



Small-angle X-ray scattering (SAXS) and nitrogen porosimetry (NP): two novel techniques for the evaluation of urinary stone hardness

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Abstract

Purpose To evaluate urinary stones using small-angle X-ray scattering (SAXS) and nitrogen porosimetry (NP). Traditionally, stones are categorized as hard or soft based on their chemical composition. We hypothesized that stone hardness is associated not only with its chemical composition but also with its internal architecture. SAXS and NP are well-known techniques in material sciences. We tested whether SAXS and NP are applicable for evaluating human urinary stones and whether they provide information at the nanoscale level that could be useful in clinical practice.

Methods Thirty endoscopically removed urinary stones were studied. Standard techniques for stone analysis were used to determine the stone composition. SAXS was used to evaluate the solid part of the stone by measuring the crystal thickness (T) and the fractal dimension (D_m/D_s), while NP was used to evaluate the porosity of the stone, i.e., the pore radius, pore volume, and specific surface area (SSA).

Results All stones were successfully analyzed with SAXS and NP. Each stone demonstrated unique characteristics regarding T , D_m/D_s , pore radius, pore volume, and SSA. Significant differences in those parameters were seen among the stones with almost identical chemical compositions. The combination of high T , high SSA, low D_m/D_s , low pore volume, and low pore radius is indicative of a hard material and vice versa.

Conclusions SAXS and NP can be used to evaluate human urinary stones. They provide information on stone hardness based on their nanostructure characteristics, which may be different even among stones with similar compositions

Keywords Stone analysis · SAXS · Nitrogen porosimetry · Urinary calculi

Abbreviations

SWL Shockwave lithotripsy
SAXS Small-angle X-ray scattering
NP Nitrogen porosimetry

SEM Scanning electron microscopy
KS Kidney stone
FTIR Fourier-transform infrared spectroscopy
SSA Specific surface area
CT Computed tomography
HU Hounsfield units

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Background

Stone composition is one of the most important parameters for the effective treatment of urolithiasis. This is especially true when extracorporeal shockwave lithotripsy (SWL) is considered for treating small-to-medium size stones in the kidney or in the ureter. Unfortunately, the common methods for stone analysis provide information only about the stone composition, and those results are taken into consideration to categorize stones as “hard” or “soft.” However, in everyday clinical practices, urologists face the scenario of stones

responding differently to SWL, even when they have the same composition [1]. Additionally, stones considered to be hard occasionally respond quite well to SWL, while stones thought to be soft do not disintegrate at all [2, 3].

We hypothesized that stone hardness is associated not only with the chemical composition of the stone but also with its internal architecture at the nanostructure level, and we tried to find a reliable method to evaluate those internal characteristics. Small-angle X-ray scattering (SAXS) and nitrogen porosimetry (NP) are well-known techniques in materials science and are used to evaluate the structures of materials [4, 5]. SAXS provides information on the average size and shape of particles and in general on the structural properties of the sample [4]. Therefore, the mean size of individual crystals and the mean size of the clusters of crystals within a stone can be measured. NP is used to measure the surface area and the porosity of solids [5]. The porosity of a solid material consists of the natural spaces (pores) between the crystals and the possible cracks occurring naturally or from external forces. Obviously, for a given volume of a stone, the existence of large pores or cracks within the material is indicative of the presence of a less solid component in the stone.

In the present study, we tried to determine whether SAXS and NP are applicable in the evaluation of human urinary stones and whether they provide information at the nanoscale level, especially regarding their hardness, which could be potentially useful in clinical practice.

Methods

Thirty kidney stones were obtained from patients treated with percutaneous nephrolithotomy. All samples were cleaned with ethyl alcohol and dried. After cleaning, the samples were stored in pre-cleaned plastic vials. The stones were crushed manually. Some small parts were collected from each sample for NP analyses. The rest of the fragments were ground to powder and sieved to obtain an average grain size of 45 microns. The samples were designated *KS(X)*, where *X* is the sample number. The study was approved by the institutional review board of the University Hospital of Alexandroupolis, Greece.

