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Urinary Peptide Patterns in Native Kidneys and Kidney Allografts

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Abstract

Background—The use of biopsies to determine kidney health after kidney transplantation is an invasive procedure with some risk to the patient. Consequently, a noninvasive test for transplanted kidney health would provide a significant advantage over current clinical practice.

Methods—Urine from kidney donors before nephrectomy, pretransplant patients with native kidney disease, and posttransplant kidney recipients were examined for protein biomarkers to diagnose or prognose kidney disease. Proteins were extracted by C4 reverse phase affinity and analyzed by matrix assisted laser desorption/ionization time-of-flight mass spectrometry.

Results—Urine from individuals with healthy kidneys showed few components other than two ubiquitous saposin B glycoisoforms. Patients with kidney disease lacked saposin B and showed new components in two patterns: the most common contained β -2 microglobulin (B2M, m/z = 11,732) plus one or more peaks at m/z = 10,350, 9480, 4337, and 4180. Pattern 2 lacked β -2 microglobulin but contained several degradation products of β -1 antitrypsin. Other pathologic components included urinary protein 1 (m/z = 15,835), transthyretin (m/z = 13,880), and a component at m/z = 13,350.

Conclusions—Patients with acute rejection showed profiles that ranged from those of kidney donors to those of advanced kidney disease. The range of patterns may be useful for analysis of transplant patients without complications and persons with developing kidney disease before or after transplant.

Keywords

Proteomics; Protein profiles; Acute rejection; Kidney allografts; Biomarkers

Kidney allograft transplantation is an effective therapy for persons with end-stage kidney disease. Typically, the health of transplanted kidneys is monitored by serum creatinine (SC) levels and calculated glomerular filtration rates (GFR). A 25% increase in SC triggers further evaluation usually including percutaneous biopsy. Biopsy is an invasive procedure

with some risk to the patient. Consequently, a noninvasive test for transplanted kidney health would provide a significant advantage over current clinical practice. A common target for a noninvasive assay is urine with anomalous protein biomarkers as indicators of kidney health.

The study of the urine proteome for biomarkers of other conditions has been extensive with several recent reviews (1–5). Initial studies attempting to identify biomarkers associated with kidney health after kidney transplant have used surface enhanced laser desorption/ionization (SELDI) to generate a list of potential biomarkers of acute rejection (AR). One group (6, 7) reported 13 features of the SELDI spectrum that increased and three features that disappeared in patients with AR. Another group reported seven features that increased with AR (8). One component that increased with AR was identified as a degradation peptide of α -1-antichymotrypsin, whereas another that declined was β -1-defensin (8). Schaub et al. (9) found that fragments of β -2 microglobulin (B2M) were better biomarkers of rejection than the intact protein (10). None of these markers was as effective as protocol biopsy (11). Oetting et al. (12) used reverse phase extraction and matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry to identify B2M in urine of patients with AR. B2M was the only biomarker that was found in more than one study.

Other studies have examined biomarkers of acute kidney transplant rejection from peptides separated by capillary electrophoresis (13). This method has been used for urinary biomarkers for several other conditions (1–5).

In this study, we have used MALDI-TOF MS, a method with some similarity to SELDI but distinct advantages. SELDI is an automated, commercial method for biologic sample extraction and MALDI-TOF analysis. This technology has been the focus of considerable controversy. Examples include substantial negative feedback and failure to reproduce (14, 15) some truly outstanding reports of biomarker discovery in several disease states (16, 17). SELDI uses a two-dimensional surface that restricts sample volume to be extracted, and the automated application of matrix virtually eliminates the use of sinapinic acid, which is optimal for larger peptide and protein analysis. SELDI also uses an automated data analysis program that attempts to identify hundreds of “features” within the SELDI spectrum. In contrast, MALDI-TOF uses manual extraction by a column format that allows larger volume extraction, allows use of sinapinic acid matrix, uses versatile instrumentation that is more commonly available in research institutions, and enables one to direct analysis to the most intense and reliable peaks of the profile, thereby avoiding a tendency of SELDI software to overinterpret features that appear just above background in the spectrum.

