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Original Paper

Urinary Biomarkers in the Prediction of Prognosis and Treatment Response in IgA Nephropathy

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Key Words
IgA nephropathy • NGAL • KIM-1 • [TIMP-2]•[IGFBP7] • Calprotectin

Abstract
Background/Aims: The addition of immunosuppression to supportive care reduces proteinuria in a subset of patients with IgA nephropathy (IgAN) but is associated with an increased rate of adverse events. The present work investigates whether urinary biomarkers are able to identify subjects who benefit from immunosuppression and to predict the progression of disease in a sub-cohort of the STOP-IgAN trial. ***Methods:*** Urinary neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), calprotectin, and the product of tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 (TIMP2•IGFBP7) were measured in all available urine samples obtained at the time point of enrollment in the STOP-IgAN trial (n=113). ***Results:*** Biomarker concentrations in both the overall study population and the subgroup with additional immunosuppression did not differ in subjects reaching vs. not reaching full clinical remission, eGFR loss ≥15, or 30 ml/min/1.73 m^2 over the 3-year trial phase (p>0.05 each). Receiver-operating characteristic curves showed a poor predictive accuracy of each biomarker for the above-mentioned parameters in the overall study population (areas under the curve ≤0.611). Accordingly, there was neither a significant correlation of any biomarker and adverse outcome in linear regression analysis, nor between biomarker concentrations at enrollment and change in the eGFR over the 3-year observation period. ***Conclusion:*** NGAL, KIM-1, calprotectin, and [TIMP-2]•[IGFBP7] had neither a prognostic value for the progression of IgAN, nor for the response to immunosuppression in the present sub-cohort of the STOP-IgAN trial. The search for appropriate biomarkers for an individualized treatment strategy in IgAN continues.

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Introduction

IgA nephropathy (IgAN) is the most common primary form of glomerulonephritis worldwide. Mesangial deposits of circulating IgA antibodies lead to inflammation and mesangial proliferation [1]. Whereas most patients reveal a benign course, some 20-30% progress to end-stage renal disease [2]. Supportive care including blockade of the renin-angiotensin system (RAS) constitutes the basis of treatment in all patients and immunosuppressive therapy is usually administered in subjects with nephrotic syndrome and crescentic glomerulonephritis. The *Supportive versus Immunosuppressive Therapy of Progressive IgAN* (STOP-IgAN) trial investigated whether addition of immunosuppressive treatment to comprehensive supportive care is superior to supportive care only [3]. The latter comprised RAS inhibition, blood pressure control (target < 125/75 mmHg), dietary counseling, cholesterol normalization, avoidance of nephrotoxins and other measures [4]. One-hundred-sixty-two IgAN patients with an estimated GFR (eGFR) ≥30 ml/min, who remained proteinuric (0.75 – 3.5 g/day) despite six months of comprehensive supportive care were randomly assigned to continue supportive treatment alone or to receive additional immunosuppression. Addition of immunosuppression was associated with a higher rate of full clinical remission but increased the number of adverse events during the 3-year study phase. Whereas additional immunosuppression transiently reduced proteinuria at 12 months, it had no effect on the decrease of eGFR during the 3-year trial phase.

Although obviously not helpful for the overall population in STOP-IgAN, immunosuppression might be beneficial for individual subsets of patients. Indeed, 17% of those subjects who were assigned to additional immunosuppression, developed full clinical remission and only 26% lost ≥15 ml/min/1.73 m^2 during the study phase. To date, there are no established markers that predict treatment responses. Neither the extent of proteinuria, presence of hematuria, nor baseline eGFR allow a reliable stratification. Urinary neutrophil gelatinase-associated lipocalin (NGAL) has been demonstrated to be an early biomarker for renal tubulointerstitial injury in IgAN [5]. Moreover, both NGAL and kidney injury molecule-1 (KIM-1) are independent predictors for disease progression and adverse outcome in IgAN [6-8]. Finally, calprotectin (also known as myeloid-related protein 8/14) blood levels have been associated with adverse outcome in a small study with 25 pediatric IgAN [9]. Calprotectin constitutes a mediator protein of the innate immune system that is released by monocytes and neutrophils as a danger associated molecular pattern protein (DAMP) and thereby may serve as a marker of inflammation [10]. We have previously demonstrated that - in contrast to prerenal acute kidney injury - urinary calprotectin concentrations are substantially increased in different forms of intrinsic acute kidney injury and may predict adverse outcome [11, 12]. These findings were consistent in adult, pediatric, and transplant populations [11-13]. In adult IgAN, however, the predictive value of calprotectin has not been systematically evaluated hitherto.

