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SECONDARILY INFECTED NON-STRUVITE UROLITHIASIS: A PROSPECTIVE EVALUATION

Mitra R. de Cógáin¹, John C. Lieske², Terri J. Vrtiska³, Pritish K. Tosh⁴, and Amy E. Krambeck¹

¹Department of Urology, Mayo Clinic College of Medicine, Rochester, Minnesota

²Department of Division of Nephrology and Hypertension, Mayo Clinic College of Medicine, Rochester, Minnesota

³Department of Radiology, Mayo Clinic College of Medicine, Rochester, Minnesota

⁴Department of Division of Infectious Diseases, Mayo Clinic College of Medicine, Rochester, Minnesota

Abstract

Purpose—To characterize patients who form non-struvite stones associated with infection (secondarily infected calculi) and to define the bacteria associated with these.

Materials and Methods—Patients undergoing percutaneous nephrolithotomy were prospectively recruited. Medical records were reviewed and stones were analyzed using microCT and infrared spectroscopy. A fragment of each stone was sent for bacterial culture. Patients were categorized by stone culture results (SC+/-) and the presence of struvite (ST+/-). Fisher's exact test was used for comparison of proportion. Sterility of intraoperative SC was established with independently collected controls.

Results—In total, 125 patients were enrolled: 24 SC+/ST-, 19 SC+/ST+ and 82 SC-/ST-. Proportions of patients with prior urologic surgery, diabetes, and immunodeficiency were similar between groups. Patients with neurogenic bladder were more likely to have SC+/ST+ stones or SC +/ST- stones than SC-/ST- stones (26% vs. 8% vs. 0%, respectively, $p < 0.01$). Among patients with metabolic evaluations, hypocitraturia was found in 31.6% (6/19) SC+/ST- patients, 46.7% (7/15) SC+/ST+ patients, and 26.0% (19/73) of SC-/ST- patients ($p = 0.28$). Approximately 40% of cultured organisms in the secondarily infected calculi possessed urease and another 40% citrate lyase activity.

Conclusions—Secondarily infected stones were detected in approximately 20% of this surgical cohort and may be more common than previously appreciated. Neurogenic bladder appeared to

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Corresponding Author: Amy E. Krambeck, M.D., Department of Urology, Mayo Clinic, 200 1st SW, Rochester, MN 55905, Tel: (507) 284-9983, Fax: (507) 284-4951, Krambeck.amy@mayo.edu.

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predispose patients to either struvite or secondarily infected stones. The role of bacterial infection in stone formation is unclear, but may include alteration of urinary components, acting as a nidus for crystallization, or inducing inflammation.

Keywords

Infection; urolithiasis; stone culture; bacteria

Introduction

Urinary tract infections have been linked to the formation of urolithiasis, and the infected stones reported in current literature are primarily composed of struvite (magnesium ammonium phosphate), with other smaller fractions of carbonate apatite and monoammonium urate often present.¹ Urease-producing bacteria including *Proteus*, *Staphylococcus*, *Klebsiella* and some *Pseudomonas* split urea into ammonia and carbon dioxide, resulting in an alkaline urinary pH and the formation of struvite and carbonate apatite crystals, subsequently leading to struvite calculi.² However, not all stones associated with infection are composed of struvite and not all are associated with urea-splitting bacteria. It is unknown whether non-struvite infected calculi result from a nidus of infection that potentially propagates stone formation, or if these stones become secondarily infected following calculus formation.

Furthermore, the role of bacteria in the growth of non-struvite stones after secondary infection has not been characterized. Multiple risk factors for the formation of struvite stones has been reported in the literature, including recurrent urinary tract infection (UTI), neurogenic bladder, and paraplegia. However, the potential causes of secondarily infected stones have not yet been delineated.³ Further, risk factors for stone formation in general, including diabetes, neurogenic bladder and bowel surgery have been reported, however, it is unknown if these patients also have a higher risk for secondarily infected urolithiasis.⁴⁻⁶ Patients with previous instrumentation of the urinary tract or immunocompromised states could also be at risk for secondarily infected stones due to bacterial colonization or difficulty warding off infection. Therefore, we sought to characterize patients who form non-struvite stones associated with infection (secondarily infected calculi) and to define the bacteria associated with these stones in a large cohort of samples sterilely removed at the time of surgical procedures.

