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## CHEMICAL COMPOSITION AND MORPHOLOGY OF RENAL STONES

**Abstract:** Two issues related to nephrolithiasis are explored: (1) does the chemical composition and morphology of renal calculi in South Poland overlap with the studies from other countries? and (2) are there possibilities to evaluate *in vivo* chemical composition of stones using computed tomography? The study was conducted on 108 renal stones. X-ray fluorescence, X-ray diffraction and Fourier transformed infrared spectroscopy were used to determine the chemical composition. The morphology of the stones was examined using micro computed tomography.

The stone chemical composition in South Poland indicate that calcium oxalate monohydrate was overwhelmingly dominant (84%) followed by hydroxyapatite (8%) and struvite (6%). The occurrence of uric acid stones was very low (2%). The relative frequency of various stone types is similar in South Poland to other industrialized countries. The studied renal stones were characterized by a large variability in the concentrations of both major and trace elements. The maximum/minimum concentration ratio exceeds two orders of magnitude. Significant morphological differences have been observed between different types of stones. The stones were composed of oxalate polyhedrons stuck together or had the phosphate core overlaid with layers oxalate and organic mater. The use of CT to identify stone type seems to be limited.

**Key words:** Nephrolithiasis, renal calculi, chemical composition, morphology.

### INTRODUCTION

Research on urinary stones has been performed continuously since the end of the 18<sup>th</sup> century, when Scheele and Bergman [1] described uric acid calculi. From physico-chemical point of view, kidney stones form when specific compounds in the urine become supersaturated, causing minerals to precipitate. Initially, the crystals are characterized by very small diameters ( $\sim\mu\text{m}$ ), but over time they can grow and/or aggregate to form a structure about a few millimeters in diameter or even bigger. Five main types [2] of renal stones may be distinguished: (1) calcium oxalate stones composed of calcium oxalate or calcium oxalate mixed with calcium phosphate, (2) pure calcium phosphate stones, (3) uric acid stones, (4) struvite stones which are the result of chronic urinary tract infections caused by specific strains of bacteria and (5) cystine stones are associated with cystinuria. The incidence of the different types of stones depends on several factors

like geographical location, climate, racial group, environmental and social characteristics as well as the incidence of urinary tract infections and chronic urinary tract obstruction [2–4].

It is commonly accepted [5–9] that the knowledge of the composition and morphology of urinary stones is important due to two reasons. Firstly, nephrolithiasis is a recurrent disease (~50% of cases results in disease recurrence in 10 years) [4, 10]. Some of the factors causing the stone formation can be influenced to prevent formation of additional stones. Secondly, medical therapy and decision about proper treatment require the knowledge of stone composition. The conviction can be found in the literature [2, 3] that every single stone taken from patient should be studied because some calculi types determinate the treatment.

Recently, significant progress in the development of micro scale *in vitro* experimental methods has been achieved facilitated a better understanding of the chemical composition and structure of renal calculi as well as the process of pathological crystallization leading to calculi formation [11–14]. Although many attempts have been made to determine the type of calculi using computed tomography [15–17], unequivocal identification of the type of stones is very difficult in clinical practice.

Two issues related to nephrolithiasis are explored in the study with the following questions. (1) Does the chemical composition and morphology of renal calculi in South Poland overlap with the studies from other countries? (2) Are there possibilities to evaluate *in vivo* chemical composition of stones using computed tomography? It should be pointed out that the paper describes the first step of large-scale investigations of the correlation between the effectiveness of different therapeutic methods and the structure of renal stones.

## MATERIAL AND METHOD

The study was conducted on 108 stones excised from patients (82 men, mean age ( $\pm$ SD)  $50 \pm 13$  years, age range 22–75 years and 26 women, mean age  $51 \pm 13$  years, age range 24–71 years) treated with percutaneous nephrolithotomy, shock wave lithotripsy or therapeutic ureterorenoscopy. The material was collected from a few hospitals in South Poland. The removed stones have been washed several times with deionised water. Next, micro-computed tomography ( $\mu$ -CT) was used in order to examine geometrical dimensions, shape and morphology of stones. After tomographic measurements, the renal stones were ground down in an agate mortar. The powder was used for the elemental composition determination by means of X-ray fluorescence (XRF) while the chemical composition was investigated using X-Ray diffraction (XRD) and Fourier transformed infrared spectroscopy (FTIR) methods.

