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FABP1 and FABP3 Have High Predictive Values for Renal Replacement Therapy in Patients with Acute Kidney Injury

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Key Words
Fatty acid binding protein 1 · Fatty acid binding protein 3 · Acute kidney injury · Renal replacement therapy

Abstract
Background/Aims: Early initiation of renal replacement therapy (RRT) is recommended in order to improve the clinical outcome of patients who develop an acute kidney injury (AKI). However, markers that guide an early RRT initiation do not really exist currently. ***Methods:*** Urine and serum samples were prospectively collected from 120 AKI patients. Depending on the necessity of initiating RRT, patients were divided into 2 different groups: dialysis (n = 52) and non-dialysis (n = 68). ***Results:*** Comparative urinary proteomic analyses identified 4 different proteins (fatty acid binding proteins 1 and 3 (FABP1 and FABP3), β-2-microglobulin (B2M), cystatin-M (CST6)) that discriminate AKI patients with high risk for RRT. Western blot analysis confirmed the proteomics data for FABP1 and FABP3 but not for B2M and CST6. Validation analysis confirmed that the FABP1 and FABP3 fulfilled the requirement of functioning as markers for AKI patients with risk to dialysis ($p < 0.001$). ***Conclusion:*** The release of high amounts of FABP1 and FABP3 in urine of AKI patients could serve as a diagnostic/prognosis marker for RRT initiation in these patients.

Introduction

The incidence of acute kidney injury (AKI) ranges from 5% in hospitalized patients to 30–50% of patients in intensive care units (ICUs) [1]. Its appearance worsens the prognosis of patients extensively [2]. The mortality of critically ill patients with severe AKI reaches up to 61% [3]. Though not completely independent from other risk factors, AKI has been shown to be independently associated with short- and long-term mortality [4–6]. Otherwise, early goal therapy, for example, sepsis treatment, justifies the need to take care of AKI early [7]. Although the risks of this complication are already known for a long time, the definition of AKI has been controversial and over 35 different definitions have been used so far [8]. Recently, the Acute Dialysis Quality Initiative published a definition, which was a consensus and evidence-based definition for ARF [9]. The so-called Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease (RIFLE) classification describes 3 grades of severity (Risk, Injury, Failure) and 2 clinical outcomes (Loss, End-stage).

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More recently, the AKI network (AKIN) supplemented the RIFLE criteria with some refinements [10]. Thus, AKI is defined as an abrupt reduction in kidney function within 48 h, currently defined as a percentage increase in urine creatinine or a reduction in urine output [11]. Similar to the RIFLE criteria, AKI is separated into 3 grades of severity, whereupon stage 3 also includes patients where a renal replacement therapy (RRT) is necessary, irrespective of the stage they are in with respect to RRT.

Through these classifications, the definition of renal failure has become more precise, but the capability to handle it is still extremely limited. There are the conventional indications for initiating RRT, for example, volume overload, hyperkalemia, metabolic acidosis, an uremic manifestation or acute intoxication with a dialyzable drug or toxin [12], but the optimal timing and dose are not well defined [11].

Despite the suggested positive impact of early RRT initiation on mortality [13–18], there are only a few studies that investigated the optimal beginning of this therapy. This may be due to lack of one or more valid biomarkers indicative for this complex structure of not having well-defined clinical and laboratory RRT indications [19]. Thus, objective criteria that may guide early RRT initiations in patients with AKI do not really exist [20, 21] and depend so far on the subjective assessment of the treating physician [19].

The aim of this study was to identify urinary protein markers that can predict the need for RRT even in the early stages of a kidney failure.

Methods

Patients

One hundred and twenty patients were recruited in 2 medical and 4 anaesthesiological ICUs as well as one medical intermediate care unit at University Medical Centre Göttingen, Germany. Patients admitted to the ICUs were prospectively screened for AKI and included in the study in case of a deterioration of kidney function, that is, a serum-creatinine rise of at least 0.3 mg/dl, which is 50% of base value, or a urine output of <0.5 ml/kg bw/h for more than 6 h. AKI was detected either on admission or during the course of hospitalization. Anuric patients or patients with a previous serum creatinine ≥2.5 mg/dl were excluded. Blood and urine samples were taken starting with inclusion, and in the sequel, depending on the physicians' clinical decision whether the patients were dialysed or not.

Clinical sample acquisition and analyses as well as data management during this study were approved by the local Ethics Committee of the University Medical Centre Göttingen, Germany (March 12, 2005). All patients or their legal guardians gave their written informed consent prior to including the patients in the study. All methods used in our experiments were carried out in accordance with approval guidelines and regulations.

Biometric data (gender, age, size, weight and body mass index), relevant past history (diabetes mellitus, coronary heart disease (CHD), chronic obstructive pulmonary disease, occlusive artery disease, congestive heart failure (CHF), chronic renal failure stadium II or III), study relevant clinical data (daily urine output, fluid balance) and laboratory values from the day of admittance were evaluated.

Dialysis

Depending on the necessity for RRT in the follow-up, patients were retrospectively divided into 2 different groups (dialysis, D vs. non-dialysis, ND). RRT was initiated by the attending physician, based on laboratory data and clinical judgment. In this regard, RRT-initiation was geared to existing, undisputed RRT-indications such as persistent hyperkalemia, uremic serositis, encephalopathy, hypervolemia and acidosis that are refractory to conservative treatment [22]. In addition, renal parameters like blood urea nitrogen, creatinine and fluid balance were taken into account although no clear-cut criteria were determined. Dialysis modalities were chosen individually including intermittent or continuous hemodialysis modalities.

