Medical Research Archives

European Society of Medicine

OPEN ACCESS

Published: September 30, 2023

Citation: Robertson, J., et al., 2023. Profiling renal dysfunction using Raman chemometric urinalysis, with special reference to COVID19, lupus nephritis, and diabetic nephropathy. Medical Research Archives, [online] 11(9). https://doi.org/10.18103/mra.v11i9.4384

DOI: https://doi.org/10.18103/mra.v11i9.4384

ISSN: 2375-1924

RESEARCH ARTICLE

Profiling renal dysfunction using Raman chemometric urinalysis, with special reference to COVID19, lupus nephritis, and diabetic nephropathy

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ABSTRACT

Background: Many systemic and urinary tract diseases alter renal structure and function, including changing the composition of urine. While routine urinalysis (physical properties, sediment evaluation, urine chemistry analytes) is useful in screening, it has limitations on separating disease processes, structural changes, and functional abnormalities. Likewise, while many individual 'biomarkers' have been used to screen for disease, they have not met with widespread clinical adoption. The recent COVID19 Pandemic and the recognition of post-acute sequelae SARS-CoV-2 infection (PASC) have highlighted the need for rapid, scalable, economical, and accurate screening tools for managing disease.

Aims: Validate a Raman spectroscopy-based screening technology for urine analysis that could be used for recognition and quantification of systemic and renal effects of acute and PASC COVID19 disease.

Methods: One hundred ten (110) urine specimens were obtained from consented adults diagnosed with COVID19 disease by RT-PCR and/or proximate (household) contact With RT-PCR-confirmed COVID19 disease. Samples were analyzed using Raman chemometric urinalysis, a technology that detects hundreds of discrete chemicals in urine and applies computational comparison-machine learning to detect COVID19-associated molecular patterns ('fingerprints').

Results: When compared with the urine multimolecular 'fingerprints' of healthy individuals and patients with known systemic diseases (diabetes mellitus, lupus) that alter renal structure and function, patients with acute and PASC COVID19 had unique 'fingerprints' indicative of alterations in renal function (i.e. – infection altered urine composition). Differences in disease severity (mild to severe) were reflected by different 'fingerprints' in urine. Roughly 20% of hospitalized patients developed a degree of renal dysfunction (decrements in eGFR) that were correlated with distinct changes in urine fingerprints.

Conclusion: Raman chemometric urinalysis may be a useful tool in management of patients with COVID19 disease, particularly in detecting patients with evolving renal dysfunction for whom there should be attention to medication use and renal health restoration/preservation.

Introducution

Urinalysis is an important tool for disease detection and management. It is used routinely to screen specimens for analytes such as glucose and protein, that are associated with common diseases such as diabetes mellitus and kidney disease. The presence of abnormal cellularity or sediments likewise provides important diagnostic information for clinicians and their patients.

Many systemic and urinary tract diseases alter renal structure and function, including changing the composition of urine. While routine urinalysis (physical properties, sediment evaluation, urine chemistry analytes) is useful in screening, it has limitations on separating disease processes, structural changes, and functional abnormalities. Likewise, while many individual 'biomarkers' have been used to screen for disease, they have not met with widespread clinical adoption. The recent COVID19 Pandemic and the recognition of post-acute sequelae SARS-CoV-2 infection (PASC) have highlighted the need for rapid, scalable, economical, and accurate screening tools for managing disease.

Here, we briefly describe current practices and limitations of routine and 'molecular' urinalysis, and then introduce and describe in detail a novel technology (Raman chemometric urinalysis - Rametrix®) that we have developed and applied to study COVID19 disease, lupus nephritis, and diabetic nephropathy. While it is recognized that the focus of this special edition of the Medical Research Archives is COVID19 disease, it was critical to place this disease process in a context of other systemic disease processes known to alter renal structure and function. As comparators, diabetic mellitus patient results were selected, as diabetes is one of the most common causes of degenerative renal remodeling and dysfunction, and lupus patients were selected as this population (lupus patients) are typically younger and do not typically have coincident renal remodeling and dysfunction associated with chronic nephropathies, such as those seen in hypertension. The specific aim of our work (and this paper is to describe the rationale for use of, and validation of a novel Raman spectroscopy-based screening technology for urine analysis that could be used for rapid recognition and quantification of systemic and renal effects of acute and PASC COVID19 disease.

This was a prospective study. Based on previous studies in our laboratory, we surmised that Raman chemometric urinalysis may be a useful tool in management of patients with COVID19 disease, particularly in detecting patients with evolving renal dysfunction for whom there should be attention to medication use and renal health restoration/preservation.

Routine urinalysis

Urine is a complex fluid. It is the product of systemic physiologic and pathologic processes, metabolism, and renal function. Urine is a constantly-forming, readily-accessible 'liquid biopsy' of the genitourinary tract and more generally of the whole body. Studies have shown that normal human urine contains more than 2,000 separate chemical entities, many of them present in extremely small amounts that can only be detected with sophisticated, laboratory-based methods (see below)[1].