Micro-computed tomography examinations were performed using a Skyscan 1172 micro-tomograph (Skyscan, Belgium). The image pixel size was 7  $\mu$ m. The

projection images were acquired over an angular range of  $180^\circ$  with an angular step of  $0.5^\circ$ . Projections were reconstructed using the cone-beam reconstruction software (Nrecon, SkyScan, Belgium) based on the Feldkamp algorithm. Subsequently, binarization was performed based on the density distribution histogram in the whole sample volume. This program allowed also for the determination of geometrical parameters of stones and the creation of 3D models. In addition, the density at each pixel was determined using the procedure described elsewhere [10].

The elemental composition of the studied stones was determined by X-ray fluorescence method. The Oxford 2000 spectrometer (Oxford Instruments, UK) was applied for XRF measurements. The working conditions were: the tube voltage 5 kV (light elements) and 35 kV (heavy elements). The tube current was 1 mA and 250  $\mu$ A, respectively. The procedure for determination of the elemental composition was described elsewhere [18].

X-ray Diffraction (XRD) and Fourier transformed infrared spectroscopy (FTIR) were used to determine the crystallographic structure and chemical compounds of the renal calculi. For each of the samples, XRD diffractograms were measured in the angular range  $10^\circ$ – $70^\circ$  at  $2\theta$  values with the measuring step of  $0.01^\circ$ . Measurements were performed on a X'Pert Pro (Philips, The Netherlands) diffractometer using copper radiation ( $\text{CuK}_\alpha$ ). The results were interpreted on the basis of material identification database using X'Pert HighScore commercial software and the literature data [19].

FTIR spectra were acquired on a Nicolet 6700 FT-IR spectrometer (Thermo Scientific, USA). Spectra recorded for KBr tablets were measured in the range of  $4000\text{ cm}^{-1}$ – $400\text{ cm}^{-1}$ . Each tablet was made from the dry powder using approximately 1 mg of renal calculi per 300 mg KBr. The analysis of the IR bands was based on the Nicolet FTIR Spectral Libraries and the published data [20]. The spectra consisting of broad absorption bands had been deconvoluted by a self-deconvolution method [21] that enhances the resolution of overlapped bands and reveals their fine structure.

Statistically relevant differences were identified with ANOVA analysis with 99% confidence level, whereas statistically significant differences between respective pairs of elements were assessed using Fisher's least significant difference (LSD) procedure with p value less than 0.01 [22].

## RESULTS

Diffractograms obtained with the use of the XRD method allowed to identify the following crystalline compounds in the stones subjected to the studies: calcium oxalate monohydrate ( $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$  — COM), calcium oxalate dehydrate ( $\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$  — COD), struvite ( $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$  — STR), hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  — HAP) and uric acid ( $\text{C}_5\text{H}_4\text{N}_4\text{O}_3$  — UA). The XRD results were fully confirmed by the measurements of the infrared spectra. Additionally, the

self-deconvolution procedure revealed the presence of Amid I and Amid II bands. A representative diffractogram and a FTIR spectrum are presented in Fig. 1 for the most common, COM mixed with COD stone. The collected results of the stone chemical composition (Table 1) indicate that COM is the overwhelmingly dominant (~80%) followed by HAP and STR calculi. The occurrence of UA stones was very low (2%).