All stones were subjected to stone analysis using Fourier-Transform Infrared Spectroscopy (FTIR). FTIR was performed with a Perkin Elmer Frontier ATR/FT-NIR/MIR spectrometer. The spectra were recorded in the range of 600–4000 cm^{-1} at room temperature. Finally, the stones were analyzed using SAXS and NP.

SAXS

SAXS was performed in a SAXS rig system provided by JJ X-ray Systems with a CuK α X-ray source ($\lambda = 0.15056$ nm). The apparatus consists of a 2D Rigaku SMAX-300 detector, vacuum chamber, pinholes to collimate the beam, and an X-ray source (Fig. 1). All measurements were corrected for background radiation and transmission. A SAXS experiment is generally simple. The sample is placed in a holder and loaded in the vacuum chamber. An X-ray source emits a beam, which is focused by a monochromator and three pin holes (Fig. 1a, b). The X-ray beam hits the sample and is scattered at small angles, and the scattering pattern is recorded by the detector (Fig. 1c). Finally, the pattern is transformed in a typical plot of diffracted intensity versus scattering vector (Fig. 1d). Two parameters can be extracted from the fractal region of the SAXS curve, D_m and D_s and one can be extracted from the Porod region, T . D_m and D_s are the mass distribution and the surface coarseness/structure compactness, respectively [4]. The fractal dimension, D , is a statistical index that shows the capacity of a pattern to fill space or its capacity to fill space while zooming down to finer and finer scales. D_m is the mass dimension of the fractal and provides information about how the mass of an object scales with the object's size. D_s is a self-similarity dimension of the object and represents the number of identical copies of the pattern divided by the scaling ratio. It refers to the surface of the object. Both parameters are directly related to the hardness of the object. T refers to the thickness of the crystals within a mineral and is calculated according to Eq. (1).

$$T = \frac{4}{\pi P} \int q^2 I(q) dq, \quad (1)$$

where P is the Porod constant, $I(q)$ is the intensity, and q is the scattering vector [6].

Studies on bones and other materials have shown that high values of fractal dimensions (D_m or D_s) indicate a soft material and vice versa, while high values of T indicate a hard material and vice versa (Table 1) [7–9].

NP

Porosities and surface area studies were obtained at 77 K in a Quantachrome Nova 4200e instrument. Each sample was outgassed at 60 °C for 16 h before the NP measurements. A typical NP experiment consists of three steps: degassing, adsorption, and desorption. The sample is placed in a special glass cell and heated in a vacuum for a certain time. When the first step is finished, the cell with the sample is placed in a Dewar flask of liquid nitrogen to maintain a constant temperature (Fig. 2a, b). Then,

