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A formal test of the hypothesis that idiopathic calcium oxalate stones grow on Randall's plaque

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Abstract

OBJECTIVE—To confirm that more than half of all idiopathic calcium oxalate (CaOx) stones grow on interstitial plaque, as CaOx stones can grow attached to interstitial apatite plaque but whether this is the usual mechanism of stone formation is uncertain.

PATIENTS AND METHODS—In nine idiopathic CaOx stone formers (ICSF) undergoing percutaneous nephrolithotomy or ureteroscopy all accessible renal papillae were endoscopically imaged using a digital endoscope. All stones were removed intact, and recorded by the operating surgeon as being attached or unattached; for all attached stones the surgeon determined if the site of attachment was to plaque. This determination was further verified by reviewing the intraoperative video record, and only instances where plaque was reliably seen on video were used for analysis. Surgical observations were further validated by a combination of microcomputed tomographic analysis and papillary biopsy. The results were analysed statistically using fixed-sample testing and group sequential sampling.

RESULTS—The nine patients had a total of 115 stones, primarily CaOx; 90 stones were attached. Of these, 81 were attached to plaque; surgeons could not visualize the site of attachment with sufficient clarity to judge in the other nine cases. Based on these data, the final point estimate for the number of stones attached to plaque was 0.754 (95% confidence interval 0.575–0.933; $P = 0.013$).

CONCLUSIONS—In ICSF most stones grow attached to papillae, on plaque, so growth on plaque is the main mechanism for stone formation in this very common group of patients.

Keywords

Randall's plaque; nephrolithiasis; stone pathogenesis

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CONFLICT OF INTEREST

None declared.

INTRODUCTION

As early as 1937 Randall (cited in [1]) described interstitial papillary and medullary deposits of apatite (Randall's plaque) in patients with stones, and showed that stones can grow attached to the plaque. We confirmed his original observations [2] and found that the fraction of attached stones on plaque was higher than he found. Our work has uncovered a previously unsuspected heterogeneity among calcium stone formers [3]. The process of forming stones on plaque appears isolated to a large but highly specific group of patients. We can best characterize these patients as those whose stones are predominantly calcium oxalate (CaOx), and who form stones for reasons other than systemic diseases. Among these so-called idiopathic CaOx stone formers (ICSF) mechanisms promoting stones are mainly effects of lifestyle and heredity that increase urinary calcium or oxalate excretions, or reduce urine volume or citrate levels [4], as opposed to bowel disease, primary hyperoxaluria, hyperparathyroidism, sarcoidosis, and other systemic diseases. We also exclude medullary sponge kidney. These ICSF constitute most of all calcium stone formers, so how they form their stones is of considerable clinical interest.

Although it has been easy to show instances in which CaOx stones grow attached to plaque in ICSF, it is not easy to show that this mode of growth is the general rule. There is the obvious problem of selection bias; 'one notices what one wishes to notice'. Generality is crucial to understanding stone pathogenesis, because if stones indeed grow on plaque as a rule, then how they do so, and how plaque itself forms and enlarges, become primary clinical research objectives. The problem is especially complicated because in other groups of calcium stone formers, apart from ICSF, growth of stones on plaque is not a prominent mechanism. For example, in patients with brushite stones the inner medullary collecting ducts (IMCD) contain apatite deposits; some stones appear attached to plugs [5], others are found free in the urinary collecting system. Patients with an intestinal bypass for obesity form CaOx stones, but have very little plaque, have IMCD filled with apatite plugs, and appear to form their stones with no attachments [1].

The purpose of the present study was to test the hypothesis that in ICSF stones grow on plaque as a rule, perhaps exclusively. The hypothesis demands that all attached stones be found attached to plaque. If this is not true, the hypothesis cannot be correct as a rule, but only as, at best, part of some more varied set of mechanisms. To test this hypothesis we needed to create an experiment in which every attached stone in a group of ICSF could be accounted for as attached to plaque or not, or attached in such a manner that the underpinning could not be seen. Until recently, with the advent of flexible digital endoscopy equipment, this experiment was not feasible; using such equipment we succeeded in our objective and report the results.