The composition and physical properties of urine in healthy people varies widely each day. For example, constantly-occurring changes in urine volume and urea content are predictably related to many physiologic/metabolic factors including the state of hydration, water intake, physical activity, and diet. We understand that urine composition is dynamic and constantly changing and that in healthy people, variations are 'normal'. For example, the presence of hemoglobin/erythrocytes in free-catch urine specimens in menstruating females and flora/fauna from the lower urinary tract of both men and women is recognized and accepted as part of 'normal' urine composition.

As noted, it has been recognized for millennia that systemic and genitourinary tract diseases frequently change the volume, physical properties (e.g., pH, specific gravity, conductivity, color, turbidity, viscosity), suspended sediments (e.g., cells, crystals, formed aggregates, sloughed/degenerate mucoproteins), and chemical components of urine. These changes can be detected by routine urinalysis which includes assessment of the physical properties, sediments, cytology, and chemical composition (usually with a point-of-care dipstick 'dry chemistry' analysis[2,3]).

Molecular urinalysis

Over the past decade, 'molecular urinalysis', urine metabolomics, and urine biomarkers for disease detection have developed and been used in clinical research practice. There has been considerable interest in the use of mass spectrometry, liquid/gas chromatography, nuclear magnetic resonance, and kinetic nephelometry methods, for detection of changes in urine analytes associated both with both normal physiology and metabolism and with disease states. These high technology approaches have had very limited clinical translation.

One example of extensive study and application has been in detection and management of diabetes mellitus, with some technological success – but limitations for use in patient care. Magalhaes and co-workers[4] using 'molecular urinalysis (capillary electrophoresis, coupled to electrospray ionization time-of-flight mass spectrometry (CE-MS)) to identify polypeptide patterns in urine of patients with kidney disease and diabetes. Patterns identified could not, however, be correlated with specific molecules (biomarkers) of physiological and pathophysiological significance.

Work by Darshi and co-workers[5], used molecular urinalysis to identify diabetic kidney disease (DKD)-related metabolomic alterations in oxidative respiration, altered mitochondrial function, amino acid utilization, and fatty acid oxidation processes, using mass spectrometry. However, their observations could not be readily applied to patient management (as they acknowledged) given the diverse spectrum of diabetes mellitus, chronic kidney disease, associated co-morbidities, lack of correlation to standard metrics (estimated glomerular filtration rate - eGFR, serum creatinine, albuminuria), limitations of generalizing "spot" sampling to ongoing, progressive disease trends, and patient demographics (age, sex, racial identity, duration of disease).

Another example (bladder cancer diagnostics) highlights both the progress made in

molecular urinalysis and the inherent limitations of high-technology approaches to diagnostics. While a substantial number of urine-based molecular biomarker tests have been developed for diagnosing bladder cancer, none of them have found wide-acceptance, due to their relatively low effectiveness (sensitivity/specificity). The American Urological Association (AUA) stated that clinicians should not rely on urine-based tumor markers for the initial evaluation of patients with microhematuria, essentially discounting the role of existing tumor markers in screening of specimen for bladder cancer[6,7]. A review of the literature on urine metabolomics for bladder cancer detection by Petrella and colleagues[8] suggested that while urine metabolomics may be a useful research tool for disease detection, results among various studies were not consistent. Progress made in the research environment has not translated to improved bladder cancer detection or patient management practices (reduction in invasive cystoscopies, for example). In practice, while many tests have no problem differentiating between healthy and bladder cancer urine specimens, they underperform when differentiating between bladder cancer and other genitourinary tract problems (other malignancies, coincident inflammatory disease) or tumor grade/stage. These assays have been criticized for low sensitivity/specificity and questionable predictive value. None of the available tests can be practically scaled for mass screening.

For these and other reasons, molecular urinalysis/urine biomarker and "-omics" technologies are rarely used for routine patient care. The complexities of many systemic and genitourinary tract diseases, and collection of large datasets for validation of technology-intensive methods (like mass spectrometry), makes their use unlikely and cost-prohibitive. The expense of purchasing and maintaining laboratory-based advanced technology (such as mass spectrometry), expertise required for interpretation of results, lack of assay validation with large datasets of normal and abnormal specimens, and expense have, and will continue to, limit clinical use.