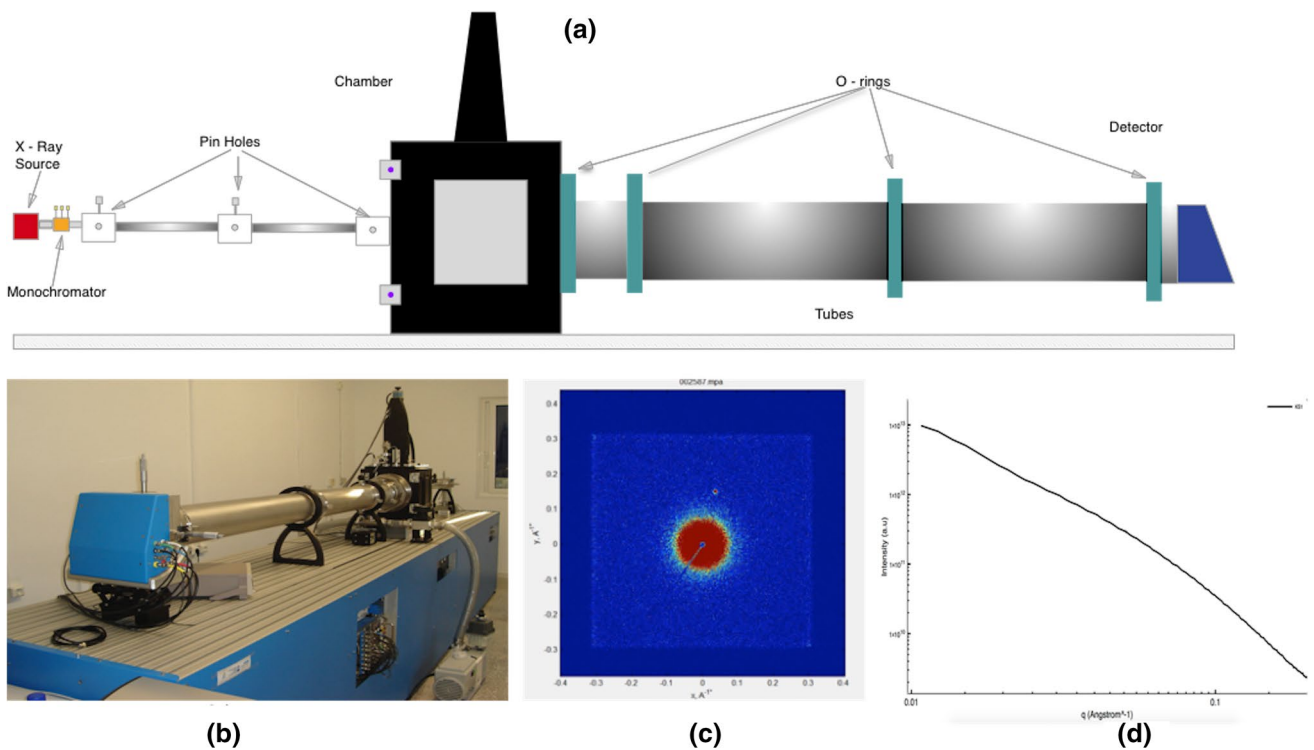


Fig. 1 SAXS instrument setup and plot. **a** Schematic diagram of a SAXS instrument, **b** SAXS instrument, **c** typical SAXS pattern, **d** an $I(q) - q$ plot

Table 1 Stone characterization (hard or soft) based on its SAXS and NP parameters

Type of stone	SAXS		NP		
	Fractal dimension D_m or D_s	Crystal thickness (T)	Pore volume	Pore radius	Specific surface area (SSA)
Hard stone	Low	High	Low	Low	High
Soft stone	High	Low	High	High	Low

SAXS small-angle X-ray scattering, NP nitrogen porosimetry

nitrogen gas is admitted partially into the sample, and nitrogen molecules stick to the surface of every pore until the relative pressure becomes one. The third step is the reverse procedure, going from a relative pressure of one to complete evacuation. During the adsorption and desorption procedure, two parameters are recorded: the relative pressure and the volume of nitrogen which are used for the plot of the NP isotherm curve for the measurement of porosity.

Three parameters can be extracted from the nitrogen porosimetry isotherm: pore radius, pore volume, and the specific surface area. Pore volume is the total volume of very small openings in the solid sample. The specific surface area (SSA) is the total area that the surface of the solid sample occupies per unit of mass. As it has been shown in studies on bones and geological materials, low pore volume

and pore radius and high SSA indicate a hard material and vice versa (Table 1) [10–12].