Materials and Methods

After gaining approval from Mayo Clinic's Institutional Review Board, patients were prospectively recruited from those undergoing percutaneous nephrolithotomy (PCNL) at Mayo Clinic, Rochester, MN by a single surgeon (AEK) between September 2009 and November 2012. Patients were excluded from the current report who did not have a stone culture performed at the time of PCNL (n=9) and those with a negative stone culture despite having a struvite stone (n=1). All patients completed urine cultures preoperatively, and received antibiotics for a minimum of 7 days before surgery appropriate for the results. If urine cultures were negative, patients still received at least one week of empiric prophylactic

antibiotic therapy (typically nitrofurantoin). Stone composition was assessed by micro computed tomography (microCT) confirmed by infrared (IR) spectroscopy.⁷ Patients completed a metabolic evaluation for stone disease, including 24 hour urine collection for supersaturation profile, at the time of postoperative assessment, typically 6 weeks after surgery. Comorbid conditions (neurogenic bladder, diabetes mellitus [DM], previous bowel surgery, prior urologic surgery, immunocompromised state) and laboratory studies including 24 hr supersaturation studies were assessed from the medical record. Cultures of stones obtained at the time of surgery were also compared to preoperative urine cultures.

In order to culture stones, a fragmented portion was removed through the PCNL access sheath using either the rigid or flexible nephroscope and placed directly into a sterile test tube containing sterile saline. As a control, five independently collected samples of surgical waste from the operative field using all equipment employed in the operating room and identical technique were similarly processed and sent for culture.

Statistical analysis was performed using Fisher's exact test for pair or group-wise comparisons using JMP 8.0 software, with a $p < 0.05$ considered significant.

Results

A total of 125 patients were prospectively enrolled in the study. Of those patients, 24 had positive stone cultures (SC) but were without struvite (ST) (SC+, ST-), 19 were typical struvite stones (SC+, ST+) and 82 had negative stone cultures and were without struvite on analysis (SC-, ST-). Control cultures were all negative for microbial growth ($n=5$), establishing the sterility of our technique. Age, gender, history of prior urolithiasis surgery, prior bowel surgery, diabetes mellitus and immunodeficiency was similar between the three groups (Table 1). Patients with a positive stone culture, whether the stone contained struvite or not, were more likely to have a neurogenic bladder than patients with negative stone cultures (SC+/ST- 8% [$n=2$], SC-/ST- 0%, SC+/ST+ 26% [$n=5$], $p < 0.01$). One patient in our study had a bladder augmentation and formed typical struvite stones; two patients had an ileal conduit and of these, one formed struvite stones and the other formed secondarily infected stones. No patient in our cohort carried a previous diagnosis of vesicoureteral reflux. Also, no one in our cohort had an indwelling stent or nephrostomy tube placed prior to their procedure.

Twenty-four hour urine studies were available for 19 SC+/ST- patients, 15 SC+/ST+ patients and 73 SC-/ST- patients (Table 2). Of these patients, the prevalence of hypocitraturia (urinary citrate < 300 mg/24H) was not significantly different between groups (SC+/ST- 31.6% [$n=6$], SC-/ST- 26% [$n=19$], SC+/ST+ 46.7% [$n=7$], $p=0.28$). Urinary citrate levels were lower in typical struvite stone formers and secondarily infected stone formers as compared to non-infected stone formers, although this was not significant (SC +/ST- 476.2 ± 352.2 , SC-/ST- 610.1 ± 395.5 , SC+/ST+ 407.7 ± 303.8 , $p=0.10$). Urinary sodium was significantly lower in the stone culture positive patients than in the non-struvite, non-infected stones (SC+/ST- 122.0 ± 56.1 , SC-/ST- 172.0 ± 93.9 , SC+/ST+ 128.9 ± 41.1 , $p=0.03$). Urinary chloride levels were also lower values in patients with culture positive stones whether they contained struvite or not, (SC+/ST- 109.2 ± 46.4 , SC-/ST- $159.6 \pm$

92.9, SC+/ST+ 105.1 $\pm$ 34.5, p 0.01). Urinary chemistry profiles were otherwise not different between groups.