PATIENTS AND METHODS

All nine patients (three women) were known to form stones that contained no brushite and 30% calcium phosphate in any analysed stone. We excluded patients with bowel resection, inflammatory bowel disease, sarcoidosis, primary hyperparathyroidism, renal tubular acidosis, and medullary sponge kidney, or any other systemic stone-forming condition.

A continuous digital recording was made from the beginning to the end of the surgical procedure without editing, pauses or discontinuities. Each film was filed as a permanent part of the study. During surgery all papillae were endoscopically visualized. All stones were removed for clinical purposes. Twenty-five stones that were obviously not attached (Table 1) were simply washed or aspirated, or removed with a grasper. Each stone that appeared to be attached was removed with an endourological stone basket. By visual inspection before,

during and after removal the surgeon determined if the stone was attached, and if the attachment was on plaque.

For the former, we required that during the removal phase kidney tissue was pulled physically toward the basket, and that the stone was clearly seen to be separated with some effort from the tissue. Failing either criterion the stone was classified as not attached, and is not included in this protocol. For the latter, we required that new images be made of the site of previous attachment and that immediate visual inspection showed white plaque underlying where the stone had been attached. Also, a subsequent review of the images, after surgery was concluded, was needed to document that white plaque was indeed present, i.e. attachment to plaque required three findings: (i) images were made after removing an attached stone; (ii) intraoperative inspection indicated white plaque at the attachment site after removal; and (iii) subsequent viewing of the images after removal confirmed that white plaque was present at the attachment site. In the event that the underpinning showed no evidence of plaque during surgery the stone would be classified as attached but not to plaque. In the event that the underpinning revealed plaque during surgery but subsequent viewing of the images failed to document plaque in the underpinning, the stone was classified as unknown in terms of the attachment site.

In four patients we obtained biopsies of attachment sites to further confirm whether plaque was present. The biopsies were taken immediately after removing an attached stone from the region where the attachment had occurred. Biopsies were prepared and assessed as described previously [1].

One stone from each patient was analysed using micro-CT (Table 1) [6]. Stones were scanned using a mCT20 system (SCANCO Medical AG, Brüttisellen, Switzerland) with voxel sizes of 20–34 μm . Three-dimensional reconstructions of stones were examined using ImageJ (<http://rsb.info.nih.gov/ij/>) and Vox2 (<http://www.nephrology.iupui.edu/imaging/vox2/>) to verify the composition. For two of the nine patients, three-dimensional reconstructions of several stones were also superimposed over the digital images collected during surgery to validate that the topo-geometric contours of plaque on stones matched that of the attachment site on the tissue.

To increase the statistical efficiency of the study, we implemented a group sequential-testing procedure [7] to allow for periodically testing of the null hypothesis that the true proportion of stones attached to plaque is 0.5, while maintaining a prescribed (one-sided) type I error rate of 0.025. The planned study design specified three equally spaced interim analyses and one final analysis, taking place after stones have been removed from a maximum of 32 patients, i.e. analyses would be after 8, 16, 24 and 32 patients had been sampled. The maximum sample size was chosen to guarantee 97.5% power to reject the null hypothesis if the true proportion of stones attached to plaque is 0.75. This sample size determination assumed that an average of three stones was collected from each patient and a within-patient correlation of 0.5. For testing, we used a Wang and Tsatis stopping rule with $\Delta = 0.1$ [8]. Generalized estimating equations assuming an exchangeable correlation structure were used to estimate the proportion of stones attached to plaque [9]. Standard errors were computed using the robust variance estimator, to provide valid inference and CIs among stones sampled from the same individual [10]. It was decided a priori that at each analysis, statistical information would be re-estimated and those estimates would be used to re-power the study via a constrained-boundaries approach [11].

RESULTS

The nine patients were aged 34–67 years; 24-h urine values for the patients (Table 2) showed the usual range of abnormalities described previously [12], including hypercalciuria, hyperoxaluria, hypocitraturia and low urine volume in many combinations. All but one patient collected two 24-h urine samples, as is our usual practice.