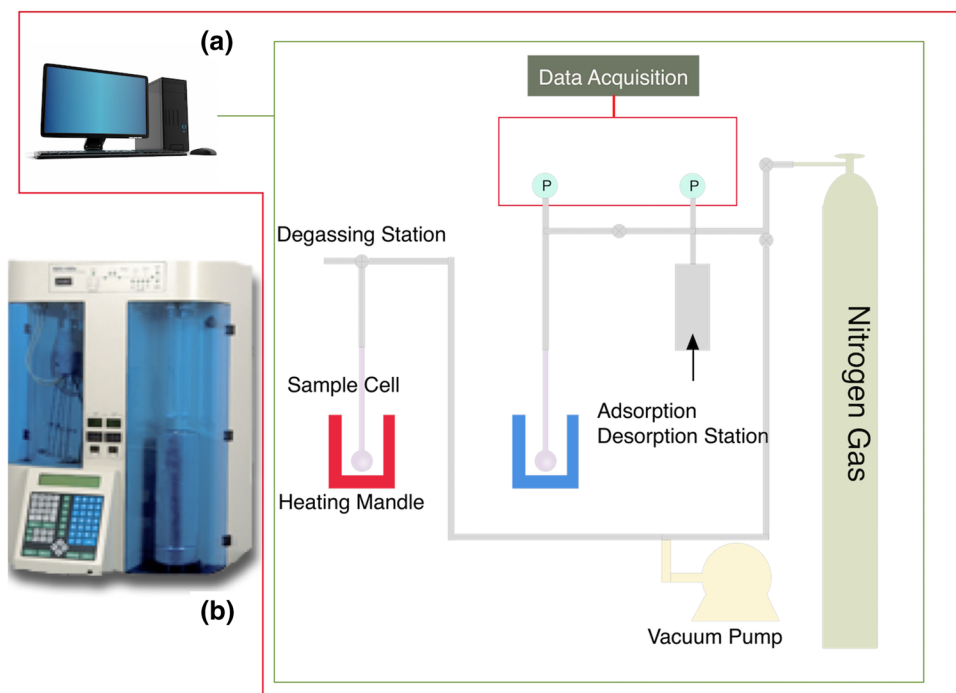
Results

The compositions of the 30 stones as determined by FTIR are presented in Supplementary Table 1. Additionally, all stones were successfully analyzed by SAXS and NP.

SAXS

The crystal thickness (T) ranged between 2.2 and 12.1 nm. The mass fractal dimension (D_m) varied between 1.64 and 2.94, and the surface fractal (D_s) varied between 1.48 and 2.94. For each stone, either the D_m or the D_s value was

Fig. 2 Nitrogen porosimeter setup. **a** Schematic diagram of an NP instrument, **b** the quantachrome poremaster porosimeter



calculated. It is noteworthy that each of the 30 stones demonstrated a unique combination of T , D_m , and D_s values. Additionally, stones with a similar composition demonstrated differences in the parameters evaluated by SAXS. For example, in the five stones composed mainly of whewellite (KS9, KS10, KS16, KS27, and KS29), the average crystal thickness T varied between 3.3 and 6.7 nm, and D_m or D_s varied between 2.12 and 2.88 (Table 2). If only SAXS results are taken into consideration, the

whewellite stone KS9 is harder than the other whewellite stones because it has both a high T value and a low D_m or D_s value. Similar results can be seen for the other stone categories (Supplementary Table 1). The two cystine stones analyzed (KS25 and KS26) demonstrated high crystal thicknesses (12.1 nm and 11.1 nm, respectively) and low D_m or D_s values (2.26 and 2.36, respectively). These values are indicative of hard stones, which is something we already know for cystine stones from clinical practice.

Table 2 SAXS and NP results for pure whewellite and whewellite/weddellite kidney stones

Sample	FTIR	Crystal Thickness (nm)	SAXS		NP		
	Synthesis (% Composition)		Fractal Dimension D_m	Fractal Dimension D_s	Surface Area (m^2/gr)	Pore Radius (nm)	Pore Volume (cc/gr)
KS9	Whewellite 99%	6.6	2.17	0.00	31.00	1.7	0.05
KS10	Whewellite 99%	6.7	0.00	2.88	24.00	1.5	0.07
KS16	Whewellite 98%	4.7	2.51	0.00	75.00	1.7	0.17
KS27	Whewellite 98%	3.4	2.33	0.00	26.00	1.7	0.04
KS29	Whewellite 98%	3.3	2.12	0.00	80.00	1.6	0.10
KS13	Whewellite 85%	7.1	0.00	2.88	23.00	1.5	0.02
	Weddellite 15%						
KS22	Whewellite 84%	7.3	2.35	0.00	5.00	2.2	0.02
	Weddellite 16%						
KS19	Whewellite 74%	4.0	1.64	0.00	4.00	1.7	0.01
	Weddellite 26%						