Kidney loss arises primarily from the process of chronic graft deterioration associated with tubular atrophy and interstitial fibrosis (TA/IF). A major risk factor for TA/IF is AR, and a method to detect AR, especially subclinical AR, would have value for early intervention to protect the patient from TA/IF. This study used a mass spectrometry method to detect intense protein biomarkers in urine of three groups of individuals: kidney recipients with normal function, persons with native kidney disease, and recipients with AR. We have identified a protein profile associated with normal kidney function and structure. We have also identified proteins that are shared between transplanted patients with AR and those with native kidney disease. These protein biomarkers could help provide the basis for a noninvasive method to determine the health of kidney allografts.

MATERIALS AND METHODS

Urine Samples

All studies were conducted with informed consent of patients and approval of the Institutional Review Board of the University of Minnesota, Minneapolis, MN and the Mayo Clinic, Rochester, MN. Urine samples were processed by centrifugation (2100 *g*) to remove cells and other insoluble material, and the supernatant was frozen at -80° within 8 hr. Urine samples from six populations were studied (A–F). Population “A” consisted of 120 urine samples from approved kidney donors and collected before nephrectomy. Population “B” contained 129 urine samples from patients with native kidney disease who were being evaluated for kidney transplantation (72 males; 57 females; 35 aged 12–40 years; 94 aged 40–74 years; 52 diabetes; 11 polycystic kidney disease; 66 other underlying diseases). Population “C” contained 41 transplant recipients with well functioning kidneys and who had a protocol biopsy exhibiting no pathologic condition. Population “D” contained serial urine samples from six healthy volunteers providing three to six urine samples over time periods ranging from 3 months to 2 years (four males and two females aged 25–62 years). Population “E” contained individuals who were referred to the clinic because of elevated creatinine and had a biopsy performed on the same day ($n = 148$, creatinine 2.27 ± 0.90 ; GFR = 34.0 ± 13.4 ; 21 AR; 90 chronic rejection [including some with AR]; and 50 no rejection). To avoid protein changes associated with the immediate side effect of the surgery, only those samples from kidney transplant recipients that were acquired more than 60 days after the surgery were analyzed. These samples were used to correlate protein components with GFR, creatinine, and fibrosis. Population “F” was used to assess the relationship between urinary markers and progressive kidney disease. These samples were from individuals with elevated creatinine and were collected at least 280 days before the final clinical data report ($n = 159$; creatinine = 2.41 ± 1.28 ; GFR = 33.8 ± 12.5 ; biopsy closest to sample date indicated the following: AR = 43; chronic rejection = 69 [some with AR as well]; and no rejection = 76). Although some individuals were included in both of populations E and F, the overall contents of the two groups were not identical.

MALDI-TOF Mass Spectrometry Analysis

The procedure for analysis of urine was adapted from the method described for diluted blood plasma (18). Briefly, urine samples were thawed, and 0.1 mL was acidified to pH less than 3.0 by sequential additions (0.5 μ L) of 10% trifluoroacetic acid (TFA). A reverse phase C4 ZipTip (Millipore Corp., Billerica, MA) containing approximately 1 μ L of reverse phase column resin was activated by drawing and extruding two 10 μ L volumes of water:acetonitrile:TFA (50:50:0.1) and washed with two 10 μ L changes of water:TFA (100:0.1). The acidified urine was slowly drawn into and extruded from the tip for 2 min. The tip was washed seven times with 10 μ L changes of water:TFA (100:0.1) and peptides eluted by aspiration and extrusion of 1.6 μ L of water:acetonitrile:TFA (25:75:0.1, eight times). The eluted proteins (0.75 μ L) were applied to a MALDI target along with 0.75 μ L of 85% saturated sinapinic acid in water:acetonitrile:TFA (50:50:0.1). The solution was stirred with the pipette tip until crystallization was fully developed. The target was dried and analyzed in a Bruker Biflex III mass spectrometer at a laser attenuation of 39–46, depending on the age of the laser. Five hundred laser shots were collected from at least 10 locations on the target. The spectra were smoothed (15 units) and background was subtracted. Peaks were labeled at the time that the spectra were collected. A defined peak had an intensity of at least 100 counts over a background of approximately 50 counts.