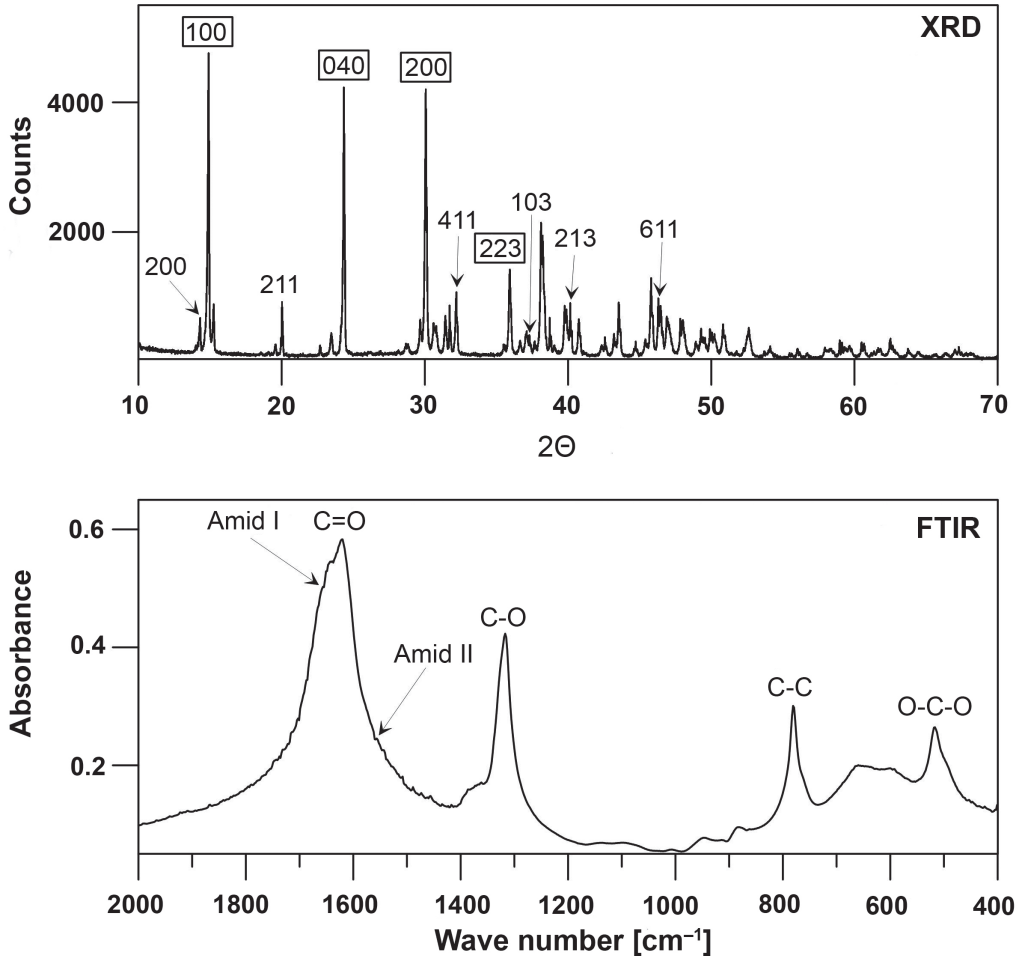


Fig. 1. Diffractogram (XRD) and infrared spectrum (FTIR) of the most common renal stone containing calcium oxalate monohydrate (COM) and calcium oxalate dehydrate (COD). The main  $d_{hkl}$  values of COM are given in boxes. For band positions of C = O and C-O depends on the concentration of COM and COD in the sample. For a pure COM stone bands are at  $1607\text{ cm}^{-1}$  and  $1311\text{ cm}^{-1}$ , respectively while for a pure COD they are at  $1644\text{ cm}^{-1}$  and  $1328\text{ cm}^{-1}$ , respectively.

In all investigated samples, concentrations of 11 elements (Mg, P, S, Cl, K, Ca, Fe, Cu, Zn, Br and Sr) were determined using XRF method. Occasionally, Rb content was also measured. The elemental composition of renal stones, divided into

Table 1

The occurrence (%) of different types of renal calculi in South Poland and other geographical areas. For abbreviation see text.

Occurrence	COM	STR	HAP	UA
South Poland	84	6	8	2
North Africa	57	8	11	24
Australia	68	17	3	12
Far East	79	2	11	8
USA*	74	7	6	12

\* 1% are classified as genetically determined (cystine) stones.

appropriate groups is presented in Tab. 2. The statistically significant differences in the elemental composition between COM and STR groups were observed for Mg, P, Cl, Zn and Sr. Concentrations of P, Zn and Sr demonstrated statistically significant difference between the HAP and COM groups. Concentrations of elements in the HAP and STR groups are not different except for the concentration of Mg. Among the 108 analyzed stones only two were built of uric acid. Hence the statistical analysis for UA stones was not performed. It should be noted that the toxic elements (Cd, Hg, Pb) were not detected in the investigated samples.

Table 2

Elemental composition of the renal stones in South Poland. The average values and standard deviations for all investigated samples are given. For abbreviation see text.