Samples Collection and Handling

Urine, plasma and serum samples were taken immediately after inclusion into this study. For all our proteomics experiments, the urine sample collection was performed according to EuroKup/HKUPP urine protocol and recommendation (http://www.eurokup.org/). For all proteomic experiments, midstream or catheter urine was used. Urine samples were collected from the examinees, centrifuged at 1,000 *g* for 10 min at 4°C to remove cell debris and casts. The supernatant was aliquoted (10 ml aliquots) without disturbing the pellets and was stored at –80°C until use. For each collected urine sample, we used 10 ml to measure routine laboratory parameters. All laboratory parameters, including protein concentration, were measured by standard routine methods in the certified laboratory of the Department of Nephrology and Rheumatology, Göttingen.

2D Gel Electrophoresis

Prior to the two-dimensional gel electrophoresis (2-DE), sample enrichment and total protein precipitation were performed. For the 2-DE, urine samples from 60 patients (D: n = 30 and ND: n = 30) were used. Three different experimental groups were generated. When generating the groups, special emphasis was laid on the cause of AKI; we included in each group, when possible, a balanced number of samples for every cause of AKI (especially in septic and cardiac cause). In each experimental group, 10 different urine aliquots per case were pooled together (online suppl. fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000447115) and 20 ml from the pooled urine were concentrated to 2 ml with an amicon column 1,000 Da cut-off filter (Beverly, Mass., USA). Subsequently, protein chloroform/methanol precipitation was carried out according to Wessel and Flugge [23] and protein estimation was performed using the Bio-Rad protein assay according to Bradford [24]. 2-DE and DIGE-analyses were carried out as previously described [25].

Protein Identification from 2-DE Gels

Manually excised gel plugs were subjected to in-gel tryptic digestion; gel plugs were excised from 2-D gels and digested as described previously [26] and the mass spectrometric sequencing was carried out according to our standard protocol. Processed data were searched against MSDB and Swissprot databases using the Mascot search engine using a peptide mass tolerance of 50 parts per million and fragment tolerance of 100 millimass unit. Protein identifications with at least 2 peptides sequenced were considered significant.

Western Blot Analysis

The validation of the proteomics data was carried out using western blot (WB) according to Towbin et al. [27]. To compensate for sample pooling error, WB analyses were carried out from single urine samples. From each experimental group (D and ND), 10 individual urine samples were included in these analyses. The urine samples were selected from the cohort (60 patients used for the 2-DE pools) using the same criteria as for 2-DE according to the AKI cause: 4 samples cardiac cause, 4 samples septic cause and 2 others. Mouse monoclonal anti-fatty acid binding proteins 1 and 3 (FABP1 and FABP3) antibodies (Abcam, UK), Mouse monoclonal anti-CST6, and rabbit polyclonal anti-β-2-microglobulin (B2M) antibodies (Sigma Aldrich, St. Louis, USA) were used as primary antibodies. Molecular Probes Alexa Fluor 647 goat anti-mouse IgG antibody or Alexa Fluor 647 goat anti-rabbit IgG were used as secondary antibodies. The blot membranes were dried in the dark, and scanned at 50 μm resolution on a Fuji FLA5100 scanner with single laser-emitting excitation light at 635 nm.

Dot Blot Analysis

To investigate the diagnostic value of identified proteins in larger patient cohorts, we performed the dot blot analysis [28]. For this validation, individual samples from the 2-DE cohort (D: n = 30 samples, ND: n = 30 samples) with a balanced number of samples for every cause of AKI (especially in septic and cardiac cause) were used. Ten micrograms from each urine sample were loaded in triplicate. Digitalize dot blot pictures were further analyzed with ImageJ software (NIH, http://rsbweb.nih.gov/ij/).

ELISA Analysis

To validate the data in an independent patient cohort and overcome the limitation of the Dot blot, the urine FABP1 and FABP3 were measured using the ELISA kit (CMIC Co., Ltd., Tokyo Japan) (HyCult Biotechnology B.V., Uden, The Netherlands) in 60 AKI urine samples (D (n = 22): cardiac (31%), septic (45%), hypovolemic (4.5%), others (18%); ND (n = 38): cardiac (78%), septic (10%), hypovolemic (2.5%), others (10%)). The kit has a minimum detection limit of 0.4 ng/ml and a measurable concentration range of 0.4–25 ng/ml. Samples were diluted 10 times before measurement. Both FABP1 and FABP3 urine concentrations were corrected for dilution using urine protein values.

Statistical Methods

Categorical patient characteristics were compared between the dialysis and non-dialysis group using either Fisher's exact or the χ^2 test, whereas metric parameters were compared by t tests. A bias of statistical different variables on investigated biomarkers was excluded by 2-way analysis of variance (ANOVA). For the ELISA test, due to their asymmetric distribution, FABP1 and FABP3 were compared by the Wilcoxon–Mann–Whitney (WMW) test. Any further parameters were compared using either the WMW tests or t tests. Analyses were separately done for days 0 and 2. All tests were conducted with a significance level of $\alpha = 0.05$. Calculations were all performed with the free software R 2.6 (www.r-project.org). For 2-DE, the digitalized images were analyzed; spot matching across gels and normalization were performed using Delta2D 3.4 (Decodon, Braunschweig, Germany). Delta2D computes a 'spot quality' value for every detected spot. This value shows how closely a spot represents the 'ideal' 3D Gaussian bell shape. Based on average spot volume ratio, spots whose relative expression is changed at least twofold (increase or decrease) between the compared samples were considered to be significant. To analyze the significance of protein regulation, Student's t test was performed, and statistical significance was assumed for p values <0.01.

All blots were quantified using the ImageJ software. The data were compiled with the software package GraphPad Prism version 4. The software was used for graphical presentation and analysis. The clinical accuracy of the examined proteins was assessed using receiver operating characteristic (ROC) curve analysis. ROC plots were constructed and the area under curve (AUC), standard errors, 95% CI, sensitivity and specificity were calculated. Cut-off values, at which the discrimination between the cases with positive and negative diagnosis was optimal, were set.