Isolation and Identification of Peak $m/z = 9746$ as Saposin B

Urine (200 mL) from a healthy donor was collected and filtered with a 0.2 μ m vacuum filter (Corning, Lowell, MA). The urine was concentrated using 4×15 mL 5 kDa molecular

weight cutoff Amicon Ultra (Millipore, Billerica, MA) spin concentrators with serial additions. The retentate (2.5 mL) was collected and passed through a 0.2- μ m syringe filter (Pall Corp. Ann Arbor, MI). It was diluted to 15 mL with water and loaded on a MonoQ 5 cm $\times$ 5 mm (Amersham, Uppsala, Sweden) anion exchange column at 1 mL/min with buffer A (25 mM Tris pH 7.35, 50 mM NaCl, 0.02% NaN₃). The column was washed with buffer A for 30 min and was then eluted by a gradient of buffer B (25 mM Tris pH 7.35, 1 M NaCl, 0.02% NaN₃) to 25% over 25 min. Fractions (1 mL) were collected and analyzed by absorbance at 280 nm and by mass spectrometry. For mass analysis, 100 μ L aliquots were acidified with small additions of 10% TFA and extracted using a C4 ZipTip (Millipore, Billerica, MA). The ZipTip eluate (0.75 μ L) was spotted on a MALDI target and mixed with an equal amount of saturated α -cyano-4-hydroxycinnamic acid (Sigma-Aldrich, St. Louis, MO) in water/acetonitrile/TFA (50/50/0.1). After the spots dried, they were subjected to a mass scan with a MALDI-QTOF mass spectrometer (Model: QstarXL, Applied Biosystems, Foster City, CA). Fractions eluting at 16 to 19 min contained a major MALDI-MS peak at 9746. These were pooled and concentrated with a 30 kDa molecular weight spin concentrator and clarified through a 0.2- μ m filter. The pooled sample was loaded on a Prosphere HP (300 Å pore size, 5 μ m bead size) 150 mm $\times$ 3 mm C4 column (W. R. Grace and Co., Columbia, MD) with 98/2/0.2 water/acetonitrile/formic acid at 0.5 mL/min. After a 5-min wash, the column was eluted with a linear gradient to 50% ACN with 0.2% formic acid (40 min) followed by 100% ACN with 0.2% formic acid over 10 min. The column effluent was split, 90% was collected as fractions of approximately 0.3 mL, and the remainder was analyzed by electrospray ionization-mass spectrometry using a Qtrap200 LC/MS/MS system (Applied Biosystems, Foster City, CA). A peak eluting at 45.5 to 46.1 min contained +8, +7, and +6 peaks corresponding to a compound with an average mass of 9746. Two fractions containing the m/z = 9746 component at high apparent purity were pooled and concentrated to approximately 40 μ L by vacuum centrifugation. The pH of the pooled solution was raised to neutrality by addition of ammonium bicarbonate to 50 mM. The sample was reduced with 10 mM dithiothreitol (DTT, Amersham) and incubated at 56°C for 1 hr. The cysteine residues were alkylated by addition of 20 mM iodoacetamide (Sigma, St. Louis, MO) followed by incubation at room temperature for 45 min in the dark. Excess iodoacetamide was neutralized by addition of 10 mM DTT. The sample was then digested with 0.2 μ g of sequencing grade trypsin (Promega, Madison, WI) at 37°C overnight. The next day, peptides generated by digestion were concentrated and purified with a C-18 ZipTip (Millipore, Billerica, MA) and were spotted on a MALDI target with α -cyano-4-hydroxycinnamic acid and analyzed in an Applied Biosystems Q-Star XL o-MALDI mass spectrometer. A prominent component at m/z = 2209 was observed. This peak was subjected to collision gas fragmentation and tandem MS/MS. Search of the fragment ions by the human NCBI database using Mascot software (Matrix Science, London, UK) found a high confidence match (score = 84 [scores >27 indicate significant homology]) to human saposin B residues 1 to 19.

Protein Digestion With Carboxypeptidase Y

For C-terminal amino acid hydrolysis, carboxypeptidase Y (Sigma Chemical Corp., St. Louis, MO) was added (final concentration of 16–25 μ g/mL) to urine samples that were buffered with 50 mM NH₄HCO₃ (pH 7.8–8.4) and then incubated at 37°C. At time points ranging from 0 and 40 min, 100 μ L aliquots were removed for MALDI-TOF analysis. In some cases, subsequent additions of carboxypeptidase Y (16–25 μ g/mL) were made and digestion continued for longer times. In either case, the aliquots that had been removed and acidified were immediately subjected to C-4 ZipTip reverse-phase adsorption and MALDI-TOF mass spectrometry analysis. The C-terminal amino acid sequence generated through this series digestion was subjected to BLAST database search to allow protein identification.

