Biomarkers in Urolithiasis

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KEYWORDS

• Urolithiasis • Biomarkers • Kidney stones • Nephrolithiasis

KEY POINTS

- Biomarker use in endourology and stone disease has seen limited use without widespread adoption to date.
- There are many potential applications of biomarkers for the study of patients with urolithiasis, both for clinical and research purposes.
- Further studies are needed to define the role of novel biomarkers in the evaluation and treatment of patients with kidney stone disease.

INTRODUCTION

Urolithiasis is one of the most common urologic diseases. It is estimated that the lifetime prevalence in the United States is approximately 1 in 11. Trend analysis has determined that stone disease is on the increase, with prevalence numbers nearly doubling over the prior 2 decades.[1] Stones that form in the kidney may detach from the renal papilla and obstruct the ureter, leading to acute obstructive episodes that require emergency care and/or intervention; this has resulted in emergency department visits and health care cost utilization estimated at more than $5 billion spending per year.[2]

Urolithiasis is the result of alterations in the renal tubular and renal pelvic pH and solute concentration. Renal handling of calcium, oxalate, phosphate, and citrate among others may be disrupted by underlying metabolic, lifestyle, and genetic factors as well as local and systemic processes. Disturbances to the regulation of excretion and secretion of these substances leads to solute crystallization and aggregation, which in turn grow into kidney stones. Given the variety of substrates handled by the renal filtration system in the convergence of multiple pathways, it is clear that different alterations may lead to different compositions of stones. Urolithiasis most commonly involves calcium oxalate stones with a degree of phosphate components. Other common types of stones are composed of struvite, uric acid, and cystine, which are estimated to represent 10%, 9%, and 1%, respectively.[3]

"Biomarker" is a broad term whose actual definition has undergone various revisions by organizations such as the World Health Organization and the National Institutes of Health, among others. Current definitions are wide encompassing, denominating a biomarker any consistent measurement that reflects underlying processes.[4] This review focuses on measurements of serum, urinary and genetic structures, substances, proteins, and bacteria. As previously mentioned, urolithiasis is the result of underlying alterations in metabolic and/or genetic processes. Therefore, biomarkers can be surrogates, reflecting underlying biological aberrations. These markers, including inflammasomes, serum and urinary proteins, RNA/DNA, and bacteria, may provide detailed insight into stone formation risk, as well as track complications such as kidney injury and sepsis.

MARKERS OF URETERAL OBSTRUCTION AND KIDNEY INJURY

Kidney stones, regardless of composition, may dislodge and cause obstruction during passage down the ureter or grow enough to partially or

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Urol Clin N Am 50 (2023) 19–29
https://doi.org/10.1016/j.ucl.2022.09.004
0094-0143/23/

completely obstruct renal outflow. In both cases, renal damage may ensue both acutely and chronically. Creatinine has been classically used as a surrogate of kidney function; however, it is nonspecific and lacks sensitivity to identify early renal damage, as serum levels may lag and not accurately depict undergoing renal injury. Various biomarkers have been proposed and studied as more accurate identifiers of ongoing renal injury with mixed success.

Kidney Injury Molecule 1

Kidney injury molecule 1 (KIM-1), a type 1 transmembrane protein in the proximal tubule, was identified to be upregulated in rat kidneys undergoing ischemic injury by Ichimura and colleagues in 1998.[5] These findings were then verified in human kidneys biopsies in 2002 by Han and colleagues.[6] They also proposed that urine levels of KIM-1 were higher in patients with ischemic acute tubular necrosis than in patients with other forms of renal injury, such as contrast nephropathy, systemic lupus erythematosus, diabetes mellitus, postrenal obstruction, chronic kidney disease, and healthy controls.[6] These findings gathered worldwide interest, resulting in more than a thousand studies that have been published on this protein in various settings. Among these studies, some have been dedicated to ureteral obstruction and urolithiasis, which found increased urinary levels of KIM-1 in obstructive nephropathy.[7–9] However, further studies attempting to determine the role and utility of KIM-1 in urolithiasis have been inconclusive.

