pH was higher in the fourth vs the third interval of COD/COM ratio, while 24-hour urinary oxalate excretion was not related with the COD/COM ratio (Table 3).

The comparison of the 24-hour urine parameters of patients belonging to different intervals of COD/COM ratio with non-stone forming controls revealed that subjects in the first interval of COD/COM ratio had a very similar urine composition than controls, exhibiting only a higher volume and excretion of phosphorus and oxalate (Table 4). Conversely, those with a COD/COM ratio >0.25 exhibited a wider range of urinary abnormalities compared to controls, including a higher calcium excretion and a higher calcium oxalate relative supersaturation index (Table 4). The 24-hour urinary calcium excretion was also unaffected by the presence of a family history of stones (Figure 1B).

A linear regression model, exploring the possible clinical and urinary parameters associated with the COD/COM ratio of stone composition, is shown in Table 5. Only 24-hour urinary calcium ($\beta = 0.124$, 95% CI

0.102-0.145, standardized β = 0.464, p<0.001) and urine pH (β = 6.402, 95% CI 1.347-11.457, standardized β = 0.103, p=0.013) were significantly associated with COD/COM ratio.

Discussion

In a group of patients with idiopathic calcium nephrolithiasis and pure calcium oxalate composition, the COD/COM ratio of stone composition, determined by FT-IR, was significantly associated with an earlier onset of the disease, higher number of urologic procedures, higher serum calcium, higher urinary excretion of calcium and pH. Among these parameters, 24-hour urinary calcium excretion exhibited the strongest correlation with COD/COM ratio. Moreover, patients with a COD/COM ratio ≤0.25, representing the majority of subjects with idiopathic calcium nephrolithiasis, showed no clinically relevant metabolic abnormalities in urine chemistry.

This is one of the first studies exploring the clinical correlates of COD/COM ratio of stone composition in idiopathic calcium nephrolithiasis with stones of pure calcium oxalate composition. Previous investigations were in fact focused on patients with known metabolic abnormalities or secondary forms of calcium nephrolithiasis, and showed an association between hypercalciuria and high COD/COM ratio [19-23]. The only study conducted on an unselected population of calcium oxalate stone formers showed the presence of a significant correlation between urinary calcium/oxalate ratio and stone COD/COM ratio, with oxalate dependence of COM crystal formation and calcium dependence of COD crystal formation [24].

In our group of pure calcium oxalate stone formers, the highest values of 24-hour urinary calcium excretion and the highest prevalence of hypercalciuria were found in those with a COD/COM ratio >0.50. Those in the fourth interval of COD/COM ratio (>0.75) also exhibited a higher urinary pH, suggesting a role of pH in determining the COD content of calcium oxalate stones in hypercalciuric patients [25].

Previous studies also suggested a significant association between hyperoxaluria and prevalent COM composition of stones [4-8, 25], that was not confirmed in our group of pure calcium oxalate stone formers with idiopathic calcium nephrolithiasis. This association is probably typical of gastrointestinal diseases with increased oxalate absorption and dietary regimens with high oxalate load [4-8]. Patients with these conditions were not included in our study, since they do not fit with the criteria for diagnosing idiopathic calcium nephrolithiasis. However, participants with a low COD/COM ratio did exhibit a significantly higher 24-hour urinary oxalate excretion than controls, although the difference was mild.

Another point of interest is the circumstance that the relative supersaturation indexes for calcium oxalate were similar between subjects with pure calcium oxalate stones and COD/COM ratio ≤0.25 and healthy controls. The supersaturation indexes are well-known predictors of recurrence of kidney stones [16, 26], and depend on

urine volume and urinary metabolic abnormalities. In clinical practice, the finding of a low COD/COM ratio at stone analysis in patients with idiopathic calcium nephrolithiasis may imply that these patients have no urinary metabolic abnormalities and a low risk of stone recurrence. This assumption is also supported by the findings of two studies performed in large groups of stone formers from the United States [27, 28]. In these studies, a large prevalence of COM composition in kidney stones from first-time stone formers was found, and this composition was associated with the lowest risk of recurrence, compared with patients with COD or other stone compositions [27, 28].

In COM stone formers, if the clinical evaluation allows to exclude the presence of a secondary cause of calcium lithiasis, such as primary hyperparathyroidism or gastrointestinal diseases, hypercalciuria and hyperoxaluria are rarely present. The urinary calcium/oxalate ratio may be involved in the pathogenesis of stones in such situations, as suggested by Daudon and colleagues [24]. However, other factors may be implied. Poor hydration may represent the most important one [29]. In fact, this is a very common risk factor for urolithiasis, although not easy to detect since patients correctly tend to increase the fluid intake after an episode of stones even before medical evaluation [29].

Nutritional imbalances, such as excessive salt intake or reduced fruit and vegetable intake, may have not a relevant role in idiopathic COM stone formers, because they are generally associated with recognizable urinary abnormalities [30, 31], that were not detected in our study. Interestingly, nutritional investigations comparing the dietary habits of idiopathic calcium stone formers with controls showed only minor differences [32, 33], supporting the assumption that nutrition plays a central role in the pathogenesis of kidney stones only in selected cases.

Family history may instead be involved. It is well known that a family history of stones is associated with an earlier onset of stone disease irrespective of urinary metabolic abnormalities [14, 34, 35] and even with stone composition [34]. In the present study, the age of onset of patients with and without family history of stones was significantly different in those with COD/COM ratio ≤ 0.25, who had few metabolic abnormalities and low urinary supersaturations (mean age of onset 35, 95% CI 33-37, vs 42, 95% CI 40-44, respectively, $p<0.001$).

This effect may depend on the urinary levels of macromolecules involved in the lithogenic process but not detected in routine 24-hour urine chemistry. These molecules may promote aggregation of calcium oxalate crystals or urinary viscosity even in the absence of high urinary calcium excretion [36-38]. Their action could

also explain the absence of a trend on the age of onset in patients with family history of stones with increasing urinary supersaturations (Figure 1A); conversely, in patients without family history of stones, with the increase in urinary supersaturations the age of onset is lowered to values close to those with family history. Higher proportions of COD in kidney stones are associated with an earlier onset of the disease, irrespective of the presence of family history.

From a clinical perspective, our findings may have relevance for defining the best management strategy for patients with idiopathic calcium nephrolithiasis. In those who have a COD/COM ratio >0.25, metabolic evaluation, i.e., 24-hour urinary collection for determination of the profile of lithogenic risk, is mandatory because the risk of metabolic abnormalities is elevated [11, 12]. Follow-ups should be scheduled every 3-6 months [39], due to the elevated risk of stone recurrence [28, 40]. Conversely, our findings suggest that, in patients with a COD/COM ratio ≤0.25, the prescription of the urinary profile of lithogenic risk should be made only in selected cases, based on a personal history suggesting the presence of risk factors for recurrence. If these risk factors are not present and the patient is a first-time stone former, metabolic evaluation could be avoided, due to the low risk of detecting metabolic abnormalities that can modify the strategy of secondary prevention [40].

The clinical relevance of the COD/COM ratio in pure idiopathic calcium oxalate stone formers should be further investigated in the future. Although our study suggests a potential usefulness of this parameter in guiding the prevention management of kidney stone formers, some limitations should be considered. The most obvious one is the observational design of the study and the absence of a follow-up, not allowing to ascertain whether the COD/COM ratio is able to predict the clinical course of stone disease. Moreover, the sample size was relatively limited, compared with other previous studies [27, 28], although focused on the most common clinical form of urolithiasis.

Conclusions

In a group of idiopathic pure calcium oxalate stone formers, the COD/COM ratio of stone composition, examined by FT-IR, was positively associated with 24-hour urinary calcium excretion and urinary pH. A COD/COM ratio ≤0.25 was associated with little urinary metabolic abnormalities, suggesting different management strategies for patients with these characteristics of stone composition. The clinical significance of COD/COM ratio in idiopathic calcium nephrolithiasis deserves further investigation in the future.