Statistical Analysis

The components of the MALDI-TOF profile were evaluated as present (+) or absent (−) from a profile. Correlations between a peak and clinical information were evaluated by Pearson uncorrected chi square test (JavaStat). Peak assignments were made by individual operators as the profiles were obtained without knowledge of clinical or laboratory information. Averaged MALDI-TOF profiles were obtained with ClinProTools (Bruker Daltonics Inc., Billerica, MA). This program was used to produce composite spectra. This program converts each spectrum to equal intensity, aligns the spectra, and averages the values from each spectrum, producing a single averaged spectrum.

RESULTS

MALDI-TOF Profiles and Their Reproducibility

Consistent methods of urine extraction produced a number of components with high intensity and reproducibility (Table 1). Reproducibility of profile components was examined by repeated assays after sample storage at -80°C . Twenty-five samples of kidney transplant patients with several additional peaks were first analyzed after one freeze-thaw cycle as the samples were collected. More than 1 year later, these samples were thawed and reanalyzed by a different staff member. A single profile was taken in each case. Ninety-three percent of the peaks were identified in both assays (data not shown). Furthermore, profiles were robust. Room temperature incubation for 24 hr did not result in appearance of pathologic markers in urine of healthy individuals or the complete disappearance of saposin B. It also did not result in loss of the pathologic markers given in the Table 1.

Profile Patterns of Individuals With Normal Kidney Function

The urine of healthy individuals with normal kidney function (pretransplant kidney donors; population A) showed two ubiquitous components at $m/z = 9073$ and 9746 (Fig. 1A). These masses have been associated with saposin B containing a HexNAc monosaccharide and saposin B with HexoseNAc₂Hexose₂deoxyHexose₁ (19, 20). The identity of the peak at $m/z = 9746$ was confirmed by isolation and mass spectrometry analysis, as described in the Materials and Methods section. Occasional but inconsistent peaks appeared below $m/z = 3000$ but were not used in any profile analyzed. Saposin B was evaluated by the ratio of peak intensities for the two major components ($m/z = 9746/9073$). Replication was excellent with an average coefficient of variation for repeated measurements of the same sample of 7% (Fig. 2, “replicate”). Serial samples from six healthy individuals (population D; Fig. 2, “serial”) gave consistent ratios over time for each person, a finding similar to protein isoform ratios found among plasma proteins (21). Each individual though had a personal ratio between the two isoforms. The range of peak ratios among healthy individuals was illustrated by the analysis of urine from 29 kidney donors (Fig. 2, “single”). The ratio between individuals ranged from approximately 0.2 to 0.6.

Individuals with kidney allografts who had biopsy-proven normal histology, by protocol biopsy, were also analyzed. The averaged profile of 41 individuals (population C) seemed similar to those from kidney donors (Fig. 1B). One difference was that the average intensity of the saposin B components was approximately half that of donors before surgery. This may reflect lower saposin B production from a single organ. Inspection of each profile showed that 44% of individuals showed at least one extraneous peak from the list of $m/z = 4303$, $10,350$, $10,840$, $11,732$, and $15,835$ (Table 1) that was at least 30% the intensity of saposin B ($m/z = 9746$). The average of each was insufficient to generate a detectable peak in the averaged spectrum. Although these components may not signify developing kidney allograft pathologic condition, their presence suggested frequent difference between the native normal kidney and the renal graft, even when the latter had normal histology.

Profile Patterns of Kidney Disease

Most (69%) profiles of individuals during evaluation for kidney transplant lacked a detectable peak for saposin B but did contain many new components (population B; Fig. 3A,B). Although the presence of new components was expected, the consistency of the components observed was surprising. The 69% of subjects without detectable $m/z = 9746$ had an average SC 6.57 ± 2.42 , whereas the 31% with detectable 9746 had an average SC of 4.05 ± 1.49 ($P = 5 \times 10^{-7}$, Student's t test). GFR was 15.6 ± 4.7 and 10.6 ± 5.0 ($P = 3.7 \times 10^{-5}$) for these, respectively. More than 90% of samples from persons with advanced kidney disease (129 individuals) were characterized by one of two peak patterns. The most common contained an intense peak at $m/z = 11,732$ (Fig. 3A, B2M, [12]) along with some combination of peaks at $m/z = 10,350, 9480, 4337, 4180$, and 3595 (Table 1). Pattern 2 lacked B2M (Fig. 3B) but contained intense peaks arising from fragments of a single region of α -1 antitrypsin ($m/z = 5324, 5007, 4860$, and 4272 , Table 1).