Fahmy and colleagues[10] analyzed KIM-1 urinary expression levels in patients undergoing retrograde intrarenal surgery (RIRS) and shockwave lithotripsy (SWL) and compared them with healthy controls. Among their findings, patients with stone disease had increased KIM-1 urinary levels compared with controls. Patients who underwent SWL had increased KIM-1 levels after treatment, whereas those who underwent RIRS did not. These findings contrast to those of Urbschat and colleagues[11] who compared urinary levels of KIM-1 in patients with acute obstructive uropathy with that of healthy controls and found no significant differences. Olvera-Posada and colleagues[7] also analyzed urinary levels of KIM-1 in patients with hydronephrosis. They found the presence of KIM-1 successfully identified hydronephrosis and decreased after treatment; however, it was not found to be a specific marker for urolithiasis, as various obstructing pathologies were included.

Bansal and colleagues[12] analyzed the ratio of KIM-1 to creatinine in urine in patients undergoing various endourological interventions for urolithiasis. They found significant correlation between KIM-1/creatinine and stone size. In addition, they found that their interventions with low stone-free rates had persistently high KIM-1/creatinine ratios after intervention, whereas RIRS and percutaneous nephrolithotomy (PCNL) had significant decreased urinary concentrations of KIM-1/creatinine in urine. These investigators concluded these combinations of KIM-1 and creatinine could be an effective indicator of underlying stone disease. KIM-1 is currently approved by the Food and Drug Administration as a marker, currently in use in various clinical trials for monitoring renal damage related to drugs.[13]

Neutrophil Gelatinase–Associated Lipocalin

Neutrophil gelatinase–associated lipocalin (NGAL) is a protein bound to gelatinase from neutrophils that mediates cellular proliferation and differentiation and exerts a bacteriostatic effect. NGAL's expression is upregulated during inflammatory events.[14] Its utility in renal damage and various other renal injuries remains contested by mixed evidence.[14–16]

Bolgeri and colleagues[15] studied NGAL as a potential renal injury marker in acute obstructive uropathy. They found both urinary and serum as well as the ratio of urinary NGAL to creatinine elevated in patients with both urolithiasis and acute obstructive uropathy compared with healthy controls. There were no differences in these values between both study groups. However, patients who experienced spontaneous stone expulsion and those who underwent surgical management also had significant reductions of these values. Hughes and colleagues[16] compared baseline and post-shockwave levels of NGAL, finding increased levels at 30 and 120 minutes post-shockwave, with levels trending toward normalization at 240 minutes. Hughes and colleagues[17] also performed a similar study by analyzing patients undergoing flexible ureteroscopy (URS) testing various biomarkers. In the setting of URS, they concluded similar findings of increased NGAL post-URS. These findings are consistent with those of Dede and colleagues.[18] Although increased markers in the setting of SWL are explained by trauma from soundwave shock, in the setting of URS increases may be explained by renal manipulation, stone movement, and/or laser heat and damage.

Olvera-Posada and colleagues[7] as well as Urbschat and colleagues[11] presented similar findings when they studied NGAL. Both studies found that although NGAL was increased in obstruction,

it was more intimately correlated to pyuria and other inflammatory processes. These results are further supported by the findings presented by Zhu and colleagues[19] in which they found the NGAL was significantly higher in patients with urinary tract infections and was a sensitive and specific predictor of systemic inflammatory response syndrome.

Carbohydrate Antigen 19-9

Carbohydrate antigen 19-9 (CA19-9) has been most widely studied as a biomarker for pancreatic and other gastrointestinal tract cancers. Prior investigators have established that serum and urinary levels of CA19-9 may have potential as biomarkers of renal injury related to obstruction.[20] Amini and colleagues'[20] analysis of patients with urolithiasis and healthy controls determined CA19-9 levels to be increased in patients with urolithiasis and hydronephrosis; however, further studies found a closer association with hydronephrosis and no relation with urolithiasis, urinary tract infections, and proteinuria. CA19-9 may also be elevated in other systemic conditions, limiting its use.[21]

N-acetyl-B-D-glucosaminidase

N-acetyl-B-D-glucosaminidase (NAG) is upregulated and found in increased concentration in tubular damage.[22] Fahmy and colleagues[10] found nonincreased levels of NAG in patients with kidney stone disease; however, they determined NAG increased shortly after SWL, suggesting that NAG may be a marker for renal damage associated to SWL.

Cystatin-C

Cystatin-C is a freely filtered protein that has been previously used as a biomarker for renal damage following surgery.[23] Hughes and colleagues[17] compared pre-URS with post-URS cystatin C levels, finding no significant differences; however, their study may be limited by a small sample size.