Raman chemometric urinalysis - Rametrix®

We recognized the limitations and challenges of associated with mass spectroscopic/chromatographic/biomarker-based technologies. To address these, we invented and extensively validated a Raman spectroscopy-based technology called Raman chemometric urinalysis (Rametrix®) for molecular urinalysis. Our Rametrix® approach has these advantages: (i) specimen analysis is rapid (<15 seconds per sample), (ii) it is inexpensive and non-destructive to the specimen, (iii) it requires no chemical labeling or physical pre-processing, (iv) it is mobile and can be performed at the point-of-care (POC), (v) analysis requires little training, (vi) data analysis is automated with Rametrix® software, (vii) specimen quality is maintained up to 8 hours at room temperature, 24 hours refrigerated, and months at -30°C prior to analysis; and (viii) freeze/thaw cycles and prolonged storage (months at <-30°C) does not adulterate the Raman signal from urine[9], and (ix) analysis has been automated for mass screening.

Raman spectroscopy is a mature, well-studied, and powerful technology that has

been applied to analysis of the chemical composition of a wide variety of solids and liquids, including biological specimens[10-16]. Irradiation of molecular mixtures (like urine), with wavelength-specific laser energy, produces weak vibrational energy (Raman scatter radiation) from deformation/relaxation of the many chemical bonds in hundreds of distinct molecules in the specimens. Different molecular constituents are visualized by 'bands' (i.e., signal intensity peaks and valleys) present in Raman spectra that are indicative of the chemical bond vibrations. These vibrations may be present in several molecules in a sample (for example, molecules with C-C, C-H, C-N and/or N-H bonds such as amino acids), meaning it can be difficult to assign individual Raman bands to specific molecules, unless they are present in abundance. This is the case for urea in urine, for example, where the C-N bond stretch at 1,004 cm-1 is dominant and can be associated with urea concentration. Using comparisons of unknown specimens with spectra (of analytical chemical samples) in reference libraries of Raman spectra, we have identified creatinine, heme, amino acids, albumin, collagen, DNA/RNA degradation products, and phospholipids in urine (to name just a few of many molecules studied). A few of these and other broad molecular assignments are shown in Raman spectra of urine from healthy volunteers[17], chronic kidney disease (CKD) 4-5 patients[18], and Surine™ urinalysis control (Dyna-Tek Industries, Lenexa, KS) in **Figure 1 A-D**.

Because it is difficult to relate individual Raman bands to specific molecules, a chemometric and computational approach is required to analyze Raman spectra of highly complex heterogenous samples, such as urine. The chemometric approach is unlike chromatographic and mass spectrometry approaches that resolve single molecules. The chemometric approach treats an entire Raman spectrum as a 'fingerprint' and then associates it with a condition (i.e., 'healthy' or end stage renal disease [ESRD], for example) using statistical models and artificial intelligence. Building an accurate model to predict the condition of an unknown sample requires a large dataset of pre-analyzed Raman spectra.

How exactly is this done? As noted previously, a Raman spectrum of human urine contains information about its metabolome, which is composed of more than 2,600 different molecules [1]. Examples of urine Raman spectra obtained from a healthy volunteer[17] and a patient receiving peritoneal dialysis for end-stage renal disease (ESRD)[18] are given in **Figure 1A**. Each of these spectra are comprised of two main parts (specified in **Figure 1A**): (i) the Raman signal and (ii) the fluorescent background[10-16]. The urine metabolome information is contained in the Raman signal, so this is separated from the non-molecule-specific fluorescent background by the process of signal baselining. To do this, first the wavenumber region containing biological information (i.e., the "fingerprint region") is extracted. While several fingerprint regions are used, the wavenumber (i.e., Raman shift) region common for biological/biomedical studies is 400-1,800 cm^{-1} [19]. Next, a spline is fit through the spectral data to subtract the Raman signal from the fluorescent background. These splines can be based on multi-order polynomials, combinations of polynomials, or other algorithms. Several different baselining algorithms are used for this currently and have been reviewed[20, 21].

Three that we have used in our studies are Savitzky-Golay[22], Goldindec[23], and ISREA[24]. Of these, we believe ISREA is most adaptable for disease detection.

An example of ISREA baselining is shown in **Figure 1B**. Multiple nodes (or knots) are aligned on a spectrum and accommodate a moving cubic spline. ISREA gives two advantages: (i) the Raman signal that falls below the baseline is set to zero and (ii) the nodes are movable along the spectrum. This provides several advantages. First, it provides a way of eliminating certain Raman signal data. Why would we ever want to do this? The Raman signal is composed of contributions from the 2,600+ urine metabolome molecules. We may ask: Are all of these associated with a disease to be detected (e.g., ESRD)? The answer is no. However, much of the urine metabolome is impacted by ESRD[18] and other diseases. Thus, the goal is to eliminate the superfluous Raman signal information and retain the signal carrying the spectral fingerprint for ESRD. When this is done, more accurate predictions of uncharacterized urine specimens can be made using models built with fewer samples. This is shown in **Figures 1C-D.** The Savitzky-Golay algorithm was used in **Figure 1C** to baseline the spectra shown in **Figure 1A.** Even though the spectra from healthy and ESRD patients look different, all information is retained, making the fingerprint for ESRD more complex. Compare this to the ISREA baselined spectra in **Figure 1D**. With superfluous information removed, the ESRD fingerprint (the major differences between the spectra) are more apparent and simpler.