Compliance with ethical standards

Conflict of interest

The authors have nothing to disclose.

Ethical standards

The study protocol was approved by the local Ethics Committee as part of a larger project on the clinical and nutritional correlates of urinary parameters in nephrolithiasis. The study was carried out according to the principles of the Declaration of Helsinki. Informed consent was obtained according to Italian law for retrospective studies.

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Table 1. Clinical characteristics of the patients with idiopathic calcium nephrolithiasis and pure calcium oxalate stone composition included in the study (n=465, 322 M and 143 F), stratified by the intervals of the ratio of calcium oxalate dihydrate (COD) and calcium oxalate monohydrate (COM) crystals in stones.

COD/COM ratio Number (%) Intervals	(0-0.25) 287 (62%) **(1)**	(0.26-0.50) 86 (18%) **(2)**	(0.51-0.75) 40 (9%) **(3)**	(0.76-1) 52 (11%) **(4)**	p* 	p** adjusted	p*** value for trend
Females,%	31	37	30	17	0.104		**0.029**
Age, years	47 ± 13	46 ± 14	46 ± 15	42 ± 16	0.085		**0.016**
Weight, kg	75 ± 16	74 ± 15	75 ± 14	76 ± 12	0.961		
BMI, kg/m^2	25 ± 4	26 ± 4	25 ± 3	25 ± 3	0.468		
Disease duration	5 [1-14]	5 [1-15]	8 [1-21]	3 [1-19]	0.373		
Family history of stones (FHS), %	52	51	60	48	0.716		
Age of onset of kidney stones	38 ± 14	37 ± 14	32 ± 13	32 ± 12	**0.007**		**0.001**
*Age of onset of kidney stones in patients **without FHS***	*42 ± 14*	*39 ± 14*	*31 ± 12*	*35 ± 13*	***0.003***		***0.003***
*Age of onset of kidney stones in patients **with FHS***	*35 ± 13*	*35 ± 13*	*33 ± 14*	*30 ± 11*	*0.315*		
Hypertensive, %	21	26	28	15	0.396	0.369	
Recurrents, %	62	68	73	62	0.495	0.634	
Stones retained, %	55	50	59	41	0.304	0.138	
Bilateral stones,%	45	52	55	43	0.473	0.585	
Extra-Corporeal Shock-Wave Lithotripsy (ESWL), number	0 [0-1]	0 [0-1]	0 [0-2]	0 [0-2]	**0.017**		
Stone rate, years	0.39 [0.16-1.00]	0.62 [0.20-1.00]	0.35 [0.16-0.97]	0.46 [0.15-1.00]	0.361		

Data reported as percentage or median and interquartile range or mean ± standard deviation. Significant p values ($p<0.05$) are indicated in bold.

*Crude p values obtained with ANOVA (dichotomous variables or continuous variables with normal distribution) or Kruskal-Wallis test (continuous variables with non-normal distribution).

**p values adjusted for sex, age, BMI and duration of disease with ANCOVA (only variables requiring adjustment for clinical reasons).

***p for trend values obtained with ANOVA (linear trends). Values are reported only if significant ($p<0.05$).

Table 2. Blood chemistry parameters of the patients with idiopathic calcium nephrolithiasis and pure calcium oxalate stone composition included in the study (n=465, 322 M and 143 F), stratified by the intervals of the ratio of calcium oxalate dihydrate (COD) and calcium oxalate monohydrate (COM) crystals in stones.

COD/COM ratio Number Intervals	(0-0.25) N.287 **(1)**	(0.26-0.50) N.86 **(2)**	(0.51-0.75) N.40 **(3)**	(0.76-1) N.52 **(4)**	p*	p**	p<0.05 Bonferroni test
Creatinine, mg/dl	0.90 ± 0.18	0.86 ± 0.18	0.90 ± 0.18	0.89 ± 0.14	0.285	**0.024**	
Uric acid, mg/dl	5.38 ± 1.27	5.02 ± 1.09	5.25 ± 0.96	5.41 ± 1.10	0.127	0.101	
Calcium, mg/dl	9.47 ± 0.38	9.46 ± 0.45	9.51 ± 0.45	9.70 ± 0.44	**0.003**	**0.014**	**(**1) and (2) vs (4)
Phosphorus, mg/dl	3.29 ± 0.53	3.29 ± 0.55	3.17 ± 0.60	3.33 ± 0.62	0.600	0.468	
PTH, pg/ml	44 ± 14	43 ± 13	39 ± 12	40 ± 14	0.226	0.282	
25-OH-D, ng/ml	22 ± 13	23 ± 15	25 ± 12	21 ± 13	0.724	0.887	

25-OH-D: 25-hydroxy-vitamin D.
Data reported as mean ± standard deviation. Significant p values (p<0.05) are indicated in bold.
*Crude p values obtained with ANOVA.
** p values adjusted for sex, age, BMI and duration of disease with ANCOVA

Table 3. Urinary chemistry parameters of the patients with idiopathic calcium nephrolithiasis and pure calcium oxalate stone composition included in the study (n=465, 322 M and 143 F), stratified by the intervals of the ratio of calcium oxalate dihydrate (COD) and calcium oxalate monohydrate (COM) crystals in stones.

COD/COM ratio Number Intervals	(0-0.25) N.287 **(1)**	(0.26-0.50) N.86 **(2)**	(0.51-0.75) N.40 **(3)**	(0.76-1) N.52 **(4)**	p*	p**	p<0.05 Bonferroni test
Volume, ml/24h	1905 ± 702	1916 ± 774	1716 ± 640	1969 ± 757	0.367	0.254	
Creatinine, mg/24h	1525 ± 426	1522 ± 452	1643 ± 455	1580 ± 404	0.367	0.125	
Sodium, mEq/24h	171 ± 61	168 ± 59	164 ± 53	166 ± 59	0.867	0.548	
Potassium, mEq/24h	55 ± 18	53 ± 20	55 ± 15	54 ± 15	0.965	0.952	
Calcium, mg/24h	194 ± 85	256 ± 84	315 ± 122	313 ± 119	**<0.0001**	**<0.0001**	(1) vs (2) vs (3) vs (4); (2) vs (3) vs (4)
Hypercalciuria (≥ 4 mg/kg/24h), %	12	28	53	48	**<0.0001**	**<0.0001**	(1) vs (2) vs (3) vs (4); (2) vs (3) vs (4)
Magnesium, mg/24h	87 ± 29	91 ± 24	101 ± 34	95 ± 29	**0.013**	**0.023**	(1) vs (3)
Chloride, mEq/24h	167 ± 62	165 ± 56	167 ± 52	168 ± 59	0.984	0.891	
Phosphorus, mg/24h	842 ± 271	848 ± 239	921 ± 235	855 ± 286	0.360	0.195	
Uric acid, mg/24h	571 ± 164	597 ± 178	595 ± 166	567 ± 144	0.518	0.186	
Oxalate, mg/24h	31 ± 11	31 ± 9	33 ± 8	30 ± 9	0.645	0.457	
Hyperoxaluria (>45 mg/24h), %	7	7	8	4	0.859	0.886	
Sulphate, mmol/24h	21 ± 7	21 ± 7	22 ± 7	20 ± 6	0.554	0.151	
Ammonium, mmol/24h	36 ± 12	37 ± 12	38 ± 10	38 ± 13	0.351	0.494	
Urea, g/24h	24 ± 7	23 ± 7	25 ± 7	23 ± 7	0.661	0.240	
Citrate, mg/24h	578 ± 257	633 ± 249	600 ± 258	591 ± 278	0.394	0.169	
Urine pH, 24h	5.88 ± 0.45	5.93 ± 0.43	5.76 ± 0.43	6.04 ± 0.46	**0.016**	**0.015**	(3) vs (4)
Calcium oxalate supersaturation	5.26 ± 2.94	6.75 ± 3.83	8.54 ± 3.42	7.22 ± 3.58	**<0.0001**	**<0.0001**	(1) vs (2) vs (3) vs (4) (2) vs (3)
Calcium phosphate supersaturation	0.66 ± 0.61	0.94 ± 0.75	1.17 ± 1.08	1.27 ± 0.83	**<0.0001**	**<0.0001**	(1) vs (2) vs (3) vs (4)

Data reported as percentage or mean ± standard deviation. Significant p values (p<0.05) are indicated in bold.
*Crude p values obtained with ANOVA. **p values adjusted with ANCOVA for sex, age, BMI and duration of disease.