Correlation of MALDI-TOF Components With Clinical Data

Averaged MALDI-TOF profiles of transplant recipients with elevated SC (population E) showed virtually all of the components found in persons with advanced native kidney disease (Fig. 4). Not all samples contained every peak. Comparison of the presence or absence of individual MALDI-TOF components with clinical results showed a strong correlation of components at $m/z = 4180, 4337, 9480$, and $13,880$ with GFR and with fibrosis (Table 1).

Prognostic Value for Kidney Failure

Although the study was of short duration, a preliminary examination of the relationship between the MALDI-TOF profiles and kidney allograft survival was determined. For this analysis, samples used were at least 60 days after surgery and at least 280 days before the end of the study (population F; $n = 159$). These MALDI-TOF profiles were from transplant recipients with elevated creatinine. In the subsequent 280 days, 26 of the 159 experienced kidney failure. Kidney failure was significantly related with the urine peptides associated with kidney disease (Table 2). In addition, the presence of a "normal profile" containing saposin B was associated with a good kidney health. Use of the optimum cutpoint for SC (2.3 mg/dL, Table 2) gave similar prognostic value over this short time period. Further studies are needed to determine the value of peak components for longer-term prognosis.

Identification of Profile Components

The proposed identities of the major components of the profile are given in Table 1. The identity of peaks attributed to α -1 antitrypsin peaks was established by analysis of mass loss on enzyme degradation with carboxypeptidase Y. A useful property that aided identification of carboxypeptidase Y digestion products was a polymorphism that appeared as a peak doublet of equal intensity separated by 19 mass units (polymorphic form had lower m/z than the peptides listed in Table 1) that appeared in approximately 20% of individuals who showed these components. This same mass-separated doublet in peptides at $m/z = 5324, 5007, 4680$, and 4272 indicated that they arose from the same core peptide. Urine of an individual who displayed this polymorphism was digested with carboxypeptidase Y as described in the Materials and Methods section. Serial analysis of the digested sample by mass spectrometry revealed sequential loss of discrete increments from the peak at $m/z = 5324$. These consisted of $-114, -99, -101, -147, -71, -129$, and -87 amu, respectively. These losses corresponded to sequential removal of the C-terminal sequence: Ser-Glu-Ala-Phe-Thr-Val-Asn-COOH. A BLAST search showed that several proteins contained this sequence. However, only α -1 antitrypsin gave this sequence in peptides of the m/z values observed, thereby providing a unique solution for these components. The $m/z = 5324$ ion

corresponding to residues 124 to 170 of α -1 antitrypsin pre-cursor L₁₂₄RTLNPDSQ LQLTTGNGLF LSEGLKLVDK FLEDVKKLYH SEAFVTN₁₇₀.

The peak at $m/z = 5007$ correlated with residues 124 to 167 (theoretical $m/z = 5008.7$), the peak at 4860 with residues 124 to 166 (theoretical $m/z = 4861.5$), and the peak at $m/z = 4272$ with residues 124 to 161 (theoretical $m/z = 4273.9$). An Arg to His mutation provides the only single change, which results in loss of 19 amu. An Arg to His polymorphism is known for this region of pre- α -1 antitrypsin (Swiss-Prot variant VAR 006991 or the variant of the coding region for this peptide p.R125H, rs709932, NCBI database). Previous studies using 2D gel electrophoresis found higher mass degradation products of α -1 antitrypsin in urine of persons with glomerular diseases (22).