Results

Patients

Within 6 months, 120 patients were recruited into this study fulfilling AKIN-criteria [29]. Fifty two patients had to undergo RRT (dialysis-group, D) during the clinical course and 68 did not (non-dialysis-group, ND). Dialyzed patients had higher AKIN-scores. Origin (pre-/intrarenal, post-renal) of AKI did not differ between the 2 different groups. When pre-/intra-renal AKI was divided into subgroups with respect to its underlying cause, significantly more patients of the dialysis group suffered from septic shock and, vice versa; cardiac shock was more frequent in the ND group but without any influence on investigated biomarkers. The SAPS-II score tended to be higher in dialysis when compared to non-dialysis patients (median 34 vs. 28). Other types of premorbid diseases did not differ between the 2 groups (table 1a, b).

Mapping AKI Urine Proteome

For the 2-DE analysis, samples from 10 patients per group (in total 60 patients, D: n = 30 and ND: n = 30) were included for the generation of urine sample pools. To compensate the disadvantage of urine pooling 3 pair pools (AKI D and AKI ND) of urine were collected together to form 3 experimental groups A–C (online suppl. fig. 1). The experimental groups were chosen according to the patient's creatinine value, which was comparable in

Table 1.

a Patient characteristics (either described by absolute and relative portions or by mean ± SE)

	Group		p value
	dialysis (n = 52)	non-dialysis (n = 68)	
Age, years	68.2±2.1	70.3±1.5	0.40
Gender, n (%)			0.83
Female	18 (35)	26 (38)	
Male	34 (65)	42 (62)	
Body mass index, kg/m^2	27.5±1.1	28.0±0.7	0.74
Previous plasma creatinine	1.87±0.95	1.45±0.49	0.003
AKIN-score, n (%)			<0.001
1	0 (0)	39 (57.4)	
2	0 (0)	20 (29.4)	
3	52 (100)	9 (13.2)	
Causes for AKI, n (%)			<0.001
Hypovolemic	1 (2.1)	3 (4.6)	
Cardiac	17 (35.4)	48 (73.8)	
Septic	24 (50.0)	8 (12.3)	
Other	10 (19.2)	9 (13.2)	
SAPS-II score	34 (9–72)	28 (6–58)	0.10
Pre-morbid diseases, n (%)			
CHD	24 (47.1)	48 (72.7)	0.008
Diabetes mellitus	16 (31.4)	27 (40.3)	0.42
Arterial hypertension	35 (68.6)	50 (75.8)	0.52
Chronic obstructive pulmonary disease	13 (25.5)	14 (21.2)	0.75
Peripheral artery occlusive disease	10 (19.6)	7 (10.6)	0.27
Chronic kidney disease (stadium 2–3)	14 (27.5)	9 (13.6)	0.10
Severity of AKI at time of biomarker sampling			
Plasma creatinine	2.9±0.2	2.1±0.1	<0.001
Daily diuresis, ml	725 (0–6,790)	2,760 (90–8,400)	<0.001
Fluid balance, ml	1,180±280	335±195	0.02

b Patients' admission diagnoses, timing of AKI diagnosis relative to admission and ventilation data

Number	120
Initial admission diagnosis (at hospitalization)	
AV-block III	1 (1)
Aortic stenosis	9 (8)
Myocardial infarction	25 (21)
Stroke	1 (1)
Cholangitis	1 (1)
CHF	6 (5)
AKI	11 (9)
Neoplasia	11 (9)
Pneumonia	6 (5)
Acute respiratory distress syndrome	3 (3)
Sepsis	6 (5)
CHD (stable)	14 (12)
Preeclampsia	2 (2)
Chronic obstructive pulmonary disease	2 (2)
Acute aortic dissection/aneurysma	2 (2)
Renal graft failure	1 (1)
Mitral insufficiency/stenosis	6 (5)

Table 1. (continued)

Arterial emboli	3 (3)
Liver cirrhosis	2 (2)
Atrial fibrillation	1 (1)
Trauma (thorax/cerebrum)	2 (2)
Systemic lupus erythematodes	1 (1)
Perforation of the bile duct	1 (1)
Hyperplasia of the prostate	1 (1)
Meningitis (herpes-mediated)	1 (1)
Pacemaker device implantation	1 (1)
Timing of AKI diagnosis relative to admission, days	4 (2–7)
Need for mechanical ventilation, n (%)	88 (73)
Duration of mechanical ventilation, h	123 (5.5–333)

Values are mean ± SD, median (interquartile range) or n (%)

AKI D and AKI ND patients from the same experimental group. The analysis of the patient data showed that both age and gender of the patients did not show any significant differences between the experimental groups (data not shown).

To map the proteome of the urine pools, protein precipitation followed with 2-DE analyses was performed as described in methods. The protein profiles of the urine pools were highly reproducible as revealed by the overlay of 5 2-DE replicates. Subsequently, the gels were post-stained with colloidal Coomassie blue for protein visualization. Delta2D analysis of the gels revealed around 267 ± 12 protein-spots in the pH 4–7 2-D gel region from urine pool samples from AKI patients. The mass spectrometric sequencing using [30] allowed the identification of 73 proteins from urine pools, which corresponded to a library of 21 non-redundant proteins (online suppl. fig. 1a and table 1).

Comparative Analyses of Urine Proteomes Using 2-D DIGE and Identification of RRT Protein Markers in AKI Urine Samples

To identify, with high reproducibility and statistical significance, proteins differentially excreted in AKI D and AKI ND urine, we performed 2-D DIGE analyses of the 2 different pool samples (D and ND). 2-D DIGE protein maps were generated in pI 4–7 range from the 2 urine pools from each experimental group (fig. 1a). The resulted gels showed a large protein release in patient's urine with AKI in low molecular weight range (fig. 1b). Qualitative and quantitative analyses of the 2-D DIGE maps with Delta2D and Prism 4 software revealed a list of 18 proteins, which were differently excreted in urine from AKI D patients compared to AKI ND (online suppl. table 2; fig. 1b and c). Among these proteins, 4 different candidates identified as FABP1, FABP3, CST6, B2M, were significantly released ($p < 0.001$) in high levels in urine in AKI D patients compared to the AKI ND (fig. 1b and c). Moreover, the 4 proteins were significantly excreted in excess in the urine of AKI patients than in the urine of healthy controls or patients with chronic kidney diseases (data not shown).