Since glomerular diseases frequently go along with secondary tubulointerstitial alterations, the present work investigates whether a defined subset of urinary candidate biomarkers of tubulointerstitial damage and inflammatory activity, i.e. the NGAL, KIM-1, calprotectin, and the product of tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 (TIMP2•IGFBP7) may be helpful in this context. Using available urine samples from the STOP-IgAN population, we examined whether these urinary biomarkers predict renal outcomes in the analyzed sub-cohort of the STOP-IgAN trial and whether they identify subjects who might respond to immunosuppression.

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Materials and Methods

Study population and Protocol

The protocol and results of the STOP-IgAN study have been published previously (*ClinicalTrials.gov* number NCT00554502) [3, 4]. Briefly, 337 subjects with IgAN started the run-in phase of the study. Inclusion criteria were biopsy confirmed IgAN, age of 18 to 70 years, a proteinuria level above 0.75 g per day plus arterial hypertension and/or impaired renal function (eGFR <90 ml/min/1.73 m^2). Prior immunosuppression, rapidly progressive crescentic IgAN and an eGFR of < 30 ml/min/1.73 m^2 were defined as exclusion criteria. During the run-in phase, all the patients received comprehensive supportive care including treatment with an ACE-inhibitor or ARB targeting a blood pressure <125/75 mmHg. Patients received dietary counselling and a cholesterol lowering therapy. If proteinuria was >0.75 g/day despite these measures after six months patients entered the 3-year study phase and were randomly assigned to continue supportive care alone or in combination with immunosuppressive treatment. Patients in the immunosuppression group with a GFR of at least 60 ml/min/1.73 m^2 received glucocorticoid monotherapy for six months [14, 15]. Patients with an eGFR of 30-59 ml/min/1.73 m^2 received a combination of prednisolone and oral cyclophosphamide followed by azathioprine.

Urine samples from randomized individuals were obtained at the beginning of the run-in phase. Measurements of NGAL, KIM-1, calprotectin, and TIMP2•IGFBP7 were performed in the available urine samples. Urine samples were centrifuged and stored frozen (-80°C) until measurements were performed. We refrained from normalization of biomarker concentrations to urinary creatinine concentrations, since our previous findings indicated no improvement of the diagnostic performance [13]. Biomarker concentrations were compared between subjects reaching vs. not reaching the following individual endpoints: full clinical remission, eGFR loss ≥15 and 30 ml/min/1.73 m^2 over the 3-year trial phase of STOP-IgAN.

Measurement of calprotectin, NGAL and KIM-1 concentrations in the urine

Urine concentrations of calprotectin were quantified using an enzyme-linked immunosorbent assay (ELISA) kit (PhiCal® Calprotectin, Immundiagnostik AG, Bensheim, Germany) according to the manufacturer's protocol as published previously [11, 12]. Concentrations of urinary NGAL were also assessed using ELISA (NGAL Rapid ELISA-Kit, BioPorto Diagnostik, Gentofte, Denmark). This assay had underwent a clinical validation prior to the current study [16]. Urinary KIM-1 concentrations were measured using a KIM-1 (human) ELISA kit (Enzo Life Sciences GmbH, Lörrach, Germany).