Finally, another major advantage of ISREA is its nodes are movable. This is how we can control which sections of the urine Raman spectra are eliminated/retained during the ISREA baselining procedure. How do we know we have chosen the right set and placement of ISREA nodes? This is done with two methods: (i) trial-and-error with cross-validation and (ii) validation of retained fingerprint bands with available literature. In the trial-and-error with cross-validation procedure, we begin by choosing and implementing a set of ISREA nodes. These nodes apply to every spectrum in our dataset (e.g., a database of healthy and ESRD urine spectra). Then, we apply Rametrix®[19, 25] to build a model capable of recognizing the disease fingerprint in urine spectra. The model is then cross-validated with leave-one-out and/or k-fold methods. Here, one or multiple spectra are left-out of the model-building process. The left-out spectra are then treated as unknown(s) and their identity predicted (i.e., healthy or ESRD) by the model based on their spectra only. This procedure is repeated until every spectrum has been left-out of the model building process. The predictions for all left-out samples are compared with their actual classifications and are used to generate prediction accuracy, sensitivity, specificity, positive-predictive value (PPV), and negative-predictive value (NPV)[26].

In designing ISREA nodes, we do so with the goal of maximizing these prediction metrics. However, the optimum ISREA nodes must also give rise to a spectral fingerprint of disease that has medical evidence from prior molecular analyses. For example, the spectral fingerprint related to bladder cancer would be expected to contain Raman bands associated with collagen and collagen degradation products due to disruption, by tumor growth,

of bladder integrity. This was, indeed, found to be the case[27]. In another example, a chromatography-based urine metabolomics study found a disproportionate level of tryptophan metabolism compounds in the urine of patients with Lyme disease[28]. We validated our ISREA spectral fingerprint for Lyme disease detection in urine by discovering that several of the bands in the fingerprint were related to tryptophan its break-down products[29].

As databases of Raman spectra of biological molecules improve in breadth and availability, our ability to cross-reference more regions of the spectral fingerprint and confirm with mined literature data will improve. Of course, the procedure of spectral fingerprint reduction with ISREA also offers the opportunity to identify new molecular contributors and urine metabolome differences due to the presence of a disease. These new metabolomic markers can be verified through other analytical means to further validate the fingerprint.