Immunological Validation of AKI Identified Protein Markers

To validate the data generated using proteomics, the excretion levels of the proteins of interest were tested using WB. For this purpose, urine samples from 10 different patients each group (D and ND) were used. From the 4 tested proteins, only in case of FABP1 and FABP3 could the immunoassays clearly confirm the 2-DE data ($p < 0.001$; fig. 2a). In contrast, analyses of B2M and CST6 excretion in single urine samples could not confirm the 2-DE data from the pooled urines ($p = 0.2359$ and $p = 0.6334$, respectively; fig. 2b).

Dot Blot and ELISA Validation of AKI D Markers

To validate the usefulness of the identified AKI D markers, we performed dot blot in a larger patient cohort (D: n = 30; ND: n = 30) and ELISA in an independent cohort of patients (n = 60: D: n = 22; ND: n = 38). The statistical analysis of the dot blot data confirmed with higher significance the increased excretion of these 2 proteins in urine samples from the AKI D group compared to AKI ND patients ($p < 0.001$; fig. 3a).

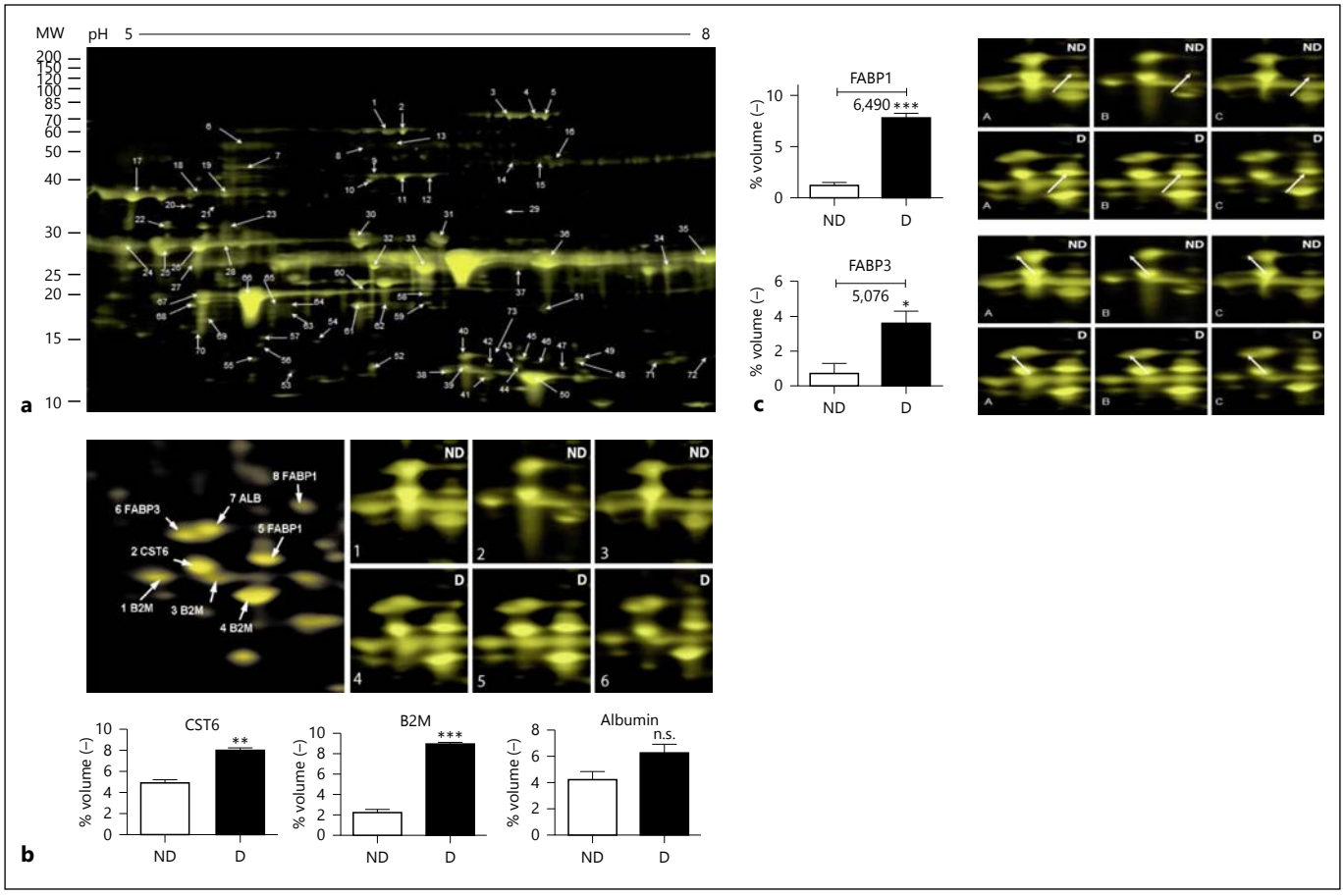

Fig. 1. Two-dimensional pattern of total protein isolated from AKI patient's urine. **a** The proteins (150 μg) were loaded and separated by 2-DE according to pI and MW. A 11-cm IPG strip with a linear pH 4–7 gradient for isoelectric focusing, and a criterion Tris-HCl linear gradient gel 10–20% for SDS-PAGE were used. The protein spots were visualized by Flamingo® (BioRad). Numeric labeling of the spots corresponds to proteins identified and listed in online supplemental table 1. Graphs represent enlargement of the gel regions of interest showing protein spots found to be differentially excreted in urine of AKI D vs. ND patients in the range of pH 4–7; **b** B2M, CYT6 and albumin; **c** FABP1 and FABP3. The names of the proteins differentially excreted are shown on gels. The protein excretion quantification for selected proteins is given in the form of bar diagrams. Results are given as the means ± SD of the percentage volume of spot as quantified by 2D-DIGE. All the protein showed present significant expression changes between AKI D and ND ($p < 0.05$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s. = non-significant.