Element	COM	STR	HAP	UA
Mg[%]	0.45 ± 0.56	1.32 ± 1.15	0.33 ± 0.06	< 0.01
P[%]	2.46 ± 1.64	10.8 ± 2.3	10.9 ± 3.3	1.04 ± 0.07
S[%]	0.19 ± 0.06	0.15 ± 0.06	0.17 ± 0.08	0.27 ± 0.11
Cl[ppm]	403 ± 121	262 ± 170	310 ± 117	220 ± 77
K[ppm]	392 ± 162	249 ± 86	261 ± 107	113 ± 42
Ca[%]	17.6 ± 6.1	12.1 ± 5.4	13 ± 2	< 0.001
Fe[ppm]	56.2 ± 24.3	47.4 ± 28.1	59.0 ± 32.5	49.8 ± 33.2
Cu[ppm]	3.48 ± 1.71	3.69 ± 1.23	4.43 ± 0.60	2.89 ± 1.15
Zn[ppm]	19.8 ± 16.2	133 ± 170	77 ± 57	4.02 ± 2.07
Br[ppm]	1.97 ± 0.94	1.38 ± 0.39	1.65 ± 0.09	1.38 ± 0.06
Sr [ppm]	22.1 ± 15.0	112 ± 86	99.4 ± 54.8	< 1

The use of  $\mu$ -CT allows the determination of the morphology of the renal calculi. The chemical compounds and the organic matrix in the samples may be identified on the basis of the density histogram. The percentage by mass of the organic matrix varied from 2% to 15%. The densities of the compounds and the organic substance differ markedly (HAP — 3.16 g/cm<sup>3</sup> COM — 2.12 g/cm<sup>3</sup>, UA — 1.87 g/cm<sup>3</sup>, STR — 1.71 g/cm<sup>3</sup>, organic matrix — ~1.1 g/cm<sup>3</sup>). The  $\mu$ -CT results confirmed that the examined stones were always a mixture of, at least, 2 chemical compounds and the organic mater (Fig. 2). The commonly accepted