Table 4. Comparison of urinary chemistry parameters between patients with idiopathic calcium nephrolithiasis and pure calcium oxalate stone composition (n=465), stratified by the intervals of the ratio of calcium oxalate dihydrate (COD) and calcium oxalate monohydrate (COM) crystals in stones, and a group of non-stone forming controls (n=486).

		Stone Formers N.465			**p**	**p < 0.05 Bonferroni test**
	Controls N.486 (c)	COD/COM (0-0.25) N. 287 **(1)**	COD/COM (0.26-0.50) N.86 **(2)**	COD/COM (0.50-1) N.92 **(3)**		
Volume, ml/24h	1516 ± 693	1883 ± 685	1895 ± 668	1843 ± 674	**<0.0001**	(c) vs (1) vs (2) vs (3)
Creatinine, mg/24h	1404 ± 293	1432 ± 290	1436 ± 283	1454 ± 285	0.351	
Sodium, mEq/24h	161 ± 57	164 ± 56	160 ± 55	155 ± 55	0.589	
Potassium, mEq/24h	57 ± 19	53 ± 18	52 ± 18	53 ± 18	**0.014**	
Calcium, mg/24h	198 ± 95	186 ± 94	247 ± 91	304 ± 92	**<0.0001**	(c) vs (2) vs (3)
Hypercalciuria,%	17	12	29	50	**<0.0001**	(c) vs (2) vs (3)
Phosphorus, mg/24h	727 ± 236	805 ± 233	809 ± 227	829 ± 229	**<0.0001**	(c) vs (1) vs (2) vs (3)
Magnesium, mg/24h	84± 29	85 ±29	89± 28	94± 29	**0.025**	(c) vs (3)
Uric acid, mg/24h	537 ± 157	550 ± 155	572 ± 151	548 ± 153	0.231	
Citrate, mg/24 h	625 ± 260	578 ± 257	626 ± 251	613 ± 253	0.095	
Sulphate, mmol/24h	20 ± 6	20 ± 6	20 ± 5	20 ± 5	0.351	
Ammonium, mmol/24h	35 ± 11	34 ± 11	36 ± 11	35 ± 11	0.687	
Oxalate, mg/24 h	27 ± 10	30 ± 10	30 ± 9	30 ± 9	**<0.0001**	(c) vs (1) vs (3)
Hyperoxaluria,%	5	6	6	4	0.935	
Urine pH, 24h	5.99 ± 0.51	5.93 ± 0.51	5.97 ± 0.49	5.95 ± 0.49	0.472	
Calcium oxalate supersaturation	5.32 ± 3.04	5.67 ± 3.00	7.18 ± 2.91	8.02 ± 2.93	**<0.0001***	(c) vs (2) vs (3)
Calcium phosphate supersaturation	0.84 ± 0.77	0.74 ± 0.76	1.02 ± 0.74	1.23 ± 0.75	**<0.0001***	(c) vs (3)

Data reported as percentage or mean ± standard deviation adjusted for age, sex and BMI with ANCOVA, or mean ± standard deviation* adjusted for age, sex, volume and BMI with ANCOVA. Significant p values ($p<0.05$) are indicated in bold.

Table 5. Linear regression model testing the relationship between calcium, oxalate and urine pH with COD/COM ratio in 465 stone formers with idiopathic calcium nephrolithiasis and pure calcium oxalate stone composition.

	β	**95%CI**	**β standardized**	**p**
Calcium, mg/24h	0.124	0.102-0.145	0.464	< **0.0001**
Oxalate, mg/24 h	-0.145	-0.370- 0.081	- 0.052	0.209
Urine pH, 24h	6.402	1.347-11.457	0.103	**0.013**

Significant p values ($p<0.05$) are indicated in bold

Figure 1. Mean and 95% CI of the age of onset of kidney stones (A) and of calciuria (B) in 461 patients stratified by family history of stones (FHS) (221 without FHS, 240 with FHS) and ratio COD/COM (0-0.25, 0.26-0.50, 0.51-1). A significant trend, for the age of onset of kidney stones, is present in patients without FHS ($p = 0.003$) increasing the COD/COM ratio and urinary supersaturations, but not in patients with FHS ($p = 0.374$). Calciuria (B) is not different in patients with and without FHS, $p = 0.798$.

Age of onset of kidney stones is adjusted for BMI and sex.
Calciuria by age, sex, duration of disease, BMI, sodium, potassium, ammonium and urinary sulfates.
Calcium oxalate supersaturation (srcaox) and calcium phosphate supersaturation (srcap) adjusted for BMI, age, volume and sex, no differences for over-saturation between patients with FHS and without FHS

Abstract

Pure calcium oxalate is the most frequent type of idiopathic kidney stone composition. Fourier transform infrared spectroscopy (FT-IR) allows to detect the ratio of calcium oxalate dihydrate (COD) and monohydrate (COM) crystals in stones, but the clinical significance of this parameter remains uncertain. The objective of this observational study was to verify the association of clinical and laboratory parameters of kidney stone disease with COD/COM ratio in a group of 465 (322 M, age 46 ± 14) patients suffering from idiopathic calcium nephrolithiasis with pure calcium oxalate stones (≥97%). Each participant underwent a complete clinical examination, serum chemistry, 24-hour urine collection for the determination of the profile of lithogenic risk, and had stones analyzed by FT-IR. Most (62%) of the stones has a COD/COM ratio ≤0.25, and the urine chemistry of the corresponding patients showed a ~~very~~ low prevalence of urinary metabolic abnormalities. With increasing ~~quartiles of~~ COD/COM ratio intervals (0-0.25, 0.26-0.50, 0.51-0.75, 0.76-1), a significant ~~positive trend~~association was observed for the number of urological procedures, serum calcium, 24-h urinary calcium excretion, prevalence of hypercalciuria and relative calcium oxalate supersaturation, and a negative trend was detected for the age of the first stone episode (all p-values ~~for trend~~ <0.05). A linear regression model showed that the only parameters significantly associated with COD/COM ratio were 24-h urinary calcium excretion (standardized β=0.464, p<0.001) and urine pH (standardized β=0.103, p=0.013). In pure calcium oxalate idiopathic stones, COD/COM ratio may reflect the presence of urinary metabolic risk factors, and ~~serve as~~represent a guide for the prescription of urinary analyses.

Key words: urolithiasis; hypercalciuria; hyperoxaluria; kidney stones; calcium oxalate.

Introduction

Under infrared spectroscopy, calcium oxalate crystals of calcium stones may appear in two distinct molecular forms: whewellite, that is, calcium oxalate monohydrate (COM), and weddellite, that is, calcium oxalate dihydrate (COD). These forms are associated with different etiology of stones [1, 2] and are also associated with different surface morphology of calculi [3].

COM depends on urinary excretion of oxalate and is typical of conditions of hyperoxaluria, such as primary hyperoxaluria, intestinal diseases or dietary regimens with a high oxalate load [34-78]. According to the surface morphology and crystallite appearance at environmental scanning electron microscopy, five different types of COM stones can be identified [3, 9]. Each of them corresponds to different pathophysiological mechanisms: low diuresis or slight intermittent hyperoxaluria (type Ia), low diuresis and slight intermittent hyperoxaluria and hypercalciuria (type Ib), primary hyperoxaluria (type Ic), hyperoxaluria with anatomical alterations (type Id) and enteric hyperoxaluria (type Ie) [9].