Among these nine patients (12 kidneys) there were 115 stones, of which 90 were attached (Table 1). Of these, 81 were attached to plaque. Nine were classified as unknown because although plaque was seen at the attachment site during surgery it could not be confirmed during review of the video images. The crude counts suggest that attachment to plaque is the rule; this is confirmed by formal statistical analysis. There were 25 unattached stones in the 12 kidneys, making clear that a large majority of all stones in the kidneys were attached.

Our design was based upon several interim analyses with stopping rules. Based on the observed data at this, the first analysis, the estimated proportion of stones attached to plaque was 0.754, with a within-subject correlation of 0.272. Using these estimates for variability and shifting the first analysis to take place at nine vs eight patients (the original first analysis point) the stopping boundaries were recomputed maintaining the specified type I error and power. With this re-computation of the stopping boundary, the observed proportion of stones attached to plaque was extreme enough to suggest that the study be stopped in favour of the alternative hypothesis (rejecting the alternative that the proportion of stones attached to plaque is 0.50). The final point estimate for the number of stones attached to plaque was 0.754 (95% CI 0.575–0.933), with a corresponding $P = 0.013$.

For the optical assessment of the attachment site at surgery, (Fig. 1, panel a) shows a 3-mm stone lying on an upper pole lateral papillum of patient no. 1, being removed with a basket (panel b) with distortion of papillary tissue (video clip in supplemental data). The underpinning (panel c) shows large amounts of white plaque directly under where the stone was attached (Fig. 1). The stone itself (panel d) has a white patch on its tissue surface; the urinary surface could be ascertained by the prominent discoloration (arrow) on the urinary side visible in panel a. This figure illustrates the procedure we used in all 115 stones. The composition of the white patch material was ascertained by micro-CT analysis.

To establish the correspondence between the tissue plaque and stone attachment site we needed to establish the orientation between the images of the papillum before and after removal. Thus we aligned an overlay of the image after removal with the original image (Fig. 2, panels a–c) using fiduciary marks consisting of plaque (arrowheads) and blood vessels (arrows). A micro-CT image of the removed stone is then aligned on to the image before removal (panel b); as the images before and after removal have already been aligned using markers, this aligns the stone onto the image taken after removal (panel d). With the micro-CT image of the stone aligned in place, one can manipulate the image to reveal the position of the plaque on the tissue side of the stone, to test whether its position matches corresponding plaque on the tissue image (panel e). Finally, the entire bulk of the stone can be removed by image processing, leaving just the stone and tissue plaque areas for inspection (panel f). In this case, the correspondence was exact.

We used this analysis for seven stones in two patients (patients no 155 and 168, Table 1) and in each case obtained similar correspondences. These stones were chosen because of good image quality of the stone and papillum after stone removal. This analysis substantiates the accuracy of the visual interpretations made during surgery, and inspection of the video images after surgery, and validates our approach.

In four patients who had percutaneous nephrolithotomy (nos 155, 168, 171, 175) we assessed renal tissue obtained at surgery to confirm that the visual impression of plaque was accurate during this experiment. As an example (Fig. 3 panels a–d), a biopsy from the underpinning of the stone shown in Figs 1 and 2 showed a large region of interstitial plaque just beneath the urothelium, confirming the visual impression of plaque during surgery. The same was true for the underpinnings of all of the other stone sites biopsied (see supplemental material for the second example). Ureteroscopy currently does not permit an adequate biopsy for research purposes. Ethical concerns limited the number of biopsies to those described here.

DISCUSSION

Growth on plaque appears to be a main mechanism of stone formation in ICSF, the most common type of patient encountered in clinical practice [4,13]. In the 12 kidneys assessed in the present study most stones were attached to papillae, and in all cases that could be fully ascertained the attached stones were attached to plaque. We are reasonably certain about our conclusions because we have validated our ascertainment of stone attachment in several and independent ways. The attachment was surgically determined by showing that the stone could be removed from tissue only with some effort and with obvious distortion of the tissue. The review of the videos reliably showed these phenomena, which were obvious to surgeons and can be seen in our supplemental videos.