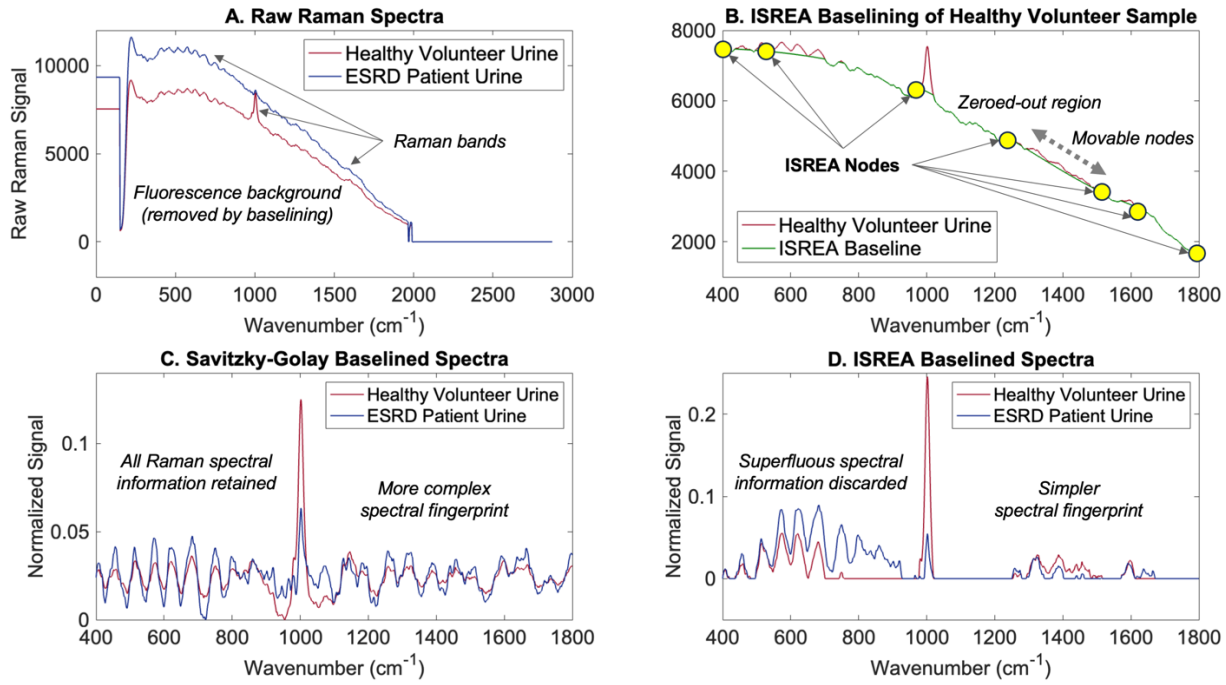

Figure 1. A-D. Urine Raman spectra of a healthy volunteer and an ESRD patient. (A) One sample unprocessed Raman spectrum obtained from each group. (B) ISREA baselining of the ESRD patient sample spectrum. (C) The Raman spectra from [A] when baselined by the Savitzky-Golay algorithm. (D) The Raman spectra from [A] when baselined by ISREA with nodes at 400, 496, 977, 1292, 1570 1613, and 1800 cm[1].

Clinical applications of Raman chemometric urinalysis – COVID19 disease, lupus nephritis, and diabetic nephropathy

COVID19 DISEASE

Nasopharyngeal swab collection/real-time polymerase chain reaction (RT-PCR) - based testing has been a fundamental tool for disease detection, containment, and assessment of public health measure efficacy – worldwide. Swab-based testing, combined with other clinical evaluations, readily detected SARS-CoV-2 infection and subsequent COVID19 disease in most people, especially in acutely-ill, symptomatic patients. However, swab-based testing and/or measurement of convalescent viral neutralizing antibodies, were not useful for detecting multiorgan systemic complications, determining severity of the disease, differentiating viral variants, or recognizing pathognomonic features of 'non-respiratory' clinical scenarios (e.g., multisystem inflammatory syndrome in children [MIS-C] [30] and post-acute sequelae of SARS-CoV-2 [PASC - Long COVID])[31].

Renal dysfunction, including acute kidney injury (AKI), is a common complication (27–50+% of hospitalized patients) of COVID19 disease[32-36]. Studies have documented a correlation between disease severity, hospitalization, and intensive care admissions as risk factors for developing AKI[37, 38]. Carlson and co-workers[39] reported an increased susceptibility to COVID19 disease in patients with chronic kidney disease, but did not specifically document deterioration of renal function after infection in their patient cohort. Hassler[40]. proposed, based on the literature, that direct viral infection of the kidney might help explain the disproportionately high incidence of AKI and collapsing glomerulopathy seen in patients with COVID19. However, they also acknowledged that prerenal azotemia and drug-associated nephrotoxicity were possible contributors to commonly observed renal dysfunction. Hassler, et. al., concluded that renal biopsies (not autopsy-derived samples) and development of urine-based screening tests would be keys to understanding the effects of COVID19 on renal function and structure, and these would be needed to improve detection and management of disease.

Based on our previous studies of several diseases[18, 25, 27, 29], including chronic kidney disease, we hypothesized that systemic inflammatory/immune responses, altered metabolism, and tissue damage associated with COVID19 disease, on multiple bodily systems including the kidneys, would alter the composition of urine. In early 2020, we began to collect urine specimens from RT-PCR-confirmed COVID19 outpatients in our community, as well as from symptomatic proximate (household) contacts. Collection of urine from forty-six (46) consented patients occurred over an 18-month period. We applied Raman molecular analysis techniques (described above) to these samples and then compared the urine spectral molecular fingerprints from these COVID19 patients, with urine spectra from 185 'healthy' individuals (college students with no evidence of renal or systemic disease), 20 patients with Stage 5 CKD, and 17 patients with active bladder cancer. Of note - all 'healthy', CKD, and bladder cancer patient urine samples were collected and analyzed from 2016-2018 (i.e., pre-COVID). The results of this study were published[41]. See **Figure 2**.

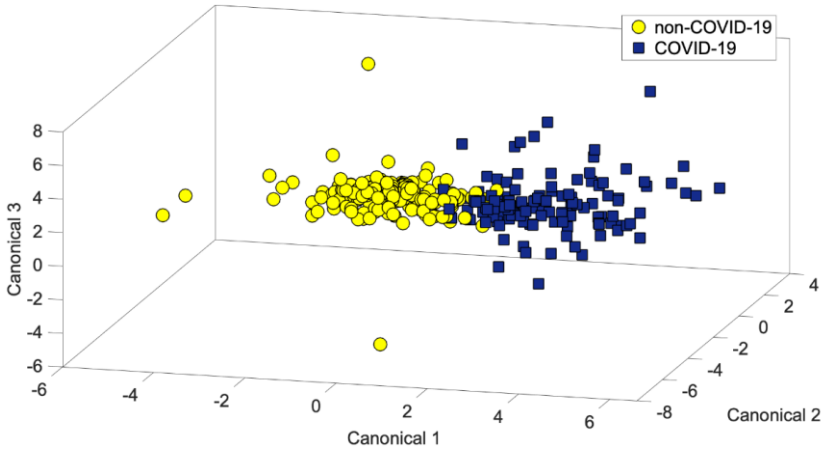

Figure 2. MANOVA clustering of COVID-19 and non-COVID-19 groups using Savitzky-Golay baselining. The COVID-19 group contained urine Raman spectra from both COVID-19 hospitalized and COVID-19 clinic assessed patients. The non-COVID-19 group contained urine Raman spectra from healthy volunteers, ESRD patients, BCa patients, and Lyme disease patients.