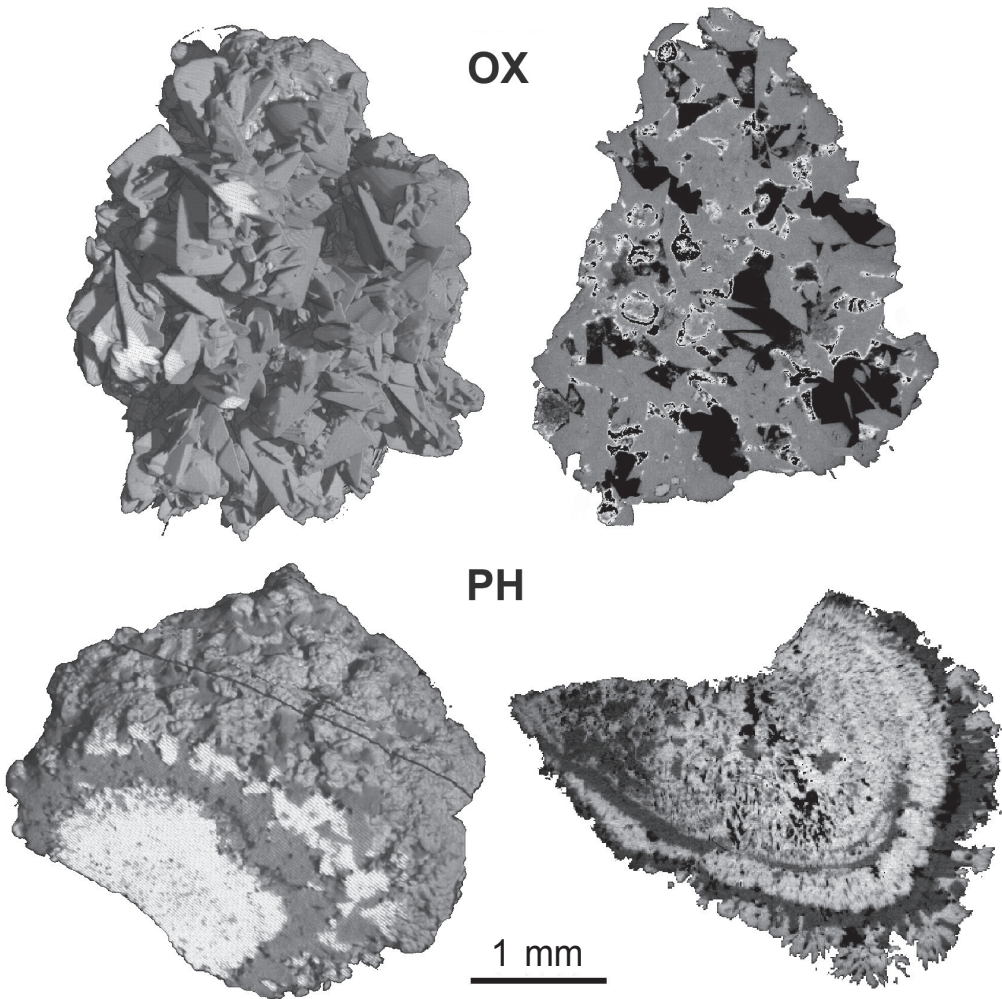


Fig. 2. Three dimensional models (right panel) and cross sections (left panel) of the COM and HAP stones extracted from  $\mu$ -CT data. Three grey scale levels are used. The white color corresponds to HAP, the grey color shows COM while the black color is correlated with the organic mater. For abbreviation see text.

classification (Table 1) indicates which compound dominates. Additionally, significant morphological differences between the COM and HAP groups were observed. As shown in Fig. 2 the COM renal stones were composed of COM polyhedrons with sharp edges stuck together. Most polyhedrons contained small clusters of HAP while the volume between polyhedrons was occupied by the organic mater. In contrast, the most common HAP stones, had the phosphate core overlaid with the layer of COM and the organic mater ("onion" structure). It should be pointed out that a trial to correlate the chemical composition with size and shape of the stone failed.

## DISCUSSION

Five chemical compounds (COM, COD, STR, HAP, UA) and an organic substance were identified in the samples subjected to the studies. The urinary stone was, in all cases, a mixture of two crystals and an unknown organic substance in different proportion. It should be pointed out that XRD and FTIR methods do not detect minor constitutes of mixed calculi. Like each spectroscopic method XRD and FTIR techniques are characterized by the detection limits (~5%). Therefore, the presence of an additional compound cannot be excluded. In all cases, however, one chemical compound dominates. The applied stone classification is based on the principal chemical compound detected in the sample.

The types of stones formed depend mainly on the composition of urine, which in turn reflects the type of diet consumed in the areas. For example, the stone problem in the tropics is compounded by low urine volumes and poor drinking. In South Poland COM stones are most common (Table 1). COM stone formation is driven by the relative concentrations of certain chemicals in the urine (to much Ca and oxalate, too little citrate) or highly concentrated urine from decreased fluid intake. Other more rare causes of COM stone formation include ingesting excessive amounts of vitamin D or calcium, hyperactive parathyroid glands, sarcoidosis, intestinal bypass surgery or chronic inflammation of the bowel.

HAP stones are formed in urine that is too alkaline, a condition often caused by renal tubular acidosis. Renal tubular acidosis can be an inherited genetic disorder or arise later in life in association with other pathological processes. Moreover alkaline urine can also result from chronic ingestion of high quantities of antacids. STR stones are almost always the result of chronic urinary tract infections caused by specific strains of bacteria. These bacteria produce enzymes that change the concentration of ammonia and the pH. The resulting ammonia-rich and alkaline environment favors the formation of magnesium ammonium phosphate crystals, the minerals composing STR stones. A few percent of stones consist of UA, a byproduct of protein metabolism. UA stones are associated with early stages of diabetes, a high-protein diet, chronic diarrhea, gout, and genetic factors that can predispose one to developing uric acid stones.

A comparison of the collected results with the chemical composition of the renal calculi from other geographical regions [12, 23–29] confirmed that the relative frequency of various stone types is similar in South Poland to other industrialized countries (Table 1). The observed discrepancies may be due to altered eating and drinking habits at various regions as well as racial differences and a family history. It should be also pointed out that there are no possibilities to evaluate the statistical significance of the observed differences (Table 1).