Myeloperoxidase

Myeloperoxidase (MPO) is an enzyme involved in the generation of oxygen radicals by neutrophils allowing for host defense. It has previously been used as a marker for inflammatory states and cardiovascular disease.[23–25] Hughes and colleagues[17] compared pre-URS with post-URS MPO levels, finding no significant differences; however, their study may be limited by a small sample size.

Urinary Genes, Proteins, and Cytokines

Various biomarkers may provide insight into renal pathophysiology when quantified. Underlying cellular changes occurring as response or in relation to stone formation and related complications may be tracked through expression and excretion of proteins and genetic material. Inflammation may be tracked through the measurement of mediators such as cytokines, of which various have been studied in the context of urological biomarkers; these have been previously used to detect acute and chronic kidney injuries, as there is often an ongoing underlying inflammatory reaction related to endothelial and tissue injury.[26,27]

Among studied cytokines are the following: interleukin-6 (IL-6) is a multifunctional cytokine that is involved in various processes, ranging from hematopoiesis, inflammation, and regulation. Increased urinary levels of IL-6 have been identified in patients with sepsis and acute kidney injury.[26,27] IL-8 targets neutrophils avidly and is also currently used as a marker of inflammation. It has previously been measured in urine of patients with pyelonephritis, hemolytic uremic syndrome, as well as graft rejection.[28] IL-10 has also been shown in the past to be elevated in infections as well as neoplasia.[29,30] IL-18 is upregulated during inflammatory responses. Although it is typically released by macrophages, it has also been determined to be present in the renal tubular epithelium. Thus, urinary concentrations of IL-18 have been confirmed to be significantly increased in patients with AKI and is able to discern between AKI and other urological pathologies such as urinary tract infections and renal syndromes.[31,32] Tumor necrosis factor alpha (TNF-α) is a multifunctional cytokine that is heavily involved in priming and sustaining inflammatory responses.[33] Hughes and colleagues[16] performed a comparative analysis in patients undergoing SWL in which they compared baseline levels of the aforementioned cytokines with various post-SWL levels and various timepoints. Among their findings, they determined IL-6 and TNF-α to increase significantly within 30 to 120 minutes post-shockwave. These findings could suggest these specific cytokines could be used to assess early renal damage.

Long noncoding RNA (lncRNA) accurately reflects intranuclear processes and is thought to provide specific insight in ongoing cellular processes. Tawfick and colleagues[34] analyzed urinary levels of RNA in patients undergoing SWL against healthy controls and also tracked changes in urinary concentrations after the intervention. They found that lncRNAs SBF2-AS1 and FENDRR-19 and messenger RNAs (mRNA) GBP1 and NLRP3 were increased in patients after undergoing SWL. They concluded that urinary markers may be potential markers for renal damage. In addition, they identified these RNA markers to be

significantly increased in urolithiasis patients as compared with healthy controls. They found absence of these 4 measured urinary RNA markers in healthy controls, whereas 40% to 80% of patients with urolithiasis had increased urinary levels. These biomarkers are reflections of established inflammasome pathways and thus may suggest underlying renal inflammation in patients with urolithiasis. An overall summary of identified biomarkers is displayed in **Table 1**.

Spontaneous stone passage

As previously mentioned, stones may dislodge from the kidney and migrate into the ureter where they may either spontaneously pass or become affected. Current guidelines endorse medical expulsive therapy (MET) for ureteral stones less than 10 mm in selected patients with varying degrees of success.[35] The role of biomarkers in this setting as possible indicators of passage has also been thoroughly studied.

Table 1
Summary of identified biomarkers related to kidney injury and acute obstruction

Biomarker	Function
Kidney injury molecule 1 (KIM-1)	Increased in renal damage and obstruction Unclear if elevated by nonobstructing stones
Neutrophil gelatinase–associated lipocalin (NGAL)	Increased in renal damage and infectious/inflammatory processes
Carbohydrate antigen 19-9/CA19-9	Increased in renal obstruction
N-acetyl-B-D-glucosaminidase (NAG)	Possibly increased in renal damage
Cystatin-C	Marker of renal function
Myeloperoxidase (MPO)	Marker of inflammation, unclear role in urolithiasis
Cytokines	Interleukins: possible markers of renal damage and infection/inflammation
Gene-related products	LncRNA/mRNA: possible markers of renal damage