This preliminary, community-based study, characterized by very mild to moderately severe respiratory disease, with patients seen in outpatient/private practice settings showed that 1) changes in molecular composition could be easily and quicky (<15 minutes) detected in voided, unprocessed urine specimens with Raman chemometric urinalysis, 2) as we hypothesized, COVID19 disease changed the molecular composition of urine, when compared to the composition of urine from healthy controls, and analyzed with Raman chemometric urinalysis, and 3) in some patients, changes in urine composition in patients with COVID19 disease persisted weeks to months after recovery. Seven patients had evidence of decreased renal function (elevated serum creatinine, eGFR <90) but we could not determine if antecedent renal disease was present or contributed to serum or urine abnormalities in these patients. We also could not correlate Raman molecular fingerprints with clinical severity, as most cases were clinically mild, resembling one another.

Subsequently (2022-2023), we conducted a more robust study involving 64 hospitalized patients suffering acutely from moderately severe to severe COVID19 disease [42]. The results of this study are currently being analyzed, but several preliminary conclusions have already been drawn from analysis of the data. First (and as before), we clearly demonstrated that Raman chemometric urinalysis can detect changes in urine composition associated with COVID19 disease. Second, the molecular composition ('fingerprints') of urine specimens from this hospital cohort study, analyzed with Raman chemometric urinalysis, differed significantly from those of healthy humans (collected and analyzed 206-2018). Third, the molecular composition of urine specimens from the hospital cohort study differed from the molecular composition of urine specimens of the previously reported community cohort study (Robertson, et. al., 2022). See **Figure 3**.

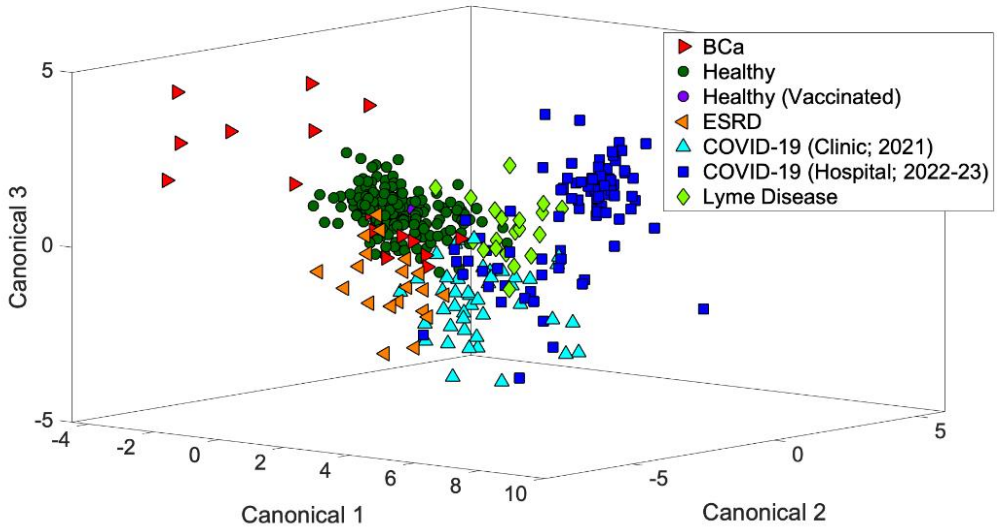

Figure 3. MANOVA clustering of independent groups given ISREA baselining with Surine nodes.

Several factors may account for these observed differences, including the prevalence of viral strains in 2020-2021 (Alpha-Gamma) or 2021-2022 (Delta-Omicron) and differences in case severity (community cohort cases relatively mild vs hospital cohort cases moderately to markedly severe). Fourth, Raman chemometric urinalysis could detect differences in urine composition related to disease severity (i.e., the 'molecular fingerprints' of moderate and severe cases were different). As noted, more thorough analysis of the hospital cohort patient data is occurring - in preparation for publication - with particular attention to whether or not the hospital cohort patients would develop significant renal dysfunction that could be predicted from a single voided sample collected on admission.