Consistent with the WB data, the dot blot analysis confirmed that the differences in excretion level of B2M and CST6 in AKI D and AKI ND were statistically not significant ($p > 0.05$; fig. 3b).

Using the ROC curve analysis, the AUC and 95% CI for examined urinary proteins were calculated. The resulting data showed that from the identified protein markers, only 2 could differentiate AKI D from AKI ND (fig. 3a; table 2) with high specificity and sensitivity. The quantification of FABP1 (95% CI, cutoff >0.2895; 88–100% sensitivity; 88–100% specificity) and FABP3 (95% CI, cutoff >0.04008; 92–100% sensitivity; 88–99% specificity) from urine from AKI D compared with urine from AKI ND reveal highly significant differences ($p < 0.001$; table 2). ROC plots of the identified proteins showed that the clinical outcome could be predicted in this cohort with good discriminatory ability (95% CI, $p < 0.001$; fig. 3a). Using logistic regression, we tested whether our identified biomarker could be a better predictor for the need for RRT than the clinically available parameters. The best single parameter for predicting the need of RRT was cystatin C with a threshold value of 2.4 mg/l, a sensitivity of 71% and a specificity of 65% (table 2). A similar result was also observed with the formula x = 7.36 + 0.005*urea-albumin + 0.59*P-creatinine + 0.05*urea-nitrogen + 0.0009*fluid balance – 0.086*P-sodium (threshold value

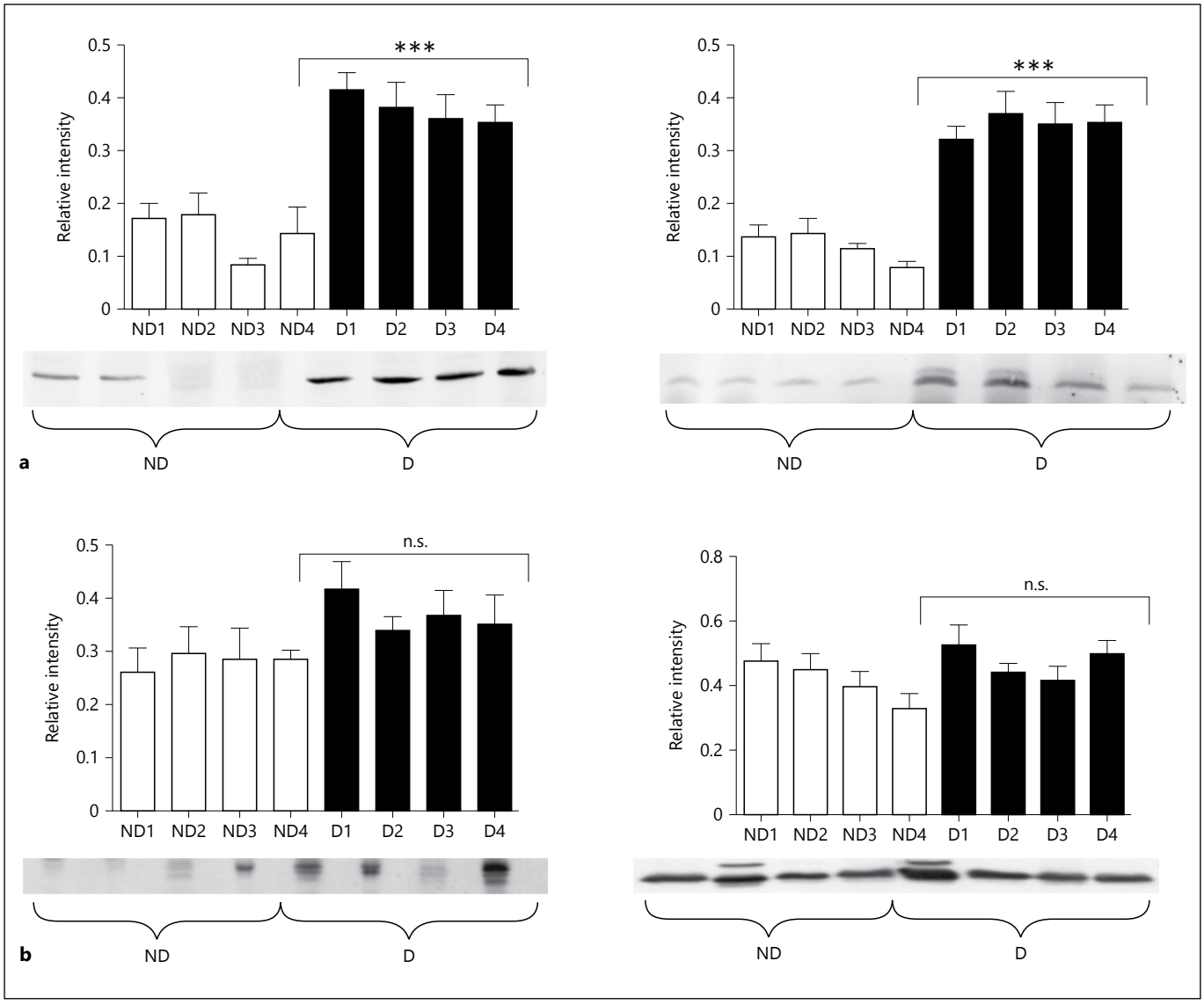

Fig. 2. WB validation of the potential biomarkers. Fluorescent WB analyses of the FABP1 and FABP3 (**a**), CST6 and B2M (**b**) that were identified to be differentially excreted between AKI D and AKI ND patients. On the y-axis, the line-volume-percentage is given and the x-axis shows distribution of the intensity thought the corresponding urine samples. Statistical analyses were performed by Prizma4 software. *** $p < 0.001$; n.s. = non-significant.