FTIR Fourier-transform infrared spectroscopy, *SAXS* small angle X-ray scattering, *NP* nitrogen porosimetry

NP

The SSA of the 30 kidney stones varied between 1 and 177 m²/g, the pore volume varied between 0.01 and 0.99 cc/g, and the pore radius varied between 1.5 and 16.2 nm. Similar to what we found from SAXS, each of the 30 stones analyzed with NP demonstrated a unique combination of pore volume, pore radius, and SSA. Significant differences in the values of those parameters were seen even for stones with the same chemical composition (Supplementary Table 1). For example, the pore radii of the five pure whewellite stones (KS9, KS10, KS16, KS27, and KS29) were similar (1.5–1.7 nm), but the pore volume varied between 0.05 and 0.17 cc/g, and the specific surface area (SSA) had an impressive distribution between 31 and 80 m²/g (Table 2). The same phenomenon was also seen in the mixed whewellite/weddellite stones. The whewellite/weddellite stones KS13 and KS22, which had almost the same percentage of each component, had the same pore volume but exhibited a significant difference in SSA and pore radius (Supplementary Table 1). Overall, KS13 is a harder stone than KS22. Similar significant differences were seen for cystine stones (Supplementary Table 1).

Combined SAXS and NP results

The characterization of a stone as hard or soft is more accurately done by combining the results of SAXS and NP. Actually, with the data currently available, we can only compare stones to each other and identify which one is harder or softer. All five parameters should be taken into consideration. Two of them are obtained from SAXS (D_m/D_s and T) and three of them are obtained from NP (SSA, pore radius, and pore volume). The additional data on calculi internal structure, which are provided by SAXS and NP, are presented schematically in Fig. 3, where the example of a Whewellite stone is presented. As shown in Fig. 3a, the typical stone analysis includes macroscopic evaluation (color, roughness) and analysis of the stone composition with FTIR. In Fig. 3b, we can see the SAXS curve from the same stone. The cluster dimension can be obtained from the first slope, while the mean size of crystal thickness is calculated from the Porod region. In Fig. 3c, the Nitrogen Porosimetry isotherm of the stone is presented, which we can use to acquire information on the surface area, the pore volume, and pore radius of the cavities within the stone. This information allows for a schematic representation of the kidney