Cilesiz and colleagues[36] analyzed serum procalcitonin levels in patients undergoing MET. They found patients who failed MET had higher procalcitonin levels than those who achieved spontaneous passage. A cutoff of 160pg/mL was proposed with an 86.7% sensitivity and 70.8% specificity. The investigators hypothesized that increased procalcitonin reflects underlying inflammation from stone impaction. Similar to procalcitonin, C-reactive protein (CRP) has been studied and demonstrated similar effects. Özcan and colleagues[37] performed a similar analysis with CRP, suggesting a cutoff of 0.506 mg/L to have significant predictive value in stone passage. These findings are supported by Aldaqadossi and colleagues[38] who proposed a cutoff of 21.9 mg/L for CRP and Jain and colleagues[39] who proposed a cutoff of 4.1 mg/L. Given the wide range of reported values, further research is needed to clarify optimal cutoff points.

It is hypothesized that inflammatory reactions are proportional to stone impaction based on the aforementioned evidence, and thus multiple other markers such as neutrophil percentage, neutrophil-to-lymphocyte ratio, and platelet-to-lymphocyte ratio (PLR) have been proposed as potential adjunct biomarkers.[40,41]

Stones and stone types

Metabolic disorders and endothelial dysfunction have long been linked to urolithiasis. Prior meta-analyses have established a significant association between diseases linked to endothelial dysfunction such as coronary artery disease, stroke and myocardial infarction, and urolithiasis.[42–44] Some have identified that patients with these disorders have lower urinary excretion of citrate and magnesium and have signaled these findings as possible related factors.[45] Certain markers such as CRP have been long used as surrogates of ongoing underlying inflammation in cardiovascular disease and other inflammatory conditions. Shoag and colleagues[46] determined increased risk of urolithiasis in young patients with elevated CRP. This database analysis of 11,033 patients found that increasing quintiles of CRP values in this population had increased odds of prevalent urolithiasis.

The triglyceride-glucose index incorporates fasting triglyceride and glucose levels and has been previously used as an accurate indicator of insulin resistance. Qin and colleagues[47] performed a large database analysis of 20,970 patients, in which they determined the triglyceride-glucose index and the prevalence as well as recurrence of urolithiasis. The mean triglyceride-glucose index was 8.71, and the investigators concluded that

there was an increase of urolithiasis incidence with an odds ratio (OR) 1.12 and an increased recurrence with an OR of 1.26 per unit increase in the index. These findings remained significant when models were adjusted for gender, age, body mass index, hypertension, and diabetes.

Urinary Proteins

Osteopontin was originally isolated from bone matrix and was later identified through many systems within the human body, as it serves various functions. In the renal epithelium it has been proposed to act as a potent inhibitor of adhesion of mineral crystals.[48] This protein is influenced by nutrition and anthropometric conformation.[49,50] Icer and colleagues[51] compared urinary levels of osteopontin in patients with urolithiasis with that in matched cohorts. Interestingly, this study found that patients in the urolithiasis cohort had significantly lower levels of urinary osteopontin when compared with the control group. The role of osteopontin in stone prevention, especially among calcium oxalate stone formers, is supported by in vitro and animal studies where it has been shown to inhibit nucleation, crystallization, and growth as well as attachment to the renal epithelium. Experiments in mice have also shown that knockout mice were more likely to form stones.[52]

Matrix Gla protein (MGP), similar to osteopontin, was first identified in the bone matrix and was later found in other tissues.[53] Because of its expression in vascular tissue, it has been proposed as an inhibitor of calcification.[54] Although in vitro and animal models have found alterations in levels of MGP, these have not been identified to be significantly altered in stone formers.[55]

Tamm-Horsfall protein (THP), also known as uromodulin, is a protein found mainly within the renal epithelium. It lines the thick ascending limb of Henle loop and serves multiple functions.[56,57] THP is normally found in urine, and although its presence has been suggested to inhibit crystallization, this finding has been contested by studies that suggest the opposite. THP's behavior has been theorized by various studies to depend on its concentration as well as that of other solutes. A recent study determined THP has concentration-dependent crystallization inhibitory effects by avidly binding calcium,[58] whereas prior studies have suggested that in settings with increased calcium concentrations, THP increases crystal aggregation.[59]

Urinary prothrombin fragment-1 (UPTF-1) is a component of the thrombin protein. In vitro findings of its inhibitory effects on crystallization have been further sustained by results from epidemiological studies that associated increased incidences of urolithiasis in populations with lower UPTF-1.[60,61]