LUPUS NEPHRITIS

Systemic lupus erythematosus, a pan-systemic inflammatory disease, can have a deleterious effect on renal function. Timely detection and management of renal dysfunction is highly desirable, since many patients develop disease at an early age and inflammatory nephropathies can be relentlessly progressive. The clinical diagnosis of lupus nephritis (LN) is made with a combination of serum analyte measurement (elevated creatinine, decreased estimated glomerular filtration rate – eGFR), detection of proteinuria and hematuria, and definitively with renal biopsy. Palazzo and co-authors[43] reviewed recent studies on the utility of serum/ plasma and urine biomarkers as adjunctive aids to diagnosis. Information gleaned from 104 studies reviewed indicated several biomarkers in urine accurately differentiated LN from other nephropathies, including neutrophil gelatinase-associated lipocalin (NGAL), a variety of microparticles, cytokines, and adhesion molecules [44-47]. From their review of the literature, they concluded that while the detection of biomarkers in urine had promise, the heterogeneity of LN and SLE – patient-to-patient, the different methodologies used in studies reviewed, and lack of correlation with renal biopsies (not all patients in all studies

had biopsy-confirmed LN), there was no biomarker or combination of biomarkers that adequately profiled LN.

As with our studies of CKD and COVID19, we hypothesized that SLE would alter urine composition and that Rametrix® analysis could detect renal dysfunction via urine molecular 'fingerprinting'.

We applied Rametrix® analysis on 587 urine specimens collected from 82 patients with biopsy-proven (80/82) and/or laboratory-validated SLE markers. Patients were 8-21 years of age (median age 14.5) and 77.5% female. Most patients were African-American (183/587), Latino (162/587) or Caucasian (129/587). Serial longitudinal urine samples were obtained on multiple individuals. A renal SLEDAI-2K score was correlated to Rametrix® findings. Using chemometric analysis of urine Raman spectra, we compared SLE urine spectra with urine spectra from urine of healthy controls (n=203), patients with CKD (n=20), COVID19 patients (n=118), bladder cancer patients (n=19) and chronic Lyme disease patients (n=20).

The results of this study are currently being analyzed, but several preliminary conclusions have already been drawn from analysis of the data [48]. First, Rametrix® molecular urinalysis distinguished SLE-associated changes in urine composition with predictive metrics (accuracy, sensitivity, specificity, PPV, and NPV) ranging between 73-97%, when urine Raman spectra from SLE patients were compared with 'healthy' controls and patients with other diseases. See **Figure 4.**

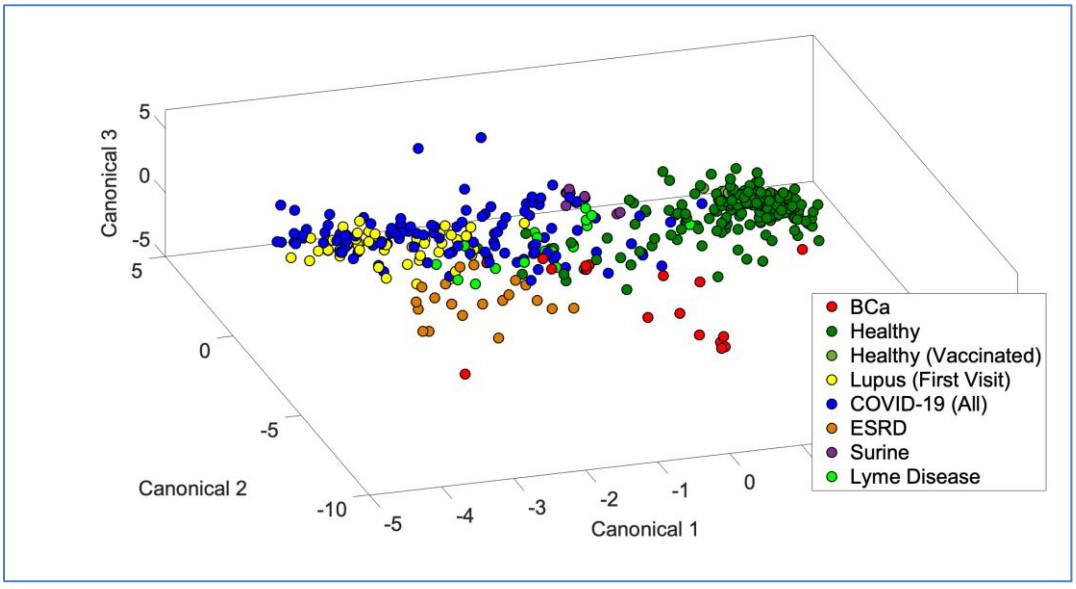

Figure 4. A comparison of urine samples from patients with biopsy-confirmed lupus nephritis, healthy volunteers, and patients with other diseases (COVID19, Lyme disease, ESRD).

Second, a weak correlation between changes in urine Raman spectra and physician assessment of disease (SLEDAI-2K) was found through computational analysis and comparison. Third, urine spectra from SLE and COVID19 patients showed notable Raman spectral similarities, suggesting common inflammatory pathways (interferonopathies).