COD is instead typically found in stones of patients who have a high urinary calcium excretion, with or without hyperoxaluria, due to primary hyperparathyroidism, Paget bone disease, prolonged immobilization, sarcoidosis, myeloma, bone metastasis, acromegaly, hyperthyroidism, renal or enteric hypercalciuria [3, 810]. In this context, the detection of prevalent COM or COD composition in stones passed by patients with calcium lithiasis may serve as a guide for detecting stone etiology and prescribing appropriated second-level diagnostic tests [911, 1012].

However, the most common etiology of calcium stones is idiopathic, accounting for around 80-85% of patients visited in stone clinics [1, 911, 1012]. In these patients, the presence of COM, COD, or a combination of the two in passed stones examined by infrared spectroscopy has uncertain significance.

The objective of this observational study was to detect the possible associations between calcium oxalate stone composition, in terms of COM, COD and their ratio, and the clinical characteristics, including 24-hour urinary parameters of lithogenic risk, of a large group of patients with idiopathic calcium nephrolithiasis (ICN).

Materials and methods

Study participants

All subjects over 18 who completed a medical and urinary metabolic evaluation at our Stone Clinic from 2009 to 2017 were eligible for study enrolment. Inclusion criteria were the presence of ICN, infrared spectroscopy analysis of stones completed at our laboratory within three months from urinary metabolic evaluation, and pure calcium oxalate stone composition (defined as calcium oxalate crystals ≥97%). Subjects with known calcium stone etiology, such as primary hyperoxaluria, enteric hyperoxaluria, primary hyperparathyroidism or other bone diseases associated with hypercalciuria, were excluded from the study. Subjects with chronic kidney disease (creatinine clearance <60 ml/min), renal tubular acidosis, recurrent urinary tract infections, congenital or acquired anomalies of the kidney and the urinary tract, spina bifida, or cystic fibrosis were excluded as well. Subjects with missing clinical or laboratory data were not considered for the final analysis.

Thus, the main study population was composed exclusively of calcium stone formers with documented pure calcium oxalate stone composition and no known etiology. From an epidemiological perspective, this circumstance represents the majority of cases with calcium nephrolithiasis [~~10~~12, ~~11~~13].

A database of urinary profiles of lithogenic risk from a group of non-stone forming controls who underwent urinary metabolic evaluation at our Stone Clinic ~~for reasons other than nephrolithiasis~~ was also considered, to compare the urine composition of patients with different COD/COM ratio in their stone composition with normal standards. These controls (mean age 42±12 years old, male:female ratio 1:2, body mass index [BMI] 24±4 kg/m^2) were selected according to the absence of episodes of renal colic in their personal history and absence of retained stones at abdominal ultrasound at the moment of urine collection. Subjects with congenital or acquires anomalies of the urinary tract, recurrent urinary tract infections, creatinine clearance <60 ml/min and suspected diseases of calcium metabolism were not considered.

Clinical and urinary metabolic evaluation

According to the clinical protocol adopted in our stone clinic [~~11~~13], a comprehensive medical history, with particular focus on the stone disease course and risk factors, was collected from all participants. Family history and age of onset of the first stone episode were carefully collected [~~12~~14]. The coexistence of kidney stones with hypertension, that represents an important risk factor for urinary metabolic abnormalities [~~13~~15], was also particularly assessed.

Height, weight, and arterial pressure were measured. Abdominal ultrasound or X-ray were performed to detect retained stones and their radio-opacity. Blood tests, including serum creatinine, calcium, phosphorus, uric acid, parathormone (PTH), and 25-hydroxyvitamin D (25-OH-D) were performed.

Each participant also collected a 24-hour urine sample for the urinary metabolic profile of lithogenic risk [~~11~~13]. During the collection, urine was equally distributed in two containers: one containing 2 ml of chlorhexidine gluconate 20% and the other 15 ml of 18% hydrochloric acid. ~~This~~ The panel of urinary analyses, performed on the same day the collection was concluded, included pH, ~~volume and urinary excretion of calcium, chloride, phosphorus, oxalate, citrate, magnesium, potassium, sodium, sulfate, ammonium, uric acid, urea and creatinine~~sodium, potassium, chloride, creatinine, ammonium, urea, uric acid, citrate (all measured from the chlorhexidine container), calcium, magnesium, oxalate, sulfate and phosphate (measured from the hydrochloric acid container). Urine volume was also assessed considering the content of both urine containers. Urinary relative supersaturations for lithogenic salts, representing an index of the risk of stone recurrence [~~14~~16], were calculated by using the Equil2 software [~~15~~17].

~~According to recent consensus, h~~Hypercalciuria was defined as a 24-hour urinary calcium excretion ≥4 mg/kg/day, while hyperoxaluria was defined as a 24-hour urinary oxalate excretion >45 mg/day ~~[9, 10]~~.

Stone analyses

Stones passed by participants or extracted during urologic procedures were examined at our stone clinic laboratory by Fourier transform infrared spectroscopy (FT-IR). This technique allows the detection and quantification of COM and COD crystals in stones.

Mixtures with different percentages of COD and COM, selected from patients' kidney stones, were prepared and used for the calibration. The pure COM and COD infrared spectra used for calibration, corresponding to the reference ones [~~16~~18], are shown in Supplementary Material (Figure S1). COM has a band with absorption peak at 1315 cm^{-1} and COD at 1325 cm^{-1}, respectively. Among kidney stones with spectra corresponding to COM [~~16~~18], the one with the lowest value of the 1325/1315 cm^{-1} ratio was chosen as the reference for pure COM. In fact, due to the additivity of the Lambert-Beer law, the presence of minimal traces of COD in the sample increases the absorbance to a greater extent at 1325 cm^{-1} than at 1315 cm^{-1}, increasing COD/COM ratio. Conversely, pure COD was selected from samples with spectra equal to COD [~~16~~18] and with the highest value at 1325/1315 cm^{-1}.

For the FT-IR analyses of stones, pellets were prepared mixing pulverized stone (1%) with potassium bromide (99%). Absorbance spectrum was recorded using a Shimadzu FTIR – 8400S spectrophotometer (Shimadzu Corporation, Kyoto, Japan), with a measurement range between 400 and 4000 cm^{-1}, resolution 4 cm^{-1}, number of scans 45. The absorbance intensity of recorded spectra ranged between 0.2 and 0.8, to avoid deviations from the Lambert-Beer law.

The relationship between the percentage of COD in the mixture (x) and the absorbance ratio at 1325/1315 cm^{-1} (y) was described by a quadratic equation ($0.078x^2 + 0.352x + 0.734$, $R^2 = 0.9995$), shown in the Supplementary Material (Figure S2). This equation was used to determine the COD/COM ratio in the calcium oxalate kidney stones of patients enrolled in this study.