Ascertainment of plaque at the attachment site relied on other criteria. In all 90 attached stones the surgeons observed white plaque after removing the stone, located in the bed from which the stone was removed. In 81 of these cases, review of the video images documented white plaque, supporting their observations, and we used only those 81 in the statistical analysis.

In two patients we used a combination of micro-CT analysis and image manipulation to further test the validity of the surgical observations. Specifically, we sought to prove objectively that the attachment site on a stone (a patch of documented apatite) fitted precisely with a corresponding area of plaque in the attachment bed. For all seven stones in two patients we were able to establish that this was true and have presented one instance in detail and the others as supplemental data.

As a final validation, we biopsied eight presumed plaque sites in four patients and found that interstitial (white) apatite plaque was present in every instance. We present one of the eight biopsies (Fig. 3) and a second is in the supplemental material. The biopsies establish that the surgeons were accurate in their appraisal that white material, presumably plaque, is present at attachment sites after stones are removed, and are accurate in assessing that the white material is indeed plaque. Overall, we are as certain as is reasonable concerning the attachment site of stones in ICSF, and when combined with our statistical analysis, we consider that the surgical observations establish that growth on plaque is indeed the general pattern for stone development in the common ICSF.

The ultrastructural details of stone growth on plaque appear to be exposure of plaque to urine via loss of urothelial integrity, overlay of plaque by organic molecules of urinary origin that include osteopontin and Tamm-Horsfall protein, nucleation of amorphous apatite species in this layer, and then a progressive layering of more matrix, nucleation of biological apatite, and finally of CaOx itself [14]. Formation of plaque [15] and production of urine supersaturation to drive the formation of apatite and CaOx overlays are both fostered by high urine calcium excretion (hypercalciuria) the treatment of which with thiazide or reduced diet protein and sodium is an established approach to reducing stone formation [4].

Also, plaque formation and urine supersaturation are fostered by low urine volume. The present study establishes that these ultrastructural and physiological details are relevant to a very large group of stone formers.

Our work does not minimize the importance of urinary oxalate, which is a crucial determinant of urinary CaOx supersaturation and therefore of the ultimate production of the main bulk of the common CaOx stone. Urinary oxalate concentration has exactly the same influence on urinary CaOx supersaturation as does urinary calcium concentration [16] and its reduction would surely aid in preventing stones.

As with any observational study, the present work might not be generally applicable. In particular, all of the patients in the current trial were recruited from the same clinic. While we think that such patients are representative of the general ICSF population, it would be valuable to replicate these results in an independent sample. In addition, we did not seek to investigate correlates of plaque attachment. This was not the goal of our work nor was our study powered to assess such associations. However, it would be beneficial for future research to investigate patient characteristics that are associated with plaque attachment. Apart from the present work and that of Randall, we cannot contrast and compare our present observations with other published research.

We state clearly that 25 stones were unattached; the origin of these stones is unknown. They might simply have detached from their original plaque anchoring site or they might have formed in free solution. Our results make it extremely improbable that they grew attached to the papillae but not on plaque. The hypothesis of development of stones in free solution does not offer, at least to us, a crucial prediction that could be tested experimentally in humans, and thus the issue of a free solution origin of stones remains unsettled.

Acknowledgments

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Abbreviations

CaOx	calcium oxalate
ICSF	idiopathic CaOx stone formers
IMCD	inner medullary collecting ducts

FIG. 1.

An attached stone before and after removal. A 3-mm stone (arrow) lying on an upper pole lateral papillum of patient no. 155 (Table 1) is visualized by digital endoscopic photography before removal (panel a) and during basket removal (panel b). Distortion during forceful removal is documented in a movie clip (supplemental data). The region where the stone was attached (panel c) contains white plaque (arrows). The removed stone has a white patch (arrow) on its tissue (attachment) surface (panel d); the urinary surface (panel e) could be identified by the prominent area of discoloration (arrow) visible in panel a.

FIG. 2.