0.48) and this showed a sensitivity of 77% and specificity of 82% (table 2) [30]. In both cases, our biomarker showed a better sensitivity and specificity in predicting RRT.

In contrast to FABP1 and FABP3, the B2M (95% CI, cutoff >0.3889; 51–80% sensitivity; 40–70% specificity) and CST6 (95% CI, cutoff >0.3773; 46–76% sensitivity; 44–74% specificity) did not show high significance in differentiating the 2 analyzed groups (fig. 3b).

The ELISA analysis of the concerned proteins in an independent patient cohort (D: n = 22 and ND: n = 38) confirmed the usefulness of FABP1 and FABP3 as urine markers that can discriminate between AKI D and AKI ND, when the marker concentration was normalized with the urine protein level. Using the ROC curve analysis, the AUC and 95% CI for examined urinary proteins were calculated. These data confirmed a clear discrimination between AKI D and AKI ND patients using both FABP1 and FABP3 (fig. 4). In one-way ANOVA, anamnesis of CHF had an influence on FABP3 levels ($p = 0.02$), but in 2-way ANOVA (CHF*D/ND) a bias of this vari-

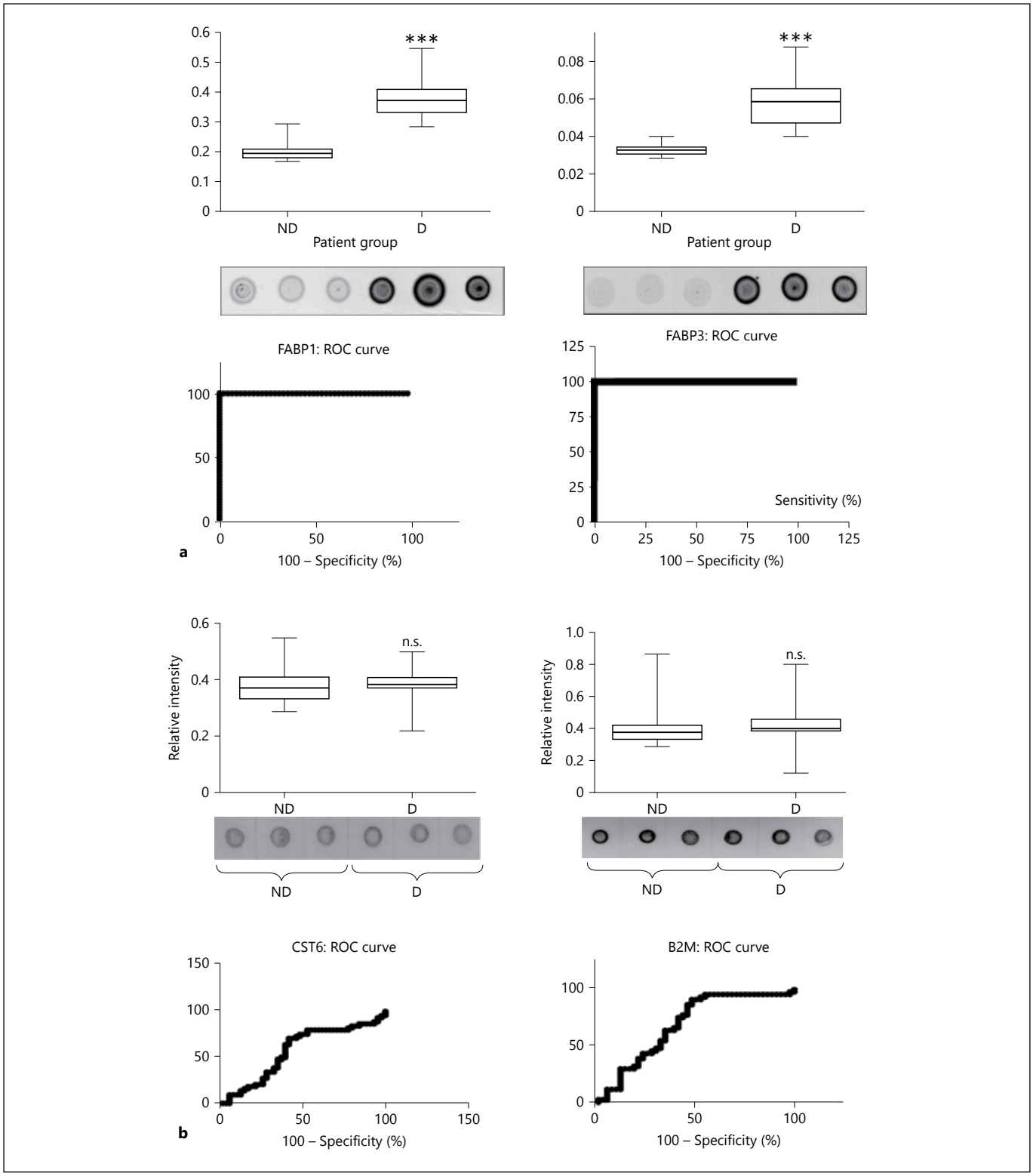

Fig. 3. Dot blot analysis of the identified biomarker in larger patient's cohort. **a** FABP1 and FABP3; **b** CST6 and B2M. On the y-axis, the line-volume-percentage is given and the x-axis shows distribution of the intensity thought the corresponding urine samples. Examples of the dot blot images are given below. Statistical analysis were performed by Prizma4 software. *** $p < 0.001$. The results of the ROC curves analysis are given in table 2. n.s. = Non-significant.