Statistical analyses

~~Data~~ Continuous variables were expressed as mean ± standard deviation or, for non-normally distributed variables, ~~mean and 95% confidence intervals (CI),~~ median and interquartile range (IQR). ~~and~~ Dichotomous variables were expressed as percentages ~~as appropriate~~. Stone formers were stratified by COD/COM ratio ~~quartile~~ intervals of their stone composition, as following: 0-0.25 (first interval), 0.26-0.50 (second interval), 0.51-0.75 (third interval), 0.76-1 (fourth interval). ~~and clinical parameters were compared among these groups and controls by using Kruskal-Wallis test, Bonferroni test for multiple comparisons, one-way analysis of variance (ANOVA), and covariance analysis (ANCOVA), as appropriate according to the variable type and distribution.~~Normally distributed continuous and dichotomous clinical and laboratory parameters were compared among groups of patients, stratified by intervals of COD/COM ratio, using one-way analysis of variance (ANOVA) for crude comparisons, and analysis of covariance (ANCOVA) for comparisons adjusted for covariates (age, sex, duration of disease, BMI). The Bonferroni test for multiple comparisons was applied if adjusted p values were <0.05. Non-normally distributed continuous variables were compared among groups of patients by Kruskal-Wallis test. ~~The relationship between urinary parameters and COD/COM ratio in stone composition was also assessed by linear regression models.~~

For comparisons between patients, stratified by intervals of COD/COM ratio, and controls, urinary parameters were handled as mean ± standard deviation adjusted for age, sex and BMI, since these variables were different between patients and controls. Urinary supersaturations were also adjusted for urinary volume. Comparisons were then made using ANCOVA. Bonferroni test was again applied if adjusted p values were <0.05.

The relationship between urinary parameters and COD/COM ratio in stone composition was also assessed by linear regression models.

All p-values ~~were two tailed and~~ were considered significant for $p<0.05$. Analyses were performed with the SPSS software v.24 (SPSS Inc., Chicago, IL, USA).

~~*Ethical statement*~~

~~The study protocol was approved by the local Ethics Committee. All data were obtained and handled in anonymous way. Informed consent was obtained and the study procedures followed the principles contained in the Declaration of Helsinki.~~

Results

From 2009 to 2017, 947 stone samples (from 677 males and 270 females) with pure calcium oxalate composition were analyzed in our Stone Clinic. However, 482 subjects were excluded from the analysis for missing clinical or laboratory data or not meeting inclusion criteria. Thus, the study was conducted on a group of 465 stone formers (322 males, 143 females, age 46 ± 14) and their stones. ~~A group of 486 non-stone forming controls who performed urine analyses at our center was also considered as a control group.~~

In stone formers, the COD/COM ratio median was 0.20 (IQR 0.10-0.40), so that most participants (62%) fell within the ~~lowest quartile~~first interval of COD/COM ratio (0-0.25). The clinical characteristics of patients, stratified by COD/COM ratio ~~quartile~~intervals, are reported in Table 1. Patients with the highest COD/COM ratio had a higher number of extra-corporeal shock-wave lithotripsy procedures (p ~~for trend~~ = 0.~~004~~017 with Kruskal-Wallis test) and a lower age of onset of kidney stone disease (p for trend = 0.001 with ANOVA) (Table 1). However, the trend for an earlier onset of the disease was confirmed only in those without a family history of stones (p for trend = 0.003 with ANOVA), and not in those self-reporting a family history of stones (Table 1, Figure 1A).

There was also a trend for an increase in serum calcium with increasing COD/COM ratio (p adjusted with ANCOVA for age, sex, duration of disease and ~~body mass index~~BMI = 0.014), while other serum parameters were not different across COD/COM ratio ~~quartiles~~ intervals (Table 2).

The analysis of 24-hour urinary parameters of lithogenic risk across COD/COM ~~quartiles~~ intervals is depicted in Table 3. With increasing COD/COM ratio, significantly higher levels of 24-h calcium excretion, calcium oxalate and calcium phosphate supersaturation (all p values adjusted with ANCOVA for age, sex, duration of disease and ~~body mass index~~BMI <0.001) could be demonstrated. Moreover, urinary pH was higher in the ~~highest~~ fourth vs the third ~~quartile~~ interval of COD/COM ratio, while 24-hour urinary oxalate excretion was not related with the COD/COM ratio (Table 3).

The comparison of the 24-hour urine parameters of patients belonging to different ~~quartiles~~ intervals of COD/COM ratio with non-stone forming controls revealed that subjects in the ~~lowest quartile~~first interval of COD/COM ratio had a very similar urine composition than controls, exhibiting only a higher volume and excretion of phosphorus and oxalate (Table 4). Conversely, those with a COD/COM ratio >0.25 exhibited a wider range of urinary abnormalities compared to controls, including a higher calcium excretion and a higher

calcium oxalate relative supersaturation index (Table 4). The 24-hour urinary calcium excretion was also unaffected by the presence of a family history of stones (Figure 1B).

A linear regression model, exploring the possible clinical and urinary parameters associated with the COD/COM ratio of stone composition, is shown in Table 5. Only 24-hour urinary calcium ($\beta = 0.124$, 95% CI 0.102-0.145, standardized $\beta = 0.464$, $p<0.001$) and urine pH ($\beta = 6.402$, 95% CI 1.347-11.457, standardized $\beta = 0.103$, $p=0.013$) were significantly associated with COD/COM ratio.

Discussion

In a group of patients with idiopathic calcium nephrolithiasis and pure calcium oxalate composition, the COD/COM ratio of stone composition, determined by FT-IR, was significantly associated with an earlier onset of the disease, higher number of urologic procedures, higher serum calcium, higher urinary excretion of calcium and pH. Among these parameters, 24-hour urinary calcium excretion exhibited the strongest correlation with COD/COM ratio. Moreover, patients with a COD/COM ratio ≤0.25, representing the majority of subjects with idiopathic calcium nephrolithiasis, showed no clinically relevant metabolic abnormalities in urine chemistry.

This is one of the first studies exploring the clinical correlates of COD/COM ratio of stone composition in idiopathic calcium nephrolithiasis with stones of pure calcium oxalate composition. Previous investigations were in fact focused on patients with known metabolic abnormalities or secondary forms of calcium nephrolithiasis, and showed an association between hypercalciuria and high COD/COM ratio [~~17~~19-~~21~~23]. The only study conducted on an unselected population of calcium oxalate stone formers showed the presence of a significant correlation between urinary calcium/oxalate ratio and stone COD/COM ratio, with oxalate dependence of COM crystal formation and calcium dependence of COD crystal formation [24].

In our group of pure calcium oxalate stone formers, the highest values of 24-hour urinary calcium excretion and the highest prevalence of hypercalciuria were found in those with a COD/COM ratio >0.50. Those in the ~~highest quartile~~fourth interval of COD/COM ratio (>0.75) also exhibited a higher urinary pH, suggesting a role of pH in determining the COD content of calcium oxalate stones in hypercalciuric patients [~~22~~25].

Previous studies also suggested a significant association between hyperoxaluria and prevalent COM composition of stones [~~3~~4-~~7~~8, ~~22~~25], that was not confirmed in our group of pure calcium oxalate stone formers with idiopathic calcium nephrolithiasis. This association is probably typical of gastrointestinal diseases with increased oxalate absorption and dietary regimens with high oxalate load [~~3~~4-~~7~~8]. Patients with these conditions were not included in our study, since they do not fit with the criteria for diagnosing idiopathic calcium nephrolithiasis. However, participants with a low COD/COM ratio did exhibit a significantly higher 24-hour urinary oxalate excretion than controls, although the difference was mild.

Another point of interest is the circumstance that the relative supersaturation indexes for calcium oxalate were similar between subjects with pure calcium oxalate stones and COD/COM ratio ≤0.25 and healthy controls. The supersaturation indexes are well-known predictors of recurrence of kidney stones [~~14~~16, ~~23~~26], and

depend on urine volume and urinary metabolic abnormalities. In clinical practice, the finding of a low COD/COM ratio at stone analysis in patients with idiopathic calcium nephrolithiasis may imply that these patients have no urinary metabolic abnormalities and a low risk of stone recurrence. This assumption is also supported by the findings of two studies performed in large groups of stone formers from the United States [~~24~~27, ~~25~~28]. In these studies, a large prevalence of COM composition in kidney stones from first-time stone formers was found, and this composition was associated with the lowest risk of recurrence, compared with patients with COD or other stone compositions [~~24~~27, ~~25~~28].