Mean urinary pH was analyzed as a continuous variable, and was not significantly different between the three groups (SC+/ST- 5.97 [Range 4.8-7.3], SC-/ST- 6.25 [Range 5.1-7.6], SC+/ST+ 6.31 [Range 5.2-7.2], $p=0.29$) (Figure 1). Predominant stone composition was significantly different between groups (Table 3). SC+/ST- patients most often formed calcium phosphate stones (54.2%, $n=13$) followed by calcium oxalate monohydrate stones (33.3%, $n=8$). SC-/ST- patients most often formed calcium oxalate monohydrate stones (61.0%, $n=50$), as expected, followed by calcium phosphate stones (24.4%, $n=20$). Patients with struvite-containing stones (SC+/ST+) formed apatite stones most frequently (47.4%, $n=9$), followed by struvite stones (36.8, $n=7$). Or, in other words, patients with culture positive stones (with or without struvite) were more likely to have calcium phosphate stones while those with culture negative stones, were more likely to have calcium oxalate monohydrate present. Subanalysis of the calcium phosphate stones demonstrated that only 1 of the 9 (11%) brushite stones was secondarily infected while 21 of the 33 (63.6%) non-brushite calcium phosphate stones was associated with infection.

The most commonly-cultured Gram negative organism from stones in the SC+/ST- patients was *Escherichia coli* ($n=5$), which typically does not hydrolyze citrate and does not produce urease, and *Proteus mirabilis* ($n=5$) which typically does hydrolyze citrate and produce urease, as determined by review of contemporary infectious diseases texts (Table 4). In the SC+/ST+ patients, *Proteus mirabilis* was most often isolated ($n=4$). *Enterococcus spp.*, which typically hydrolyze citrate, were the most prevalent Gram positive organism in the SC+/ST- patients ($n=6$), while *Staphylococcus* was most often identified in the SC+/ST+ patients ($n=6$). Other pathogens, including *Ureaplasma* ($n=2$), *Mycoplasma* ($n=3$) and *Gardnerella* ($n=1$) were identified in the SC+/ST- patients, while one SC+/ST+ had *Ureaplasma*. *Candida* was identified in 3 SC+/ST- patients and 3 SC+/ST+ patients. Overall, 43% (16/37) of all organisms identified in the SC+/ST- group (some cultures were polymicrobial) produced urease and 43% (16/37) hydrolyzed citrate, while 56% (15/27) in the SC+/ST+ group were urea splitting organisms and 48% (13/27) were citrate hydrolyzers.

Preoperative urine cultures and urinalysis results were also compared to the stone culture results. A UA was considered concordant with a positive culture if it contained greater than three WBCs per high powered field on microscopic examination, which would be indicative of an underlying UTI when a formal urine culture was not available. In the SC+/ST- group, 12 (50%) had concordant cultures, 6 (25%) had discordant cultures, and 6 (25%) had a negative UA or urine culture ($n=6$). In the SC+/ST+ group, 8 (42%) had concordant cultures and 5 (26%) had discordant cultures, while 6 (32%) had a negative UA or urine culture. There was no statistically significant difference in concordance or discordance rates between the SC+/ST- and SC+/ST+ groups ($p=0.85$).