Identities of several other components of the profile were suggested by patterns of m/z values or changes after chemical treatment. The peaks at $m/z = 3770$, 3441, and 3485 corresponded to the well-known triad of human neutrophil peptides (23). Components at $m/z = 9422$ and 9713 correlated with the two major glycoisoforms of apolipoprotein C3 (18). Human urinary protein 1 (HUP1) is synonymous with Clara cell protein that was previously identified in reverse phase extracts of bronchoalveolar lavage fluid (24). Evidence for the identity of HUP1 was obtained by reduction of the sample with DTT (1 mM at 37°C for 30 min) followed by acidification and ZipTip extraction. The peak at $m/z = 15,835$ was lost and a new intense peak appeared at $m/z = 7919$, appropriate for the monomeric form of HUP1 (24). Evidence for the identity of transthyretin was obtained by reduction of a sample with DTT. Transthyretin that is disulfide linked to cysteine (18) appears at $m/z = 13,880$. Reduction resulted in loss of the peak at $m/z = 13,880$ and appearance of a new component at $m/z = 13,761$. This corresponded to transthyretin with a free sulfhydryl. A peak at $m/z = 13,350$ was also observed in many pathologic samples.

DISCUSSION

The first goal of this study was to develop a noninvasive method for diagnosis and prognosis of native and posttransplant kidney disease. The identification of components associated specifically with normal kidney function, and several components associated with kidney disease, provided proof of concept for prognosis of kidney deterioration or failure based on the presence or absence of specific protein biomarkers. A less obvious goal was to determine the nature of variation and change in the urinary proteome across the spectrum from healthy individuals to kidney allograft recipients. A relatively small number of components offered insight into these several issues. We limited our analysis to peaks that appear to best differentiate the different states of kidney health. These components appeared in a large number of samples, making them useful in group comparisons. Other peaks were found in only a few samples and were therefore of low utility.

To optimize experimental descriptions and accessibility of the method, this study used an inexpensive, readily accessible method and evaluated only the most intense peaks of the profiles. The method shared some properties with an automated commercial system (SELDI). The peak identified as B2M ($m/z = 9073$ and 9746) correlated with SELDI peaks reported at $m/z = 9.0$, 9.7, and 9.8 kDa (6–11, 25). Loss of saposin B was associated with kidney disease.

Two properties of protein profiles of healthy individuals were identified. First, this method and analysis detected a small number of major components among healthy persons, making every new peak a potential biomarker. Second, the ratio of saposin B isoforms was stable in each individual over time. This correlated with the suggestion that longitudinal change in a protein ratio of an individual may detect a change in health status even though the actual

value (peak ratio in this case) remained within the range found among healthy individuals (21).

Proteins or peptides associated with pathologic conditions appeared in a sequence that correlated with value in prognosis. Components at $m/z = 4303$ and $10,350$ appeared frequently but showed little value for diagnosis or prognosis over the time of this study. B2M had some value for prognosis but much less than the component at $m/z = 9480$. Optimum prognosis of kidney failure was provided by components found in advanced native kidney disease ($m/z = 4272, 4337, 4860, 5007, 13,350, \text{ and } 15,835$). These findings might be rationalized by the suggestion that early, reversible markers occur far from the endpoint, and are subject to intervening, unpredictable events that have impact on the outcome. Markers associated with advancing disease seemed more tightly correlated with final outcome and, therefore, had better prognostic ability. If this general property is applicable to protein biomarkers, it follows that optimum diagnosis/prognosis will use a complex pattern of proteins that are evaluated in sequential samples from each individual. For example, although a single detection of B2M had relatively modest value, its continual or repeat presence may provide an early indication of developing disease. Although the findings did not seem to improve on creatinine, comparable outcomes served to validate the method and provide proof of principle that proteome analysis can work. This new technology can be improved by a nearly unlimited potential for additional discovery of additional peaks to increase the sensitivity and specificity for this approach.

For the current study, limited data at longer times suggested that kidneys producing pathologic components may survive for 2 years or longer but with impaired function. It is possible that the markers of advanced disease described in this study will be most useful for management of transplanted kidneys with impaired but useful function. It is possible that future combination of protein biomarkers with laboratory and biopsy results will improve diagnostic and prognostic value in kidney disease and transplant. Identification of a profile that reduces the need for a biopsy would be a valuable outcome even if applicable to a subpopulation of healthy transplant patients.

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FIGURE 1.