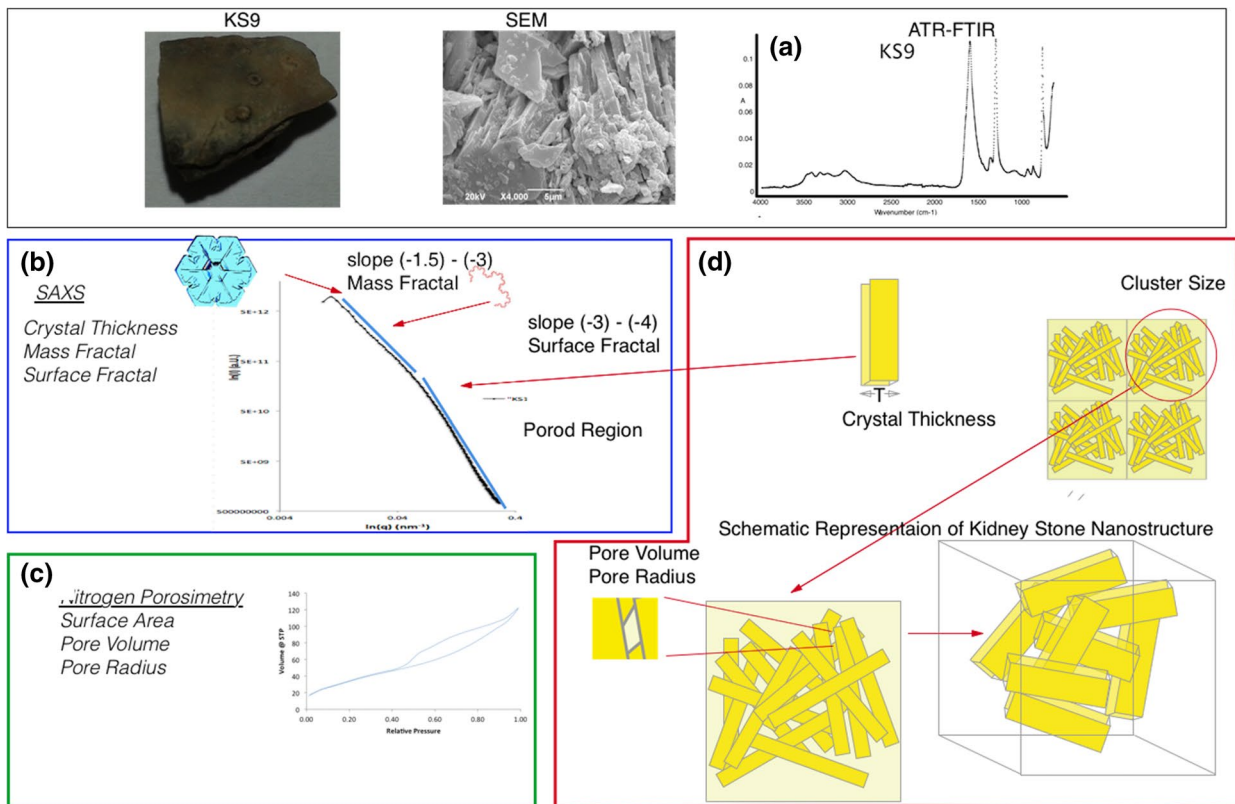


Fig. 3 **a** Stone analysis with standard methods (optical, SEM, and FTIR), **b** SAXS analysis provides information on the solid component of the stone, **c** nitrogen porosimetry isotherm provides information on

the cavities within the stone, **d** schematic representation of the stone nanostructure based on SAXS and NP results

stone nanostructure, as seen in Fig. 3d. The knowledge of all these parameters allows for better overall characterization of the stone.

Overall, significant differences in the internal structure of the stones were identified even for those with similar composition. For example, KS9 and KS10 have similar composition according to FTIR. T , pore radius, and pore volume of the stones are similar (Table 2). For each stone either D_m or D_s can be calculated and therefore only one value will be obtained (these values are directly comparable). D_m/D_s are pretty close for KS9 and KS10. However, SSA is significantly higher for KS9 (Table 2). Therefore, overall KS9 is harder than KS10 (Table 1). Another example is KS27 and KS29 (Table 2). Both are Whewellite stones with similar values for T and Pore radius. Although pore volume is slightly lower for KS27 compared to KS29 (0.04 cc/g vs. 0.10 cc/g), the D_m value is lower for KS29 (2.12 vs. 2.33) and the SSA value is significantly higher for KS29 (80 m²/g vs. 26 m²/g) (Table 2). Overall, KS29 is harder than KS27 despite their almost identical chemical composition (Table 1).

Discussion

In the present study, we were able to demonstrate that human urinary calculi can be analyzed by SAXS and NP. The combined results of this analysis can provide information on the nanostructure of human calculi, which seems to be related to their hardness. It was clear that calculi with a similar chemical composition had different structural parameters on the nanolevel. The FTIR method give quantitative and qualitative characteristics, while SAXS and NP provide information about the internal architecture of the stones, specifically about the solid component (SAXS) and the cavities within the stone (NP) [13].