Discussion

The current understanding of the role of bacteria in urolithiasis formation is quite limited. Struvite stones are unequivocally related to underlying UTI with urease producing

organisms. However, the association of bacterial pathogens with non-struvite urolithiasis is not well delineated. Our study demonstrates that calcium-based calculi may be associated with infection, as reflected by positive stone cultures, even in patients with negative preoperative urinalysis and/or culture. However, whether these stones become colonized with bacteria following crystal formation or if, in fact, bacteria propagate stone formation, is yet to be determined.

In the late 1990's, by Kajander and Ciftçioğlu speculated that nanobacteria, purported atypical gram negative life forms smaller than typical bacteria, could be found in concentrated within the kidney and formed an apatite shell that could serve as a nidus for calculus formation.⁸⁻¹² More recent studies suggest that nanobacteria are not a true life-form, but represent a novel assembly of proteins that can self-propagate and promote calcification.¹³⁻¹⁵

The role of more typically cultured organisms in chronic disease states, especially those linked to pathologic calcification, is largely unknown and unstudied, particularly in the case of kidney stone disease. However, most urine samples contain bacterial colonies as demonstrated by 16S rRNA gene sequencing, despite sterile cultures.^{16,17} It appears that bacterial communities vary by gender and bladder functionality (i.e. the presence or absence of a neurogenic bladder). Therefore, the bacteria colonizing men and women with and without urolithiasis may differ as well.

Our study suggests that non-struvite, infected urolithiasis may be more prevalent than previously appreciated, with *E. coli* and *Enterococcus* being the most commonly identified organisms. Further, in a recent study by Tavichakorntrakool and associates, 36% of patients in a cohort of Thai stone formers had positive stone cultures, with 78% of those forming calcium based or uric acid calculi, while only 22% of patients with positive stone cultures had struvite or other infection-related stones.¹⁸ In that study, *E. Coli* was the most frequently isolated bacterium, and only 31% of all organisms isolated from stones produced urease.

Potentially, organisms cultured from stones, with or without a clinically apparent UTI, could initially cause kidney cell injury and inflammation, which could in turn potentiate crystal retention and stone formation.¹⁹⁻²¹ Alternatively, the presence of bacteria could change the local microenvironment by metabolic activity. Nearly 40% of cultured organisms in the secondarily infected patients possessed urease and another 40% citrate lyase activity. Therefore, enzymatic urease could increase the local pH, carbonate and ammonia concentration to favor struvite, calcium carbonate and calcium phosphate growth, or citrate lyase could reduce the level of this crystal inhibitor to favor calcium phosphate or calcium oxalate crystal growth. We did note a trend towards lower urinary citrate in patients with stones harboring infection; however, this finding was not significant probably due to the timing of the 24 hour urine studies, which were performed after stone removal and antibiotic treatment. We also found that those patients with positive stone cultures had lower urinary sodium and chloride compared to noninfected stones. We cannot explain why these two urinary analytes would be influenced by infection and further investigation is necessary to investigate their role in secondarily infected stone pathogenesis. Interestingly, in our study only one brushite stone was associated with infection, while over half of the non-brushite

calcium phosphate stones were associated with infection. The brushite stone that was associated with infection was mixed with octacalcium phosphate, indicating that brushite stone composition alone, in this study, is not generally associated with urinary infection. An alternative theory for the role of bacteria in stone pathogenesis is bacteria produce a biofilm that serves as a matrix or biofilm for stone growth, as has been demonstrated for geological mineralizations.²² Widespread and systematic screening of kidneys, urine and stones obtained from diverse populations of stone formers for the presence of microbial RNA or DNA will be necessary in order to determine if previously unrecognized pathogens are in fact present in these patients.

Most urinary tract infections are thought to arise via an ascending route of bladder colonization from the periurethral region.²³ Although not all of our SC+/ST- patients had a history of clinical cystitis, concordance rates between preoperative urine cultures and stone cultures were 50% in this group. The other 50% may have had subclinical infection that cleared by the time of evaluation. It is also possible that bacteria could have arrived in the kidney via a systemic route, although this does not seem likely given the mix of cultured organisms. This finding underscores the importance of performing a stone culture at the time of PCNL, as the results may significantly alter postoperative antibiotic therapy.