Measurement of the product of tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 [TIMP2•IGFBP7] in the urine

The urine concentrations of TIMP-2 and IGFBP7 were measured using the NephroCheck™ Test (Astute Medical, San Diego, CA, USA). [TIMP-2]•[IGFBP7] indicates the product of the respective urinary concentration of both biomarkers that is automatically calculated by the ASTUTE140® Meter. The product is divided by 1, 000 to report a single numerical test result with a unit of $(ng/mL)^2/1000$, the unit for all [TIMP-2]•[IGFBP7] values in this report.

Statistical analysis

Data are presented as median and interquartile range (IQR). Biomarker concentrations of those subjects reaching or not reaching an endpoint were compared by Mann-Whitney U test. Receiver-operating characteristic (ROC) curves were formed in an attempt to determine the accuracy of the individual biomarkers in predicting an endpoint in the overall study population. The change of eGFR during the study population was analyzed for a potential association with urinary biomarkers separately in the supportive care and immunosuppression group by Spearman correlation analyses. Using logistic regression analyses with the individual biomarker as continuous influence factor, odds ratios (OR) were calculated to indicate the relative risks for each endpoint. Eventually, ORs were displayed as likelihood to reach the individual endpoint by an increase in the NGAL unit of 500 pg/ml, in the KIM-1 unit of 500 pg/ml, in the calprotectin unit of 500 ng/ml, and in the [TIMP-2]•[IGFBP7] unit of 1 ng^2/ml^2. All statistical analyses were performed with SAS (Version 9.4, SAS Institute Inc, Cary, NC, USA).

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Results

Urine samples at the beginning of the run-in phase were available from 113 STOP-IgAN participants, i.e. 70% of patients who were eventually randomized into the 3-year trial phase. Table 1 gives demographic and clinical characteristics of the analyzed cohort that were largely similar to the entire STOP-IgAN cohort [3]. Urinary NGAL, KIM-1, calprotectin, and [TIMP-2]•[IGFBP7] were detectable in all these patients. Table 2 provides the urinary biomarker concentrations of the overall study population, the supportive care group and the group with additional immunosuppression. In the overall study population, the concentrations of none of these biomarkers differed in subjects reaching vs. not reaching full clinical remission or a eGFR loss ≥15 or 30 ml/min/1.73 m² over the 3-year trial phase of STOP-IgAN, respectively (p>0.05 each). Separate analyses of the supportive care and the immunosuppression group also did not yield significant differences in biomarker concentrations (p>0.05 each).

Using logistic regression analyses with the respective biomarker as continuous variable, odds ratios (OR) were calculated. Table 3 presents the results and shows that none of the biomarkers was significantly associated with any of the three endpoints. This finding was consistent in the overall study population, the supportive care group, and the group with additional immunosuppression.

The prognostic accuracy of the four biomarkers in predicting renal

Table 1. Characteristics of analyzed patients at the beginning of the run-in phase

Characteristics	Supportive care (n=58)	Supportive care plus immunosuppression (n=55)
Female sex - %	21	27
Smoker - %	17	15
Age - yr	45 ± 12.5	43.5 ± 12.7
Body-mass index	27.9 ± 4.5	27 ± 4.5
Blood pressure – mmHg		
systolic	132.7 ± 14.8	129.8 ± 12
diastolic	84.3 ± 10.8	80.9 ± 9.3
Serum creatinine - mg/dl	1.5 ± 0.5	1.5 ± 0.5
eGFR - ml/min/1.73 m²	60.9 ± 24.7	62.9 ± 28
Urinary protein excretion rate – g/day	2.3 ± 1.2	2.4 ± 1.6
Protein-to-creatinine ratio – g/g	1.6 ± 1.7	1.6 ± 1.1
Cholesterol - mg/dl	213 ± 45.8	212 ± 58.4

Table 2. Urinary biomarker concentrations and the occurence of endpoints during the 3 year study period of the STOP-IgAN study. Neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), product of tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 (TIMP2•IGFBP7). Endstage renal disease (ESRD). Full clinical remission, defined as urinary protein-creatinine ratio < 0.2 and loss of eGFR < 5 ml/min per 1.73 m² of body-surface. Numeric data are presented as median and interquartile range (IQR). Biomarker concentrations were compared by Mann-Whitney U test. P<0.05 was regarded statistically significant