Correspondence between tissue plaque and attachment site on stone. Panel a: Endoscopic image of a CaOx stone on a papillum. Panel b: the same papillum as panel a, with a micro CT image of the stone superimposed. Plaque (arrowheads) and blood vessels (arrows) are marked for orientation purposes. Panel c: Portion of an image after removal, of the same papillum superimposed on panel a, orientated to match plaque (arrowheads) and blood vessels (arrows) as in panel b; this alignment was done by making the image after removal partially transparent and rotating and sizing it so that the orienting markers coincided. Panel d: Correctly orientated CT image of stone (panel b) over the post-removal image of papillum (panel c) combined. Panel e: Micro CT image of stone manipulated (ghosted) to show the

position of the apatite region on the tissue side of the stone (arrowheads). Panel f: Apatite region on the tissue side of the stone outlined by arrowheads is shown within stone outline, against post-removal image of papillum; the apatite region on the stone aligns with a small area of haemorrhage on the plaque surface (arrow), presumably the site where the stone was attached to the papillum.

FIG. 3.

Histopathological validation of visual sites of Randall's plaque. Panel a: A renal papillum from patient no. 155 immediately after stone removal shows a whitish irregular underpinning (arrow) presumed to be Randall's plaque. Panel b: The same site after biopsy (note the pit, arrows). Panel c: Biopsied tissue stained with Yasue shows typical interstitial plaque deposits (arrows), which is also visualized by micro-CT (panel d, arrows). Original magnification (c) $\times 100$.

TABLE 1

Characteristics of the stones removed at surgery

Patient no./age/sex	Surgical procedure	Total	Attached	Plaque	Unknown	Analysis
155/67/M	PCNL	9	8	8	0	99/1
		2	1	1	0	
168/52/M	PCNL	11	10	10	0	99/1
171/71/M	PCNL	20	12	5	7	94/6
		5	3	2	1	
173/34/F	PCNL	6	2	2	0	100
175/67/F	PCNL	37	30	29	1	100
180/41/M	URS	9	8	8	0	99/1
183/62/M	URS	2	2	2	0	88/12
		10	10	10	0	
185/59/F	URS	2	2	2	0	70/30
187/66/M	URS	2	2	2	0	
All/—/—	-	115	90	81	9	-

PCNL, percutaneous nephrolithotomy; URS, ureteroscopy; Attached, stones attached to papilla; Plaque, stones attached to plaque; Analysis is %CaOx/% CaP from one removed stone per patient.

TABLE 2

The 24-h urine measurements and body weight

Patient	Volume, L	Weight, kg	Ca, mg/day	Ox, mg/day	Citrate, mg/day	pH	Na, mmol/day	Creatine, mg/day	SS CaOx	SS CaP
155	0.84	81.1	167	39	577	5.35	120	1707	15.7	0.92
	2.15		279	52	1039	5.53	223	2262	8.4	0.58
168	2.19	95.5	144	66	485	5.91	149	2074	6.85	0.49
	1.13		117	44	429	5.69	100	2012	8.61	0.57
171	1.76	86.4	310	41	382	5.61	175	1314	9.81	0.76
	1.37		367	40	536	5.77	121	1468	13.0	1.48
173	0.33	63	58	9	165	5.50	38	512		
175	0.77	100	138	20	111	5.20	121	1129	8.77	0.5
	0.92		172	22	153	5.32	147	1285	8.36	0.64
180	2.22	75	222	23	851	5.96	184	1528	3.85	0.62
	1.41		235	26	1020	5.73	155	1732	6.87	1.2
183	1.62	99.1	186	86	49	6.75	128	2137	11.8	1.79
	2.19		134	43	66	6.88	116	1268	4.34	0.76
185	0.6	77	38	37	12	5.82	83	1010	6.77	0.6
	1.69		106	35	34	5.89	218	1018	4.44	0.4
187	2.37	109	294	55	1152	5.66	275	2007	8.56	0.5
	2.25		412	46	1013	5.43	293	2021	9.13	0.5

SS, supersaturation; CaP, calcium phosphate.