In COM stone formers, if the clinical evaluation allows to exclude the presence of a secondary cause of calcium lithiasis, such as primary hyperparathyroidism or gastrointestinal diseases, hypercalciuria and hyperoxaluria are rarely present. The urinary calcium/oxalate ratio may be involved in the pathogenesis of stones in such situations, as suggested by Daudon and colleagues [24]. ~~From a physio-pathological perspective, this means that~~However, other factors may be implied. Poor hydration may represent the most important one [~~26~~29]. In fact, this is a very common risk factor for urolithiasis, although not easy to detect since patients correctly tend to increase the fluid intake after an episode of stones even before medical evaluation [~~26~~29].

Nutritional imbalances, such as excessive salt intake or reduced fruit and vegetable intake, may have not a relevant role in idiopathic COM stone formers, because they are generally associated with recognizable urinary abnormalities [~~27~~30, ~~28~~31], that were not detected in our study. Interestingly, nutritional investigations comparing the dietary habits of idiopathic calcium stone formers with controls showed only minor differences [~~29~~32, ~~30~~33], supporting the assumption that nutrition plays a central role in the pathogenesis of kidney stones only in selected cases.

Family history may instead be involved. It is well known that a family history of stones is associated with an earlier onset of stone disease irrespective of urinary metabolic abnormalities [~~12~~14, ~~31~~34, ~~32~~35] and even with stone composition [~~31~~34]. In the present study, the age of onset of patients with and without family history of stones was significantly different in those with COD/COM ratio ≤0.25, who had few metabolic abnormalities and low urinary supersaturations (mean age of onset 35, 95% CI 33-37, vs 42, 95% CI 40-44, respectively, $p<0.001$).

This effect may depend on the urinary levels of macromolecules involved in the lithogenic process but not detected in routine 24-hour urine chemistry. These molecules may promote aggregation of calcium oxalate crystals or urinary viscosity even in the absence of high urinary calcium excretion [~~33~~36-~~35~~38]. Their action

could also explain the absence of a trend on the age of onset in patients with family history of stones with increasing urinary supersaturations (Figure 1A); conversely, in patients without family history of stones, with the increase in urinary supersaturations the age of onset is lowered to values close to those with family history. Higher proportions of COD in kidney stones are associated with an earlier onset of the disease, irrespective of the presence of family history.

From a clinical perspective, our findings may have relevance for defining the best management strategy for patients with idiopathic calcium nephrolithiasis. In those who have a COD/COM ratio >0.25, metabolic evaluation, i.e., 24-hour urinary collection for determination of the profile of lithogenic risk, is mandatory because the risk of metabolic abnormalities is elevated [~~9~~11, ~~10~~12]. Follow-ups should be scheduled every 3-6 months [~~36~~39], due to the elevated risk of stone recurrence [~~25~~28, ~~37~~40]. Conversely, our findings suggest that, in patients with a COD/COM ratio ≤0.25, the prescription of the urinary profile of lithogenic risk should be made only in selected cases, based on a personal history suggesting the presence of risk factors for recurrence. If these risk factors are not present and the patient is a first-time stone former, metabolic evaluation could be avoided, due to the low risk of detecting metabolic abnormalities that can modify the strategy of secondary prevention [~~37~~40].

The clinical relevance of the COD/COM ratio in pure idiopathic calcium oxalate stone formers should be further investigated in the future. Although our study suggests a potential usefulness of this parameter in guiding the prevention management of kidney stone formers, some limitations should be considered. The most obvious one is the observational design of the study and the absence of a follow-up, not allowing to ascertain whether the COD/COM ratio is able to predict the clinical course of stone disease. Moreover, the sample size was relatively limited, compared with other previous studies [~~24~~27, ~~25~~28], although focused on the most common clinical form of urolithiasis.

Conclusions

In a group of idiopathic pure calcium oxalate stone formers, the COD/COM ratio of stone composition, examined by FT-IR, was positively associated with 24-hour urinary calcium excretion and urinary pH. A COD/COM ratio ≤0.25 was associated with little urinary metabolic abnormalities, suggesting different management strategies for patients with these characteristics of stone composition. The clinical significance of COD/COM ratio in idiopathic calcium nephrolithiasis deserves further investigation in the future.

Compliance with ethical standards

Conflict of interest

The authors have nothing to disclose.

Ethical standards

The study protocol was approved by the local Ethics Committee as part of a larger project on the clinical and nutritional correlates of urinary parameters in nephrolithiasis. The study was carried out according to the principles of the Declaration of Helsinki. Informed consent was obtained according to Italian law for retrospective studies.

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Table 1. Clinical characteristics of the patients with idiopathic calcium nephrolithiasis and pure calcium oxalate stone composition included in the study (n=465, 322 M and 143 F), stratified by the ~~quartiles~~ intervals of the ratio of calcium oxalate dihydrate (COD) and calcium oxalate monohydrate (COM) crystals in stones.

COD/COM ratio Number (%) ~~Quartiles~~Intervals	(0-0.25) 287 (62%) **(1)**	(0.26-0.50) 86 (18%) **(2)**	(0.51-0.75) 40 (9%) **(3)**	(0.76-1) 52 (11%) **(4)**	~~P~~p*	p** adjusted	~~P~~p*** value for trend ~~(reported only if significant)~~
Females,%	31	37	30	17	0.104		**0.029**
Age, years	47 ± 13	46 ± 14	46 ± 15	42 ± 16	0.085		**0.016**
Weight, kg	75 ± 16	74 ± 15	75 ± 14	76 ± 12	0.961		
BMI, kg/m^2	25 ± 4	26 ± 4	25 ± 3	25 ± 3	0.468		
Disease duration	5 ~~([~~1-14~~)]~~	5 ~~([~~1-15~~)]~~	8 ~~([~~1-21~~)]~~	3 ~~([~~1-19~~)]~~	0.373		
Family history of stones (FHS), %	52	51	60	48	0.716		
Age of onset of kidney stones	38 ± 14	37 ± 14	32 ± 13	32 ± 12	**0.007**		**0.001**
*Age of onset of kidney stones in patients **without FHS***	*42 ± 14*	*39 ± 14*	*31 ± 12*	*35 ± 13*	***0.003***		***0.003***
*Age of onset of kidney stones in patients **with FHS***	*35 ± 13*	*35 ± 13*	*33 ± 14*	*30 ± 11*	*0.315*		
Hypertensive, %	21	26	28	15	0.396	0.369	
Recurrents, %	62	68	73	62	~~0.634*~~0.495	0.634	
Stones retained, %	55	50	59	41	~~0.138*~~0.304	0.138	
Bilateral stones,%	45	52	55	43	~~0.585*~~0.473	0.585	
Extra-Corporeal Shock-Wave Lithotripsy (ESWL), number	~~0.67 ± 1.28~~0 [0-1]	~~0.80 ± 1.71~~0 [0-1]	~~0.94 ± 1.41~~0 [0-2]	~~1.42 ± 2.34~~0 [0-2]	~~**0.022***~~**0.017**		~~**0.004**~~
Stone rate, years	~~0.79 ± 1.20~~0.39 [0.16-1.00]	~~0.93 ± 1.40~~0.62 [0.20-1.00]	~~0.73 ± 0.77~~0.35 [0.16-0.97]	~~0.61 ± 0.52~~0.46 [0.15-1.00]	~~0.948*~~0.361		

Data reported as percentage or median and interquartile range or mean ± standard deviation. Significant p values (p<0.05) are indicated in bold.
*Crude p values obtained with ANOVA (dichotomous variables or continuous variables with normal distribution) or Kruskal-Wallis test (continuous variables with non-normal distribution).
~~p~~** p values adjusted for sex, age, BMI and duration of disease with ANCOVA (only variables requiring adjustment for clinical reasons).