Some investigators have hypothesized that urolithiasis generates a constant inflammatory environment in the kidney and thus may elevate some of the damage markers previously mentioned. In children, Kovacevic and colleagues[62] compared the levels of NGAL, cystatin C, and lysozyme C in patients with urolithiasis against healthy controls. Out of these 3 markers, cystatin C and NGAL were found to be significantly increased in patients with urolithiasis. Further proteomic analysis revealed that these markers were elevated within patients with urolithiasis regardless of urinary levels of calcium and citrate. Lastly, both cystatin C and NGAL were elevated in patients with a history of stones regardless of stone status at the time of urine collection. Fan and colleagues[63] analyzed the urinary protein components' differences between patients with unilateral and bilateral urolithiasis. They found patients with higher urinary NAG-to-creatinine ratios had increased odds of bilateral stones.[63]

Cadieux and colleagues[64] analyzed various urinary proteins through a surface-enhanced laser desorption/ionization–time-of-flight mass spectrometry in patients with urolithiasis and compared them with healthy controls. They found patients with urolithiasis had increased levels of proteinuria, oxalate, p67:p24, and albumin while having similar levels of osteopontin. Zhu and colleagues[65] performed a similar proteomics analysis, in which they found that in patients with calcium oxalate urolithiasis, fibrinogen alpha chain precursor and apolipoprotein A-1 were accurate markers.[65] Wang, and colleagues[66] identified altered metabolism of involved caffeine, phenylalanine, galactose, and tyrosine metabolism.

Metabolomics

Duan and colleagues[67] performed a nuclear magnetic resonance–based metabolomic analysis to create a metabolomic profile for stone formers. They found various pathways, notably glyoxylate and dicarboxylate metabolism, as well as the metabolism of glycine, serine, threonine, phenylalanine, and the Krebs cycle to be associated with urolithiasis. Primiano and colleagues[68] further analyzed the urinary amino acid signatures of patients with urolithiasis and compared them with healthy controls by quantifying the amount of certain amino acids in the urine. This analysis involved 15 stone formers and 12 controls who were tested using a panel of 25 amino acids and derivatives. Resulting urinary amino acid profiles revealed stone formers had lower urinary levels

of α-aminobutyric acid, asparagine, ethanolamine, isoleucine, methionine, phenylalanine, serine, tryptophan, and valine. These findings are consistent with prior studies analyzing altered metabolic pathways in urolithiasis and its traces in urine that have found low urinary levels of ethanolamine, serine, tryptophan, oxalate, calcium, citrate, and cystine.[64,66,67] It is theorized that alterations in the glycine metabolism may result in increased oxalate production and thus increased risk of urolithiasis.[67] Other amino acids such as alanine, tryptophan, and threonine are proposed to have inhibitory effects on crystallization.[68,69] Similarly, Wen and colleagues[70–73] performed a metabolomic analysis on children with urolithiasis and compared it with a control group. They identified 40 metabolites related to retinol metabolism, steroid hormone biosynthesis, porphyrin, and chlorophyll metabolism, and these translated to lower levels of serum bilirubin, with increased levels of retinal, all-trans retinoic acid, progesterone, and prostaglandin E2.[70] Overall findings are summarized in **Table 2**.

Table 2
Summary of identified biomarkers and proposed applications for kidney stone detection

Marker	Function
CRP	Possibly increased in younger patients with urolithiasis. Possibly related to underlying metabolic disorders
Triglyceride-glucose index	Possibly increased in patients with urolithiasis in relation to underlying metabolic disorders
Osteopontin	Thought to be decreased in stone formers
Matrix Gla protein (MGP)	In vitro evidence of possible mineralization inhibitor
Tamm-Horsfall protein (THP)	Concentration-dependent function. Possibly decreased in stone formers. In high-calcium scenarios increases mineralization
Urinary prothrombin fragment 1	Possible inhibitor of crystallization, decreased in stone formers
Amino acid and other metabolic pathways	Various metabolic pathways altered identified in patients with urolithiasis including • Glyoxylate and dicarboxylate metabolism • Glycine, ethanolamine, serine, and others

SEPSIS

Sepsis is an immune phenomenon resulting from dysregulated inflammatory response to infectious insults. It is termed urosepsis when the original infectious insult originates from within the urinary tract. Urolithiasis may result in sepsis when stones become infected, and obstruction occurs with concomitant infection or as a postoperative complication of a urologic procedure. Procedures such as PCNL may carry up to 5% rates of postoperative sepsis.[74] Prior literature is abundant in modeling attempts to determine risk factors in order to better predict and prevent sepsis.