Table 2. The results of ROC curves analysis from dot blot data of the 4 identified urinary markers in AKI ND and AKI D samples

Marker	AKI D vs. AKI ND				
	cutoff	sensitivity, %	specificity, %	difference AUCs	p value
B2M	>0.3889	51–80	40–70	0.6296	<0.05
CST6	>0.3773	46–76	44–74	0.5521	n.s.
FABP1	>0.2895	88–100	88–100	0.9995	<0.001
FABP3	<0.04008	92–100	88–99	1	<0.001
Serum cystatin C [32]	>2.4	71	65	0.74	<0.001
Logistic regression model* [32]	>0.53	77	83	0.53	<0.001

* Probability (person in dialysis group) = 7.36 + 0.005*urea-albumin + 0.59*P-creatinine + 0.05*BUN + 0.0009*fluid balance – 0.086*P-Na.

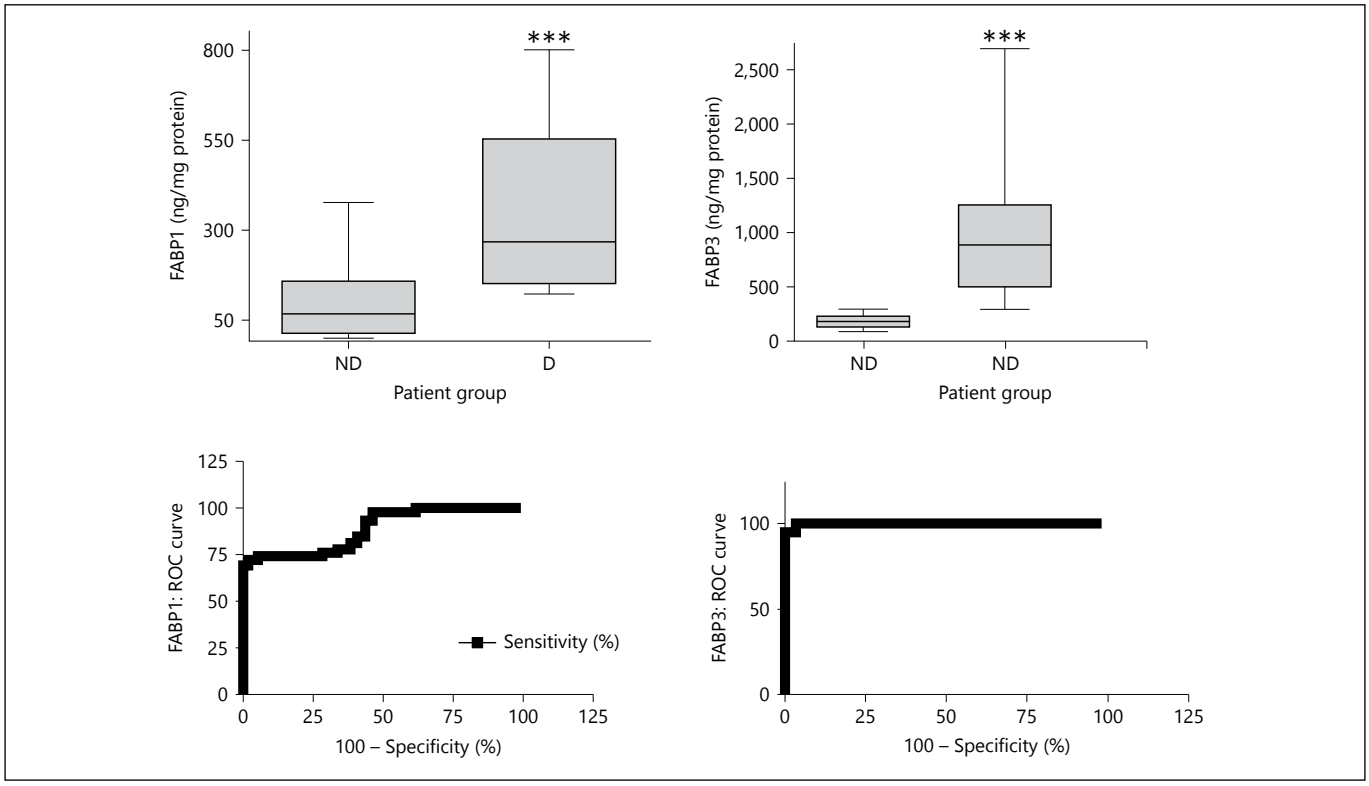

Fig. 4. ELISA analyses of the FABP1 and FABP3 in larger cohort of patients. Urine samples from 22 AKI D and 38 AKI ND were tested for the excretion level of the identified biomarker using ELISA. On the y-axis, the measured protein concentration in ng/mg protein is given and the x-axis shows patients group. Statistical analyses were performed by Prizma4 software (*** $p < 0.001$). The ELISA analysis showed a clear and highly significant difference in FABP1 and FABP3 excretion between D and ND patients group. In both cases, the AKI D patients excreted high levels of both proteins compared to healthy donors.

able could be ruled out ($p = 0.08$). Even if each AKIN-group would be separately compared to the HD group, both biomarkers revealed a significant difference (all $p < 0.05$).

Using our markers, the necessity of RRT was predictable with a mean of 3.6 days (median 1 day, range 0–24 days) before initiation.

Discussion

In AKIN and RIFLE criteria, the AKI stages are based on gradual changes in the maximum serum creatinine level from baseline or on hourly urine output. However, serum creatinine only detects advanced stages of AKI process resulting in a delay of diagnosis and therapy.

Due to the rapid development in biomarker research, a high number of potential biomarkers for early diagnosis of AKI have been identified and characterized. However, many exert substantial limitations: some of them are not only associated with kidney damage but also associated with the underlying conditions causing AKI; some report higher values for the biomarker in patients with AKI, but with a substantial overlap between AKI and non-AKI patients, hampering discrimination in the single case, and, at least, some of the proposed biomarker show increasing sensitivity, but at the expense of specificity and positive predictive value [31]. As most promising candidates for early AKI detection cystatin C, neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1) and interleukin-18 (IL-18) have been identified. However, most of the potential markers were not tested or when tested, they failed for prediction of RRT [20, 21].