***p for trend values obtained with ANOVA (linear trends). Values are reported only if significant ($p<0.05$).
~~Significant p values ($p<0.05$) are indicated in bold.~~

Table 2. Blood chemistry parameters of the patients with idiopathic calcium nephrolithiasis and pure calcium oxalate stone composition included in the study (n=465, 322 M and 143 F), stratified by the ~~quartiles~~ intervals of the ratio of calcium oxalate dihydrate (COD) and calcium oxalate monohydrate (COM) crystals in stones.

COD/COM ratio Number ~~Quartile~~Intervals	(0-0.25) N.287 **(1)**	(0.26-0.50) N.86 **(2)**	(0.51-0.75) N.40 **(3)**	(0.76-1) N.52 **(4)**	~~P~~p*	p*~~*~~	p~~*~~<0.05 Bonferroni test
Creatinine, mg/dl	0.90 ± 0.18	0.86 ± 0.18	0.90 ± 0.18	0.89 ± 0.14	0.285	**0.024**	
Uric acid, mg/dl	5.38 ± 1.27	5.02 ± 1.09	5.25 ± 0.96	5.41 ± 1.10	0.127	0.101	
Calcium, mg/dl	9.47 ± 0.38	9.46 ± 0.45	9.51 ± 0.45	9.70 ± 0.44	**0.003**	**0.014**	**(**1) and (2) vs (4)
Phosphorus, mg/dl	3.29 ± 0.53	3.29 ± 0.55	3.17 ± 0.60	3.33 ± 0.62	0.600	0.468	
PTH, pg/ml	44 ± 14	43 ± 13	39 ± 12	40 ± 14	0.226	0.282	
25-OH-D, ng/ml	22 ± 13	23 ± 15	25 ± 12	21 ± 13	0.724	0.887	

25-OH-D: 25-hydroxy-vitamin D.
Data reported as mean ± standard deviation. Significant p values (p<0.05) are indicated in bold.
*Crude p values obtained with ANOVA.
** p values adjusted for sex, age, BMI and duration of disease with ANCOVA
~~Data reported as mean ± standard deviation. p* adjusted for age, duration disease, sex and BMI with ANCOVA. Significant p values (p<0.05) are indicated in bold. 25-OH-D: 25-hydroxy-vitamin D.~~

Table 3. Urinary chemistry parameters of the patients with idiopathic calcium nephrolithiasis and pure calcium oxalate stone composition included in the study (n=465, 322 M and 143 F), stratified by the ~~quartiles~~ intervals of the ratio of calcium oxalate dihydrate (COD) and calcium oxalate monohydrate (COM) crystals in stones.

COD/COM ratio Number Intervals~~Quartile~~	(0-0.25) N.287 **(1)**	(0.26-0.50) N.86 **(2)**	(0.51-0.75) N.40 **(3)**	(0.76-1) N.52 **(4)**	~~P~~p*	p**	p*<0.05 Bonferroni test
Volume, ml/24h	1905 ± 702	1916 ± 774	1716 ± 640	1969 ± 757	0.367	0.254	
Creatinine, mg/24h	1525 ± 426	1522 ± 452	1643 ± 455	1580 ± 404	0.367	0.125	
Sodium, mEq/24h	171 ± 61	168 ± 59	164 ± 53	166 ± 59	0.867	0.548	
Potassium, mEq/24h	55 ± 18	53 ± 20	55 ± 15	54 ± 15	0.965	0.952	
Calcium, mg/24h	194 ± 85	256 ± 84	315 ± 122	313 ± 119	**<0.0001**	**<0.0001**	(1) vs (2) vs (3) vs (4); (2) vs (3) vs (4)
Hypercalciuria (≥ 4 mg/kg/24h), %	12	28	53	48	**<0.0001**	**<0.0001**	(1) vs (2) vs (3) vs (4); (2) vs (3) vs (4)
Magnesium, mg/24h	87 ± 29	91 ± 24	101 ± 34	95 ± 29	**0.013**	**0.023**	(1) vs (3)
Chloride, mEq/24h	167 ± 62	165 ± 56	167 ± 52	168 ± 59	0.984	0.891	
Phosphorus, mg/24h	842 ± 271	848 ± 239	921 ± 235	855 ± 286	0.360	0.195	
Uric acid, mg/24h	571 ± 164	597 ± 178	595 ± 166	567 ± 144	0.518	0.186	
Oxalate, mg/24h	31 ± 11	31 ± 9	33 ± 8	30 ± 9	0.645	0.457	
Hyperoxaluria (>45 mg/24h), %	7	7	8	4	0.859	0.886	
Sulphate, mmol/24h	21 ± 7	21 ± 7	22 ± 7	20 ± 6	0.554	0.151	
Ammonium, mmol/24h	36 ± 12	37 ± 12	38 ± 10	38 ± 13	0.351	0.494	
Urea, g/24h	24 ± 7	23 ± 7	25 ± 7	23 ± 7	0.661	0.240	
Citrate, mg/24h	578 ± 257	633 ± 249	600 ± 258	591 ± 278	0.394	0.169	
Urine pH, 24h	5.88 ± 0.45	5.93 ± 0.43	5.76 ± 0.43	6.04 ± 0.46	**0.016**	**0.015**	(3) vs (4)
Calcium oxalate supersaturation	5.26 ± 2.94	6.75 ± 3.83	8.54 ± 3.42	7.22 ± 3.58	**<0.0001**	**<0.0001**	(1) vs (2) vs (3) vs (4) (2) vs (3)
Calcium phosphate supersaturation	0.66 ± 0.61	0.94 ± 0.75	1.17 ± 1.08	1.27 ± 0.83	**<0.0001**	**<0.0001**	(1) vs (2) vs (3) vs (4)

Data reported as percentage or mean ± standard deviation. Significant p values (p<0.05) are indicated in bold.
*Crude p values obtained with ANOVA.

**p values adjusted with ANCOVA for sex, age, BMI and duration of disease.

~~p crude, p* adjusted for age, duration disease, sex and BMI with ANCOVA. P<0.05 in bold.~~

Table 4. Comparison of urinary chemistry parameters between patients with idiopathic calcium nephrolithiasis and pure calcium oxalate stone composition (n=465), stratified by the ~~quartiles~~ intervals of the ratio of calcium oxalate dihydrate (COD) and calcium oxalate monohydrate (COM) crystals in stones, and a group of non-stone forming controls (n=486).

		Stone Formers N.465				
	Controls N.486 (c)	COD/COM (0-0.25) N. 287 **(1)**	COD/COM (0.26-0.50) N.86 **(2)**	COD/COM (0.50-1) N.92 **(3)**	~~P~~p	**p < 0.05 Bonferroni test**
Volume, ml/24h	1516 ± 693	1883 ± 685	1895 ± 668	1843 ± 674	**<0.0001**	(c) vs (1) vs (2) vs (3)
Creatinine, mg/24h	1404 ± 293	1432 ± 290	1436 ± 283	1454 ± 285	0.351	
Sodium, mEq/24h	161 ± 57	164 ± 56	160 ± 55	155 ± 55	0.589	
Potassium, mEq/24h	57 ± 19	53 ± 18	52 ± 18	53 ± 18	**0.014**	
Calcium, mg/24h	198 ± 95	186 ± 94	247 ± 91	304 ± 92	**<0.0001**	(c) vs (2) vs (3)
Hypercalciuria,%	17	12	29	50	**<0.0001**	(c) vs (2) vs (3)
Phosphorus, mg/24h	727 ± 236	805 ± 233	809 ± 227	829 ± 229	**<0.0001**	(c) vs (1) vs (2) vs (3)
Magnesium, mg/24h	84± 29	85 ±29	89± 28	94± 29	**0.025**	(c) vs (3)
Uric acid, mg/24h	537 ± 157	550 ± 155	572 ± 151	548 ± 153	0.231	
Citrate, mg/24 h	625 ± 260	578 ± 257	626 ± 251	613 ± 253	0.095	
Sulphate, mmol/24h	20 ± 6	20 ± 6	20 ± 5	20 ± 5	0.351	
Ammonium, mmol/24h	35 ± 11	34 ± 11	36 ± 11	35 ± 11	0.687	
Oxalate, mg/24 h	27 ± 10	30 ± 10	30 ± 9	30 ± 9	**<0.0001**	(c) vs (1) vs (3)
Hyperoxaluria,%	5	6	6	4	0.935	
Urine pH, 24h	5.99 ± 0.51	5.93 ± 0.51	5.97 ± 0.49	5.95 ± 0.49	0.472	
Calcium oxalate supersaturation	5.32 ± 3.04	5.67 ± 3.00	7.18 ± 2.91	8.02 ± 2.93	**<0.0001***	(c) vs (2) vs (3)
Calcium phosphate supersaturation	0.84 ± 0.77	0.74 ± 0.76	1.02 ± 0.74	1.23 ± 0.75	**<0.0001***	(c) vs (3)