Our study has certain limitations. Although data was gathered prospectively, some patients only had urinalyses and not urine cultures, which could still have been positive in the face of normal urine microscopy. Also, all patients were treated with antibiotics preoperatively, and this may have altered the flora cultured from stones, but we cannot determine how the microbial profile changed before and after preoperative antibiotic administration. However, based on the fact that stone culture positivity was found in multiple patients, it appears that preoperative antibiotics do not completely sterilize all stones, further supporting the practice of complete stone removal in the presence of recurrent urinary tract infections. In addition, we did not test individual isolates for citrate hydrolysis and urease production, but instead relied on published historical data in the infectious disease literature regarding organism characteristics. Nevertheless, this is the largest report of consecutively cultured stones removed at the time of surgery. The results suggest a surprising percentage harbor bacteria, whether or not they are composed of struvite. Due to these findings, we will continue our current practice of obtaining stone cultures on all patients undergoing percutaneous nephrolithotomy and providing appropriate antibiotic therapy to those with positive cultures.

Conclusion

To date, only struvite stones have been definitively linked to causation by bacterial infection. However, our study suggests that microbes are present in a significant portion of non-struvite stones, whether the patients have symptomatic UTIs or not. The presence of a neurogenic bladder appeared to predispose patients to struvite and secondarily infected calculi. However, no other risk factors for secondarily infected stones were identified. Since a large percentage of non-struvite stones were secondarily infected, even in some patients with negative preoperative cultures or urinalyses, it is imperative that stone cultures be performed at the time of PCNL, in order to ensure adequate treatment and clearance of any

underlying, previously unappreciated infection. Further studies are necessary to delineate the exact role, if any bacteria play in stone formation.

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Table 1
Characteristics of patients undergoing percutaneous nephrolithotomy by group

	Stone Culture + Struvite – (n=24)	Stone Culture – Struvite – (n=82)	Stone Culture + Struvite + (n=19)	p value
Average Age (SD)	55.6 (17.2)	55.2 (14.0)	54.7 (14.4)	0.98
Female Gender	79.2%(19)	56.1% (46)	63.2% (12)	0.12
Prior Stone Surgery	50% (12)	51.2% (42)	57.9% (11)	0.85
Diabetes Mellitus	17% (4)	21% (17)	21% (4)	0.90
Neurogenic Bladder	8% (2)	0	26% (5)	<0.01
Bowel Surgery	17% (4)	15% (12)	26% (5)	0.47
Immunodeficiency	17% (4)	12% (10)	5% (1)	0.51

Table 2
24 hour urine chemistries by group (mean $\pm$ SD)

	Stone Culture + Struvite – (n=19)	Stone Culture – Struvite – (n=73)	Stone Culture + Struvite + (n=15)	p value
Sodium (mmol/24H)	122.0 $\pm$ 56.1	172.0 $\pm$ 93.9	128.9 $\pm$ 41.1	0.03
Potassium (mmol/24H)	54.4 $\pm$ 28.7	69.7 $\pm$ 49.4	61.9 $\pm$ 16.0	0.36
Calcium (mg/24H)	199.8 $\pm$ 116.7	221.5 $\pm$ 121.9	192.6 $\pm$ 92.9	0.50
Magnesium (mg/24H)	99.4 $\pm$ 55.1	112.7 $\pm$ 51.6	108.4 $\pm$ 37.6	0.58
Chloride (mmol/24H)	109.2 $\pm$ 46.4	159.6 $\pm$ 92.9	105.1 $\pm$ 34.5	0.01
Phosphorus (mg/24H)	772.1 $\pm$ 360.9	941.9 $\pm$ 383.6	818.3 $\pm$ 331.5	0.15
Sulfate (mmol/24H)	16.3 $\pm$ 8.1	19.9 $\pm$ 10.0	18.0 $\pm$ 6.9	0.30
Citrate (mg/24H)	476.2 $\pm$ 352.2	610.1 $\pm$ 395.5	407.7 $\pm$ 303.8	0.10
Hypocitraturia (<300 mg/24H), %(n)	31.6% (6)	26.0% (19)	46.7% (7)	0.28
Oxalate (mmol/24H)	34.8 $\pm$ 35.7	33.2 $\pm$ 18.7	36.2 $\pm$ 17.4	0.87
Uric Acid (mg/24H)	493.7 $\pm$ 227.8	592.4 $\pm$ 256.3	552.0 $\pm$ 160.3	0.27
Volume (ml/24H)	1852.6 $\pm$ 956.7	2028.6 $\pm$ 817.1	2134.4 $\pm$ 899.7	0.61
pH (range)	6.1 (4.8-7.3)	6.2 (5.1-7.6)	6.4 (5.2-7.2)	0.29