In our study, we identified 4 potential markers for differentiation and risk stratification for RRT. B2M and CST6 failed the validation process, whereas FABP1 and FABP3 revealed an accuracy of 99.95 and 100%, respectively. The prediction value of FABP1 and FABP3 is significantly better than the rate we reported recently (88% accuracy in predicting RRT for AKI patients) and better than the best single parameter, cystatin C (correct classification rate 74%) [32]. Despite plasma creatinine and urine output differed between the dialysis and non-dialysis group even at baseline, the AUC of each of these parameters were with 70 and 73% [32], respectively, clearly below the predictive value of FABP1 and FABP3. This reflects that indication for RRT is complex and actually cannot be based solely on one clinical or laboratory parameter. Our study, however, has one relevant limitation; that is, discovery and validation were performed in the same cohort. Therefore, results need to be validated in an independent validation cohort under real-life conditions. Additionally, the population studied was older than 65 years at mean. Whether these findings are suitable for younger age cannot be derived from the present data.

FABPs are members of the superfamily of lipid-binding proteins with a low molecular weight between 14 and 15 kD. So far, nine different FABPs have been identified with tissue-specific distribution [33] with liver-type FABP1 highly expressed in liver (L-type), especially in hepatocytes and heart FABP3 in the muscle and heart muscle (H-type). Both isoforms, FABP1 and FABP3, were also identified in renal tissue with FABP1 expressed in proximal tubular cells and FABP3 in the distal tubule [34].

Clinical studies have identified the urinary FABP1 as a promising biomarker for several clinical settings, whereas data on urinary FABP3 are less reliable in showing the decline of renal function in proteinuric, diabetic nephropathy [35] or idiopathic membranous glomerulopathy [36]. Increased urinary FABP1 levels were associated with chronic kidney diseases with progressive course [37–39] as well as septic and non-septic AKI [40–45]. In addition, urinary FABP1 levels were correlated with the degree of renal tubular damage in different studies [41]. Furthermore, studies investigated the level of urine FABP1 in patients undergoing cardiac surgery. The excretion level of FABP1 in urine was found to correlate with the AKI development in pediatric and adult patients after cardiac surgery, and could serve as marker risk marker for AKI [42, 43]. Compared to other AKI biomarkers, FABP1 was found to be as good as NGAL and KIM-1 and statistically better than IL-18 [46].

The statements concerning the prognosis value of FABP1 in the onset of RRT are contradictory. Nakamura et al. [47] reported that the level of FABP1 in urine does not correlate with the need for RRT. Otherwise, there are studies that provide evidence that in particular the FABP1 can be used for deciding whether an RRT should be performed or not. Ferguson et al. [46] investigated 92 patients with AKI of various origins, and compared this population with 68 patients without AKI. The urine level of FABP1 ($p = 0.02$) correlated well with the need of an RRT. Another notable point of this study is that because of the medical records, the various causes of acute renal failure of each patient were determined. Despite the heterogeneity of the patient population (acute tubular necrosis due to ischemia, post-cardiac surgery or pigment nephropathy ($n = 28$), sepsis ($n = 30$), a nephrotoxic exposure ($n = 6$), contrast induced nephropathy ($n = 5$), and other causes ($n = 23$) obstruction, acute intestinal nephritis, acute glomerulonephritis, multiple myeloma), no difference in urinary FABP1 level was found between the disease categories [46]. In our study, when the concentration was normalized with urine protein both marker, FABP1 and FABP3 levels correlated the need of RRT in AKI patients irrespective of AKI cause. Elevated serum FABP1-levels have already shown to be prognostic in patients with sepsis [48] as well as in patients with acute heart failure/CHD [49, 50]. They correlate with the all-cause mortality in both conditions, whereas in acute heart failure, it can indicate the occurrence of AKI even on admission. Despite elevated urinary levels of FABP1 in several conditions as sepsis and CHD/acute heart failure, and a higher prevalence of CHD and acute heart failure

in the non-HD group or sepsis in the HD-group, respectively, urinary FABP1-levels were able to distinguish patients who are likely to need dialysis from those who do not. Otherwise, different medical situations, like sepsis, acute heart or liver failure, lead to an increase of serum FABP1 and/or FABP3 [48–50]. As both are small molecules, we cannot exclude the condition whether increased urinary levels derive from a spillover of these proteins and/or reduced tubular reabsorption. As organ damages are comparable in the HD- and non-HD-group, reflected by an equal SAPS II-score, it is tempting to speculate that irrespective of the underlying pathomechanism, elevated urinary FABP1 and FABP3-levels may reflect a certain degree of renal damage.

Conclusions

Our study added more evidence to the usefulness of FABP, especially FABP1 as a predictor marker in AKI outcome. Although urinary FABP1 and FABP3 may be promising biomarkers for early detection of AKI and prediction of RRT, their potential values need to be validated in large studies and across a broader spectrum of clinical settings [44].

Disclosure Statement

The author(s) declare no competing financial or nonfinancial conflict of interests for any of the authors.

Author Contributions

H.D. conceived and designed the study, performed part of the experiments, and coordinated and drafted the manuscript. M.J.K. recruited the patients, collected the samples, participated in the draft of the manuscript and performed the analysis of patient's serum parameters. R.R.D. prepared the urine samples, and performed the majority of the 2D experiments. K.J. performed the statistical analysis of all data and revised the manuscript. M.W. performed patients recruitment, sample collection and has been involved in revising the manuscript critically. D.H. was involved in patients' recruitment and sample collection. G.H.D. was involved in WB analysis and ELISA and I.M. in dot blot analysis. A.R.A. carried out the mass spectrometric analysis; G.A.M. participated in its design. All authors have given approval to the final version of the manuscript.

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