Traditionally, stones are categorized as hard or soft based on their chemical composition, a notion that is partly but not entirely correct. Williams et al. [3] in an in vitro study measured the number of shock waves to complete comminution for 195 human stones, representing six major stone types. They found that the variability in the stone fragility to shock waves is large, even within groups of stones with similar mineral composition. They concluded that knowing the major composition of a stone may not allow adequate prediction of its fragility in lithotripsy treatment. Variations in the internal stone structure, including the secondary mineral composition, may be a significant cause of this variability in stone fragility [3]. If stone fragility could be predicted before treatment, stones that would not break easily by SWL could be treated by other means, thereby avoiding the exposure of patients to an unnecessary and potentially injurious treatment [14]. This concept is the driving force behind the efforts of several groups to use helical computerized

tomography (CT) to determine the stone composition or stone fragility [15, 16]. In a recent study, Christiansen et al. [17] tried to evaluate whether the internal morphology of calculi defined by CT bone window influences the SWL outcome in vivo. They used CT to categorize stones as homogeneous or inhomogeneous based on the differences in their internal structure as seen in the CT scans. Unfortunately, there was no correlation between the stone appearance in CT and the final outcome with SWL. Therefore, it seems that CT is not the ideal method to evaluate the internal stone structure. Similar problems have been seen with the use of Hounsfield units (HU) to predict stone fragility because the overlap of ranges precludes accurate identification of the stone composition [15]. One of the problems with HU is that this parameter has been related mainly to stone composition, and ignores the potential differences in the stone architecture at the nanoscale level that definitely exist even among stones with the same chemical constituents, as we have seen in the present study using SAXS and NP.

The hardness of a solid material depends not only on its chemical composition but also on the arrangement of the atoms at the atomic level, which eventually affects its nanostructural characteristics. Urinary stones are not completely solid and consist of a solid part and cavities (pores) filled with air that are either natural or in the form of cracks caused by external forces. To completely characterize a solid material such as calculus, it is necessary to determine characteristics such as color, volume, weight, chemical composition, and internal architecture. The latter depends on the arrangement of the solid part and the cavities within the stone. At the nanoscale level, the solid part is created by many primary particles with a mean size of α , which create a cluster. Many clusters generate crystal formations with fractal characteristics. When the fractal dimensions increase, the hardness decreases [9]. Hardness also varies based on the orientation and thickness of the crystals [18]. Additionally, hardness is related to the material porosity. It is reasonable to assume that increased porosity correlates with decreased hardness because more dead spaces exist among the solid parts of the stone. In the present study, we demonstrated that SAXS was able to characterize the solid part of the stone by measuring the average crystal thickness and the fractal dimension, while NP provided information on porosity, such as pore radius, pore volume, and specific surface area (Table 1). The most interesting finding, however, is the significant differences in those parameters among stones with almost identical chemical compositions. Clearly, although chemical composition plays a pivotal role, it is not the only parameter affecting stone hardness.

The present study should be viewed as the basis for future research in the field. The cut-off values for every parameter analyzed by SAXS and NP and most importantly the combined evaluation of these values need to be standardized in

order to categorize a stone as hard or soft. Although cut-off values are not available yet, the data of the present study allow the comparison between stones and their classification as harder or softer from another stone with similar chemical composition. Furthermore, the most demanding issues here are how to incorporate these two techniques into clinical practice and how to take advantage of their information for more effective stone treatment. Obviously, the results of SAXS and NP are available after the stone has been removed and therefore cannot help us with the pretreatment evaluation of the stone. It is necessary to relate these results to the clinical and radiological characteristics of the stones. Currently, we are planning studies to relate the results of SAXS and NP with the features of the stones in CT scan and with the success rate of SWL. These might offer some new parameters that are readily applicable in clinical practice and potentially useful for better evaluation of stone hardness before treatment is applied.

Conclusions

SAXS and NP can be used to evaluate the internal structure of human urinary calculi. They provide information on stone hardness based on its nanostructure characteristics, which may be different even among stones with similar compositions. These new insights into the characteristics of human urinary calculi extracted from these techniques could be important for the selection of an appropriate treatment leading to overall better disease management. Further studies are needed in order to incorporate these techniques in the routine management of stone disease.

Author contributions NV, SG, and ST conceived the study, participated in its design. NV, SG, JW, and EV analyzed the data. NV, SG, DB, IK, CK, and AM participated in interpretation of data, as well as in drafting the manuscript and revising it critically. All authors have read and approved the final manuscript.

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval The study was approved by the institutional review board of the University Hospital of Alexandroupolis, Greece.

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