Data reported as percentage or mean ± standard deviation adjusted for age, sex and BMI with ANCOVA, or mean ± standard deviation* adjusted for age, sex, volume and BMI with ANCOVA. Significant p values ($p<0.05$) are indicated in bold.

Table 5. Linear regression model testing the relationship between calcium, oxalate and urine pH with COD/COM ratio in 465 stone formers with idiopathic calcium nephrolithiasis and pure calcium oxalate stone composition.

	β	**95%CI**	**β standardized**	**p**
Calcium, mg/24h	0.124	0.102-0.145	0.464	< **0.0001**
Oxalate, mg/24 h	-0.145	-0.370- 0.081	- 0.052	0.209
Urine pH, 24h	6.402	1.347-11.457	0.103	**0.013**

Significant p values ($p<0.05$) are indicated in bold

Figure 1. Mean and 95% CI of the age of onset of kidney stones (A) and of calciuria (B) in 461 patients stratified by family history of stones (FHS) (221 without FHS, 240 with FHS) and ratio COD/COM (0-0.25, 0.26-0.50, 0.51-1). A significant trend, for the age of onset of kidney stones, is present in patients without FHS ($p = 0.003$) increasing the COD/COM ratio and urinary supersaturations, but not in patients with FHS ($p = 0.374$). Calciuria (B) is not different in patients with and without FHS, $p = 0.798$.

Age of onset of kidney stones is adjusted for BMI and sex.
Calciuria by age, sex, duration of disease, ~~body mass index~~BMI, sodium, potassium, ammonium and urinary sulfates.
Calcium oxalate supersaturation (srcaox) and calcium phosphate supersaturation (srcap) adjusted for BMI, age, volume and sex, no differences for over-saturation between patients with FHS and without FHS

Figure 1

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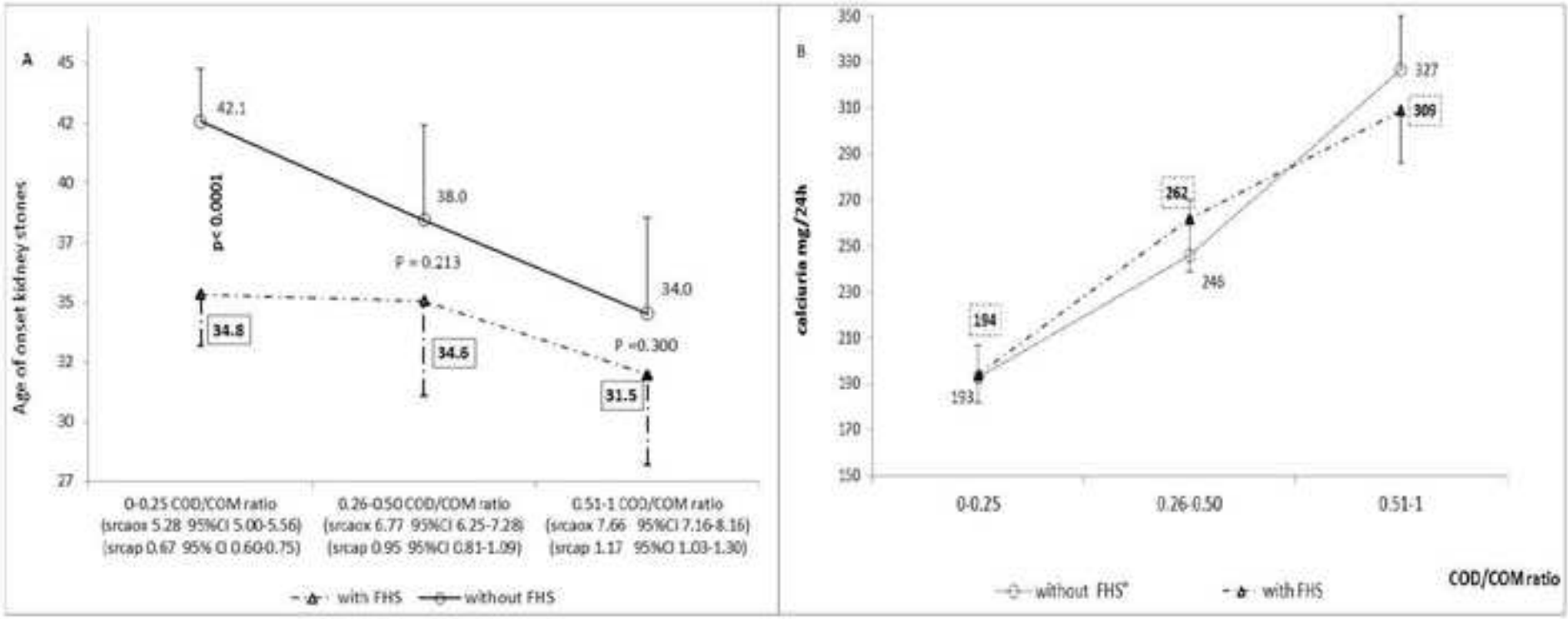

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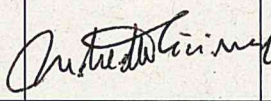
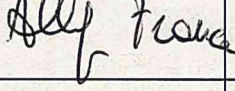
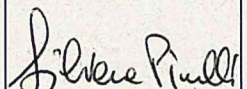
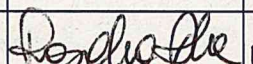
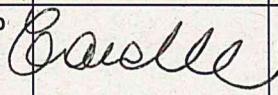
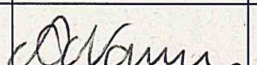
2nd author	ANDREA	TICINESI	I agree to the proposed new authorship shown in section 4 /~~and the addition/removal*of my name to the authorship list.~~		UNIVERSITY HOSPITAL OF PARMA	6/28/2019
3rd author	FRANCA	ALLEGRI	I agree to the proposed new authorship shown in section 4 /~~and the addition/removal*of my name to the authorship list.~~		UNIVERSITY HOSPITAL OF PARMA	6/28/2019
4th authors	SILVANA	PINELLI	I agree to the proposed new authorship shown in section 4 /~~and the addition/removal*of my name to the authorship list.~~		UNIVERSITY HOSPITAL OF PARMA	6/28/2019
5th author	ROSALIA	ALOE	I agree to the proposed new authorship shown in section 4 /~~and the addition/removal*of my name to the authorship list.~~		UNIVERSITY HOSPITAL OF PARMA	6/28/2019
6th author	TIZIANA	MESCHI	I agree to the proposed new authorship shown in section 4 /~~and the addition/removal*of my name~~ to the ~~authorship list.~~		UNIVERSITY HOSPITAL OF PARMA	6/28/2019
7th author	ANTONIO	NOUVENNE	I agree to the proposed new authorship shown in section 4 /and the ~~addition~~/removal*of my name to the authorship list.		UNIVERSITY HOSPITAL OF PARMA	6/28/2019

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