Table 3
Predominant stone composition by group (Mean)

	Stone Culture + Struvite – (n=24)	Stone Culture – Struvite – (n=82)	Stone Culture + Struvite + (n=19)	p value
				<0.01
Calcium Oxalate Monohydrate	33.3% (8)	61.0% (50)	10.5% (2)	
Calcium Oxalate Dihydrate	4.2% (1)	8.5% (7)	0	
Calcium phosphate/hydroxyapatite	50.0% (12)	14.6% (12)	47.4% (9)	
Brushite	4.1% (1)	9.8% (8)	0	
Uric Acid	4.2% (1)	4.9% (4)	0	
Struvite	0	0	36.8% (7)	
Other (2.8 Dihydroxyadenine, cystine)	0	2.4% (2)	0	
Mixed	4.2% (1)	0	5.3% (1)	

Table 4
Pathogens identified in stone cultures

Results expressed as positive (+) or negative (–) for enzyme activities, as based on typical species characteristics, or % (n) for culture isolates.

Gram Negative Pathogen	Urease production	Citrate hydrolysis	Stone Culture+ Struvite– (n=24)	Stone Culture+ Struvite+ (n=19)
<i>Pseudomonas spp.</i>	+	+	8.3% (2)	5.3% (1)
<i>Escherichia coli</i>	–	–	20.8% (5)	0
<i>Klebsiella spp.</i>	+	+	4.2% (1)	5.3% (1)
<i>Proteus mirabilis</i>	+	+/-	20.8% (5)	21.1% (4)
<i>Providencia rettgeri</i>	+/-	+	0	5.3% (1)
<i>Enterobacter cloacae</i>	–	+	4.2% (1)	0
<i>Citrobacter spp.</i>	+/-	+	0	5.3% (1)
<i>Unknown spp.</i>	n/a	n/a	4.2% (1)	0
Gram Positive Pathogen				
<i>Enterococcus spp.</i>	–	+	25% (6)	26.3% (5)
<i>Micrococcus spp.</i>	+/-	–	4.2% (1)	0
<i>Streptococcus spp.</i>	–	n/a	4.2% (1)	5.3% (1)
<i>Aerococcus spp.</i>	–	n/a	0	5.3% (1)
<i>Staphylococcus</i>	+/-	n/a	16.6% (4)	31.6% (6)
<i>Corynebacterium spp.</i>	–	–	8.3% (2)	0
<i>Lactobacillus spp.</i>	–	–	0	5.3% (1)
<i>Propionibacterium spp.</i>	–	+	4.2% (1)	0
<i>Propionimicrobium spp.</i>	n/a	n/a	0	5.3% (1)
Other Pathogen				
<i>Ureaplasma</i>	+	n/a	8.3% (2)	5.3% (1)
<i>Mycoplasma</i>	+	n/a	4.2% (1)	0
<i>Candida</i>	–	–	12.5% (3)	15.8% (3)
<i>Gardnerella</i>	–	n/a	4.2% (1)	0