The studied renal stones were characterized by a large variability in the concentrations of elements (Table 2). Taking into account the variation coefficient VC, defined as (standard deviation)/(mean value), it may be concluded that the VC values were spread over broad ranges (e.g. STR — VC(Zn) > 1, HAP — VC(Ca)  $\approx$  0.15). It should be noted that big VC values were observed both for major and trace elements. In selected cases the observed differences were directly correlated with the chemical compositions (e.g. Mg concentration for STR and COM/HAP or P concentration for COM and STR/HAP). In other cases it is possible to postulate a mechanism responsible for the discrepancies (e.g. Ca-Sr and Mg-Sr substitutions take place in HAP and STR, respectively while Ca-Sr substitution is hampered). There are, however, cases for which the explanation of differences awaits further studies (e.g. Zn concentration for COM and STR/HAP).

The biggest differences in the elemental composition were observed for trace elements. It should be pointed out that the trace element concentrations of renal stones were extensively investigated [24–29]. The broad ranges of trace element concentrations have been observed for all investigated stone types collected at different geographical regions. As an example, the ranges of trace element concentrations for three regions are given in Table 3. It is clearly visible that the maximum/minimum concentration ratio exceeds two orders of magnitude. It should be noted that the accumulation of a trace element in the human tissue that exceeds the physiological level by factor of 100 is a marker of a severe pathology.

Table 3

Comparison of trace element concentrations (ppm) of renal calculi in South Poland and two other. All types of calculi were pooled. Ranges of concentrations are given.

Element	East Africa	South Africa	South Poland
Fe	39 ÷ 200	25 ÷ 103	20 ÷ 106
Cu	3 ÷ 9	1 ÷ 8	1 ÷ 6
Zn	1 ÷ 145	1 ÷ 572	4 ÷ 503
Br	1 ÷ 10	1 ÷ 9	1 ÷ 4
Sr	1 ÷ 151	7 ÷ 185	1 ÷ 240



The  $\mu$ -CT technique enables the unequivocal description of the renal calculi morphology. Two findings are of fundamental importance. Firstly, the renal stones were, in most cases, a mixture of two chemical compounds and the organic matter. The proportion of components was sample dependent. Secondly, two patterns of the stone spatial architecture were distinguished (Fig. 2): (1) the aggregate of polyhedrons, typical for COM-dominated stones, and (2) the “onion” structure, the most common for HAP stones. The explanation of the observed differences of the stone spatial architecture awaits further studies.

The collected results showed that  $\mu$ -CT is an excellent method allowing for the creation of a new, more precise classification of renal stones than those currently used in clinical practice. On the basis of a single  $\mu$ -CT measurement, it is possible to determine both the morphology and chemical composition of renal calculi. Using  $\mu$ -CT data it is also possible to answer the question; are there possibilities to evaluate in vivo chemical composition of stones using computed tomography?

Currently, CT provides accurate determination of stone location, size, number and CT density (Hounsfield unit). Moreover, CT permits the creation of 3-dimensional reconstructions with the spatial resolution of  $\sim 1$  mm. It should be kept in mind that CT technique differentiates the tissue structure on the basis of the linear absorption coefficient value which, in turn, is linearly related to the structure density. Therefore, using the appropriate calibration it is possible to determine the density of the structure within the human body (quantitative computed tomography). Assuming, average X-ray energy 50 keV, the stone diameter 5 mm (smaller stones are ejected spontaneously) and the soft tissue thickness 20 cm, it is possible to calculate the relative contrast ( $C = (\text{the change of the image intensity caused by the lesion})/(\text{average image intensity in the vicinity of the lesion})$ ) of the CT image.  $C$  values equal to 0.55, 0.29, 0.13 and 0.07 for pure HAP, COM, STR and UA stones, respectively. Therefore, it may be concluded that pure renal stones (composed of one chemical compound) should be distinguished using routine CT examination. Unfortunately, the renal stones are always a mixture of a few compounds in different proportion (Fig. 2). Theoretically, the minimal density of the mixture equals to  $\sim 1.1$  g/cm<sup>3</sup> (organic matrix) while the maximal value corresponds to 3.16 g/cm<sup>3</sup> (pure HAP stone). Since the proportion of the compounds cannot be extracted from the CT image (spatial resolution limit), the unequivocal determination of the chemical composition of renal stones using the CT examination is impossible. In contrast to routine CT technique the  $\mu$ -CT method has significantly better spatial resolution (factor 100). Therefore, the  $\mu$ -CT method may be used to distinguish the chemical compounds of the renal stones. The transfer of the  $\mu$ -CT results from laboratory to the clinical level is the goal of our future research.

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## CONFLICT OF INTEREST STATEMENT

None declared.

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