Profiles of kidney donors and transplant recipients with histologically normal kidney allografts. (A) Profiles of 29 kidney donors before nephrectomy. (B) Profiles of 41 kidney allograft recipients with stable kidney and normal histology determined by protocol biopsies. Profiles were averaged in ClinProTools (Bruker Daltonics, Inc.). Peaks of saposin B are identified (m/z 9073 and 9746).

FIGURE 2.

Reproducibility of saposin B isoform ratios. Values shown above “replicate” are the average and standard deviation for three analyses of each of 13 samples. Data points above “serial” are the average and standard deviation of three to six longitudinal samples from six different individuals over 3 month to 2-year intervals. Data points above “single” represent single analysis of 29 kidney donors before nephrectomy.

FIGURE 3.

Profiles of urine associated with kidney disease. (A) Average profile of 22 spectra with β -2-microglobulin (pattern 1). (B) Average profile of 11 spectra without β -2-microglobulin. Profiles were averaged in ClinProTools. Important m/z values are labeled.

FIGURE 4.

Averaged matrix assisted laser desorption/ionization time-of-flight profiles of individuals returning for evaluation because of elevated creatinine level. The averaged profile is shown for 159 kidney transplant recipients who returned to the University-Fairview Transplant Center for evaluation of an elevated creatinine level. The major areas of the profile ($m/z = 4000-5400$ and $m/z = 8000-12,000$) are presented. The spectra were averaged with ClinProTools.

TABLE 1

Components of the profiles and correlation with biopsy/laboratory results^a

Profile (m/z)	Suggested protein (n = plus)	Peak present or absent (GFR, mL/min)	P (GFR)	Peak present or absent (number with fibrosis)	P (fibrosis)
3370, 3441, 3485	HNP (n = 21)		0.6		0.59
4303	Unidentified (n = 74)		0.4		0.56
4272, 4860, 5007	α -1 antitrypsin (n = 35)		0.5		0.6
4180, 4337	Unidentified (n = 43)	Present (28.6 $\pm$ 12.1)	0.02	Present (31 of 43)	0.009
		Absent (35.6 $\pm$ 13.4)		Absent (51 of 105)	
9480	Unidentified (n = 73)	Present (28.7 $\pm$ 12.4)	0.0004	Present (48 of 73)	0.012
		Absent (37.9 $\pm$ 12.8)		Absent (34 of 75)	
9073, 9746	Saposin B (n = 134)	Present (34.8 $\pm$ 13.0)	0.009	Present (74 of 135)	0.64
		Absent (21.3 $\pm$ 11.2)		Absent (8 of 13)	
9422, 9713	Apolipoprotein CIII	Infrequent			
10350	Unidentified (n = 89)	Present (31.8 $\pm$ 13.1)	0.04	Present (50 of 89)	0.07
		Absent (37.2 $\pm$ 13.2)		Absent (42 of 59)	
11732	β -2 microglobulin (n = 82)		0.49		0.63
13350	Unidentified (n = 12)		0.06		0.64
15835	Urinary protein I (n = 24)		0.79		0.40
15126, 15880	Hemoglobin	Infrequent			

^aData from 148 individuals with samples obtained >60 d after surgery and with biopsy on the same day. P values are for differences between those that are plus vs. minus detected amount of the component in column 1.

HNP, human neutrophil peptide; GFR, glomerular filtration rate.

TABLE 2

Prognosis of kidney allograft failure based on urine profile components^a

Peaks present	Failed (n)	<i>p</i> ^b	Sensitivity	Specificity	PPV ^c	NPV ^d
Any one of <i>m/z</i> = 4272, 4337, 4860, 5007, 13350, 15835						
No (n = 78)	3	<0.0005	88%	56%	28%	96%
Yes (n = 81)	23					
<i>m/z</i> = 9480						
No (n = 89)	6	<0.0005	77%	62%	28%	93%
Yes (n = 70)	20					
B2M						
No (n = 77)	7	0.016	73%	53%	23%	91%
Yes (n = 82)	19					
Creatinine						
<2.3 (n = 98)	4	<0.0005	85%	71%	36%	96%
>2.3 (n = 61)	22					

^aTime of MALDI-TOF analysis = 0.

^bPearson's uncorrected χ^2 value.

^cPositive predictive value.

^dNegative predictive value.

B2M, β -2 microglobulin; MALDI-TOF, matrix assisted laser desorption/ionization time-of-flight.