Although leukocytosis is common in infections and other inflammatory processes, attention has been paid to the degree in which various specific cell counts increase or decrease in relation to another. Although in the past, specific cell counts have been used as biomarkers in the evaluation of oncology patients, it has only recently been explored in urolithiasis.[75] Kriplani and colleagues[76] analyzed a cohort of patients undergoing PCNL and determined that patients who developed sepsis had significantly higher leukocyte count, higher neutrophil-to-lymphocyte ratio (NLR), higher PLR, and lower lymphocyte-to-monocyte ratio (LMR). They established various cutoffs for these findings. At a cutoff of 2.45, NLR was found to have an 87% sensitivity and on receiver operating characteristic curve analysis, an area under the curve (AUC) of 0.639. PLR had a sensitivity of 80.2% at a cutoff of 110, with an AUC of 0.663. Lastly LMR had a sensitivity of 87.5 with a cutoff of 3.23 and an AUC of 0.649.

Some of the previously mentioned biomarkers such as NGAL, cystatin-C, MPO, and others were analyzed in the context of infectious complications due to a urologic source. Hughes and colleagues,[17] although limited by a small sample size, found that there may be a role for these biomarkers in monitoring infections. Patients with urinary tract infections had increased levels compared with the rest of the cohort of cystatin-C; however, the patient who developed sepsis had average levels of cystatin C. NGAL was average in 2 patients with urinary tract infections and normal in the patient with sepsis and was elevated only in 1 of the 3 patients with a urinary tract infection. On the other hand, MPO was increased in the patient with sepsis and elevated in 1 of the 3 patients with urinary tract infections. The investigators encouraged future experiments

involving these biomarkers in larger cohorts. Qi and colleagues [77] performed similar measurements in patients undergoing PCNL and found that serum IL-6 drawn within 2 hours of the operation was found to have an AUC of 1 at identifying postoperative urosepsis. Procalcitonin was also found to be accurate with an AUC of 0.954 when drawn on the third postoperative day. Thus, the investigators suggest that IL-6 may be a very accurate early marker of sepsis in patients undergoing PCNL.[77,78] In a similar analysis, Liu and colleagues[79] analyzed sepsis rates after endourological surgery and found that procalcitonin levels of greater than 0.1 ng/mL had significantly increased odds of postoperative gram-negative sepsis. Studies have also found ratios of procalcitonin to albumin to be accurate identifiers of patients with urosepsis after endourological stone procedures.[80]

GENETICS

Genetic analysis may provide insight into the multiple pathways that may be altered and ultimately increase the risk of urolithiasis. CD44 is a cell adhesion molecule that is known to play a role in various biologic processes and has been associated with cancer pathogenesis. Among its notable functions, it has been established as an important receptor for osteopontin. Qiao and colleagues[81] studied the possible role of CD44 rs13347 locus polymorphisms in Chinese patients with urolithiasis. The investigators identified 4 genotypes for this gene, cytosine cytosine (CC), cytosine thymine (CT), thymine thymine (TT), and cytosine thymine + thymine thymine(CT + TT). In their analysis, CT, TT, and CT + TT had increased odds of urolithiasis compared with CC (OR 1.98, OR 2.69, and OR 2.21, respectively). Further stratified analysis revealed CT and TT to demonstrate an increased risk of stone recurrence. They also concluded markedly increased risk of urolithiasis in male populations. Jabalameli and colleagues[82] analyzed SLC25A25 variants in European, theorizing that mutations in this transporter altered mitochondrial ATP production and renal solute transport.

Liu and colleagues[83] analyzed subsets of patients with urolithiasis to determine if their genetics played a role as a predisposition to renal damage from stone disease. They analyzed various genes in patients relative to the quantification of several markers, such as NAG. Their analysis concluded that rs4880 and rs5746135 of manganese superoxide dismutase could increase susceptibility to renal damage in patients with urolithiasis. Similarly, Mehdi and colleagues[84] analyzed polymorphisms in human transcription factor 7-like 2, β-defensin, and CD14 as possible links between these genes and urolithiasis. Further studies by Liang and colleagues[85] support the possible polygenic causes of urolithiasis, as they found alterations in the expression of 9 microRNAs, 883 mRNAs, and 1002 lncRNAs in patients with calcium oxalate urolithiasis.