Based on our preliminary (and ongoing) study of this large analytical and clinical dataset, we believe Raman chemometric urinalysis can be useful to detect and manage SLE and renal dysfunction, possibly reducing the need and number of renal biopsies for definitive diagnosis of LN.

DIABETIC NEPHROPATHY

In patients with diabetes mellitus (DM), it can be difficult to differentiate diabetic kidney disease (DKD) from other causes of glomerular damage. While it is common in clinical practice, it should not be assumed that all DM patients with decreased eGFR and/or proteinuria have DKD. As with lupus nephritis, renal biopsy is the standard for definitive diagnosis, although it is used infrequently in patients with DM due to concerns over potential biopsy complications (i.e., bleeding)[49]. Other less invasive methods may provide clinical benefit. We hypothesized that Raman chemometric urinalysis of DM patient urine, with statistical and chemometric modeling, will detect DKD-associated changes in in urine composition when compared to the composition of urine from healthy individuals, patients with other diseases, and with urine analytical control solution. In order to test our hypothesis, 263 urine samples were collected from renal biopsied and non-biopsied patients presenting with CKD secondary to DM and non-diabetic kidney disease. Samples were analyzed by Raman spectroscopy, detailed above. The results of this study were published previously [50].

We showed that Raman chemometric urinalysis was a useful tool in screening urine for the presence of molecular fingerprints indicative of diabetic kidney disease. Our analysis showed 1) urine samples of DKD patients and those with immune-mediated nephropathy (IMN) could be distinguished from one another with 82% sensitivity, specificity, positive-predictive value (PPV), and negative-predictive value (NPV), 2) among urine samples from all biopsied CKD patients, renal neoplasia was identified in urine with 100% sensitivity, specificity, PPV, and NPV, and membranous nephropathy was identified with 66.7% sensitivity, 96.4% specificity, 80.0% PPV, and 93.1% NPV, and 3) DKD was identified among a population of 150 patient urine samples containing biopsy-confirmed DKD, other biopsy-confirmed glomerular pathologies, un-biopsied non-diabetic CKD patients (no DKD), healthy volunteers, and Surine™ urine analytical control with 36.4% sensitivity, 97.8% specificity, 57.1% PPV, and 95.1% NPV. This computational model was used to screen the Raman urine spectra of un-biopsied diabetic CKD patients and identified DKD in more than 8% of this population.

IMN in diabetic patients was identified among a similarly sized and diverse population with 83.3% sensitivity, 97.7% specificity, 62.5% PPV, and 99.2% NPV. Finally, IMN in nondiabetic patients was identified with 50.0% sensitivity, 99.4% specificity, 75.0% PPV, and 98.3% NPV.

We concluded that Raman chemometric urinalysis may be able to differentiate between DKD, IMN, and other glomerular diseases, possibly reducing the need and number of renal biopsies for definitive diagnosis of DN. Future work will further characterize CKD stages and glomerular pathology, while assessing and controlling for

differences in factors such as comorbidities, disease severity, and other lab parameters.

Summary

In summary, we have described a novel technology – Raman chemometric urinalysis (Rametrix®) – that enables evaluation of the molecular complexities of urine and the development of disease-associated molecular fingerprints. The technology is simple, economical, rapid, robust and validated.

Using this Raman spectroscopy-based platform technology, we have focused on several important clinical applications, including disease/renal profiling in patients with COVID19 disease, in a large cohort of patients with SLE, and in a population of patients with diabetes and complications of diabetic nephropathy. In each clinical application, comparisons were made with potentially coincident comorbidities (such as chronic kidney disease). It was fortuitous for us that we had conducted a large-scale analysis of urine specimens before the Pandemic – so that our comparator baseline controls were clearly not affected by the presence and evolution of COVID19 disease.

We fully understand that this technology is designed to be an aide to patient management, not a single, stand-alone test for 'making a diagnosis'. The ease of collection and analysis of specimens encourages repetitive sampling during treatment and may be a potential means to see if treatment is efficacious (i.e., Does the Raman urine spectral fingerprint of this patient change over time in response to treatment and does this correlate to clinical progress?). We feel the technology has 'proved its mettle' and we would strongly encourage collaboration and widespread use. We are especially interested to see if Rametrix® technology could provide an objective, quantifiable means to detect, profile, and manage Long COVID19 (PASC) and will continue to pursue this line of investigation.

Conflict of Interest Statement:

None

Disclosure:

John Robertson and Ryan Senger are co-inventors and have patented the Rametrix® technology described in this paper. They are co-founders of Rametrix Technologies, Inc. (https://rametrixtech.com) and are intending to commercialize this technology. Their interests in commercial development have not in any way influenced the information contained herein. Mr. Sayed Issa is an employee of Rametrix Technologies, Inc.

Funding Statement:

None

Acknowledgement Statement:

None

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