Endpoint	NGAL (ng/ml)	KIM-1 (ng/ml)	Calprotectin (ng/ml)	TIMP2•IGFBP7 (ng²/ml²)
Overall study population	n=112 21.1 (20.5)	n=113 1.6 (2.8)	n=111 54.4 (204.7)	n=105 0.13 (0.22)
eGFR loss ≥ 15 ml/min/1.73 m² (yes vs. no)	n=22 vs. 85 20.3 (19.9) vs. 21.1 (22.8), p=0.36	n=22 vs. 86 1.4 (2.9) vs. 1.7 (2.8), p=0.70	n=22 vs. 84 48.9 (113.5) vs. 58.6 (270.5), p=0.39	n=19 vs. 81 0.07 (0.16) vs. 0.15 (0.22), p=0.23
eGFR loss ≥ 30 ml/min/1.73 m² (yes vs. no)	n=12 vs. 95 14.4 (18.3) vs. 21.1 (219.0), p=0.35	n=12 vs. n=96 1.2 (3.9) vs. 1.7 (2.8), p=0.64	n=12 vs. 94 1231.6 (3869.6) vs. 59.5 (268.1), p=0.19	n=10 vs. n=90 0.06 (0.09) vs. 0.15 (0.22), p=0.09
Full clinical remission (yes vs. no)	n=14 vs. 85 13.8 (24.1) vs. 22.8 (20.2), p=0.19	n=14 vs. n=86 2.0 (2.9) vs. 1.5 (2.6), p=0.38	n=14 vs. 84 49.4 (448.4) vs. 57.7 (187.0), p=0.84	n=13 vs. 80 0.17 (0.19) vs. 0.12 (0.26), p=0.23
Supportive-care group	n=58 24.9 (23.8)	n=58 1.5 (3.3)	n=58 50.9 (153.4)	n=54 0.13 (0.27)
eGFR loss ≥ 15 ml/min/1.73 m² (yes vs. no)	n=10 vs. 45 16.8 (20.2) vs. 25.1 (30.7), p=0.58	n=10 vs. 45 1.3 (3.7) vs. 1,7 (3,1), p=0,69	n=10 vs. 45 22.4 (272.5) vs. 58.0 (150.7), p=0.42	n=9 vs. 42 0.07 (0.14) vs. 0.13 (0.27), p=0.55
eGFR loss ≥ 30 ml/min/1.73 m² (yes vs. no)	n=5 vs. 50 13.5 (4.0) vs. 25.8 (26.0), p=0.18	n = 5 vs. 50 0.9 (4.8) vs. 1.5 (3.1), p=0.85	n=5 vs. 50 16.7 (6.6) vs. 58.9 (161.3), p=0.18	n=4 vs. 47 0.04 (0.06) vs. 0.13 (0.27), p=0.02
Full clinical remission (yes vs. no)	n=3 vs. 48 35.5 (29.7) vs. 25.1 (28.0), p=0.48	n=3 vs. 48 1.3 (4.0) vs. 1.5 (3.2), p=0.69	n=3 vs. 48 140.4 (476.9) vs. 31.0 (145.4), p=0.19	n= 3 vs. 45 0.28 (0.55) vs. 0.12 (0.27), p=0.19
Immunosuppression group	n=54 20.2 (18.2)	n=55 1.7 (2.5)	n=53 57.4 (272.5)	n=51 0.15 (0.20)
eGFR loss > 15 ml/min/1.73 m² (yes vs. no)	n=12 vs. 40 21.8 (15,9) vs. 19.2 (19.5), p=0.57	n=12 vs. 41 1.6 (2.4) vs. 1.7 (2.5), p=0.94	n=12 vs. 39 61.5 (83.1) vs. 59.2 (395.1), p=0.73	n=10 vs. 39 0.08 (0.15) vs. 0.18 (0.22), p=0.31
eGFR loss ≥ 30 ml/min/1.73 m² (yes vs. no)	n=7 vs. 45 22.8 (22.3) vs. 19.4 (18.2), p=0.92	n=7 vs. 46 1.5 (3.0) vs. 1.8 (2.5), p=0.69	n=7 vs. 44 57.4 (108.2) vs. 60.3 (357.4), p=0.66	n= 6 vs. 43 0.09 (0.36) vs. 0.17 (0.19), p=0.80
Full clinical remission (yes vs. no)	n=11 vs. 37 12.4 (11.4) vs. 21.1 (16.6), p=0.05	n=11 vs. 38 2.0 (3.1) vs. 1.4 (2.0), p=0.41	n=11 vs. 36 37.7 (449.8) vs. 71.3 (254.8), p=0.29	n=10 vs. 35 0.16 (0.15) vs. 0.11 (0.25), p=0.60