Current evidence suggests there are a variety of involved genes and pathways in urolithiasis; however, a study by Halbritter and colleagues[86] determined that 14 monogenic genes were responsible for 15% of diagnosed patients with nephrolithiasis. Although germline genetic analysis is not truly a biomarker, per se, and is beyond the scope of this review, current evidence suggests a possible future role in the identification and screening of stone formers.[86]

Gastrointestinal and Urinary Microbiota

The human intestinal and urinary tracts are the hosts of complex, dynamic symbiosis with microbial organisms. In particular, gut microbiota have been extensively linked to metabolic diseases, autoimmune diseases, and urolithiasis. Some of these organisms, such as *Oxalobacter formigenes* play an important role in the downstream prevention of stones by regulating intestinal absorption of oxalate through degradation.[87] Although deficiency of this bacterial population was thought to increase the risk of stones, further studies found normal populations in stone formers.[88–91] However, clinical studies have suggested that *O formigenes* colonization can significantly reduce the risk of calcium oxalate stone recurrence[88]; this is probably due to the complex interplay of various pathways related to stone formation.

As the human intestinal tract hosts more than thousands of species of bacteria, scientists have studied the balance and proportions of these bacteria and their possible impact on measured outcomes. Decreased microbial diversity and alterations of specific bacterial populations have been identified in patients with urolithiasis by various investigators.[92–95] Among them, *Lachnospiraceae, Ruminiclostridium*, *Dorea*, *Christensenellaceae*, and *Enterobacter* have been found to be reduced, whereas *Bacteroides*, *Bifidobacterium*, and *Faecalibacterium* were found to be increased.[95] Studies attempting to determine the accuracy of gut bacteria in identifying patients with nephrolithiasis have returned mixed results.[92] However, Tang and colleagues[95] concluded *Escherichia coli* and *Pseudomonas aeruginosa* could identify patients with urolithiasis correctly with statistical significance.

Chen and colleagues[96] performed a similar study by analyzing the gut flora of patients with calcium oxalate urolithiasis and compared them with healthy controls. The investigators determined that the urolithiasis groups had decreased populations of *Firmicutes*, *Verrucomicrobia*, *Akkermansia* spp, *Faecalibacterium* spp, and *Lactobacillus* spp while having higher populations of *Bacteroidetes* and *Phascolarctobacterium*. Through Spearman correlation analysis, they determined that renal calculi had a negative correlation with *Akkermansia* spp, *Faecalibacterium* spp, *Streptococcus* spp, and *Lactobacillus* spp, whereas *Phascolarctobacterium* spp, *Blautia* spp, *Lachnospiraceae*, and *Bacteroides* spp were positively correlated with urolithiasis. Ultimately, the investigators concluded that *Lactobacillus* spp contributed the most (76%) to reducing the risk of kidney stone disease. These 5 bacteria had an AUC of 0.871 and 95% confidence interval (CI) (0.785–0.957) for predicting patients with calcium oxalate kidney stone disease.

Additional analysis of short-chain fatty acid contents determined that urolithiasis was correlated with valeric acid positively and with propionic acid, acetic acid, and butyric acid negatively. These short-chain amino acids were associated with various bacteria such as the aforementioned *Roseburia* and *Megamonas.* Lastly, the investigators concluded that these variations in microbiota can be related to tea consumption and thus may represent an opportunity for effective intervention at normalizing gut microbiota.[96]

Cao and colleagues[97] analyzed the gut microbiomes of uric acid stone formers with and without gout and compared them with normal controls. They identified increased colonization of *Bacteroides* and *Fusobacterium* in patients with uric acid stones, identifying a correlation between the population of this bacteria and serum uric acid levels. These bacteria have been found to be proinflammatory.

SUMMARY

Biomarker research in urolithiasis represents an array of potential applications ranging from diagnosis and detection, assessment of risk of stone development, kidney injury, ureteral obstruction, postsurgical alterations, infection, and stone passage. Even with such potential utilities, biomarkers currently face limited adoption and use stemming from an incomplete understanding of their application in these settings and require further studies to better define their role in the evaluation and treatment of patients with kidney stone disease.

CLINICS CARE POINTS

- Biomarkers are underutialized in the evaluation of stone patients.-Some biomarkers which may be useful are included in the standard evaluation of stone patients while others are not.
- The future utilization of biomarkers may help in understanding various aspects of stone disease including risk of stone formation and recurrence, risk of infection related to stones, and risks of surgical procedures.

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