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outcome parameters was additionally tested by ROC analyses in the overall study population. Fig. 1 illustrates the corresponding curves for full clinical remission. NGAL achieved an area under the curve (AUC) of 0.611, KIM-1 an AUC of 0.574, calprotectin an AUC of 0.483, and [TIMP-2]•[IGFBP7] an AUC of 0.604. The ROC curves for the prediction of a loss of eGFR ≥30 ml/min/1.73 m², are provided in Fig. 2. AUCs were 0.564 for NGAL, 0.527 for KIM-1, 0.560 for calprotectin, and 0.411 for [TIMP-2]•[IGFBP7]. All ROC curves failed to reach statistical significance (p>0.05 each).

Finally, we tested the hypothesis that the concentration of a urinary biomarker might associate with eGFR-loss rates by Spearman correlation analyses, which were performed separately for the supportive care group (Fig. 3) and the group with additional immunosuppression (Fig. 4). None of the chosen biomarkers showed a significant association with eGFR-loss rates in neither group.

Table 3. Association of urinary biomarkers and endpoints in the individual groups. Biomarker concentrations were assessed at baseline in the overall study population Logistic regression analysis for the individual binary endpoints. Neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), product of tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 (TIMP2•IGFBP7). Endstage renal disease (ESRD). Protein-to-creatinine-ratio (PCR). Full clinical remission: PCR < 0.2 and loss of eGFR < 5 ml/min per 1.73 m² of body-surface. P<0.05 was regarded statistically significant. Odds ratios (OR) were calculated with a NGAL unit of 500 pg/ml, a KIM-1 unit of 500 pg/ml, a calprotectin unit of 500 ng/ml and a TIMP2•IGFBP7 unit of 1 ng²/ml² (i.e. with each increase of the indicated serum level in this marker the likelihood to reach this endpoint is equal to the indicated OR)

Endpoint	NGAL		KIM-1		Calprotectin		TIMP2•IGFBP7	
	OR	p-value	OR	p-value	OR	p-value	OR	p-value
Overall study population (n=164)								
eGFR loss ≥ 15 ml/min/1.73 m²	0.99	0.20	0.99	0.82	0.64	0.30	1.06	0.40
Progression to ESRD	1.00	0.61	1.03	0.61	0.39	0.36	1.04	0.69
Full clinical remission	1.00	0.46	1.06	0.20	0.95	0.75	1.09	0.23
Supportive care group								
eGFR loss ≥ 15 ml/min/1.73 m²	0.99	0.34	0.98	0.81	0.74	0.61	1.15	0.18
Progression to ESRD	0.98	0.34	1.10	0.42	0.71	0.73	0.23	0.31
Full clinical remission	1.00	0.81	1.02	0.88	1.10	0.86	1.09	0.54
Immunosuppression group								
eGFR loss ≥ 15 ml/min/1.73 m²	0.99	0.40	0.99	0.89	0.55	0.33	0.99	0.92
Progression to ESRD	1.00	0.92	1.00	0.96	0.14	0.38	1.11	0.26
Full clinical remission	0.99	0.42	1.06	0.26	0.91	0.66	1.09	0.32

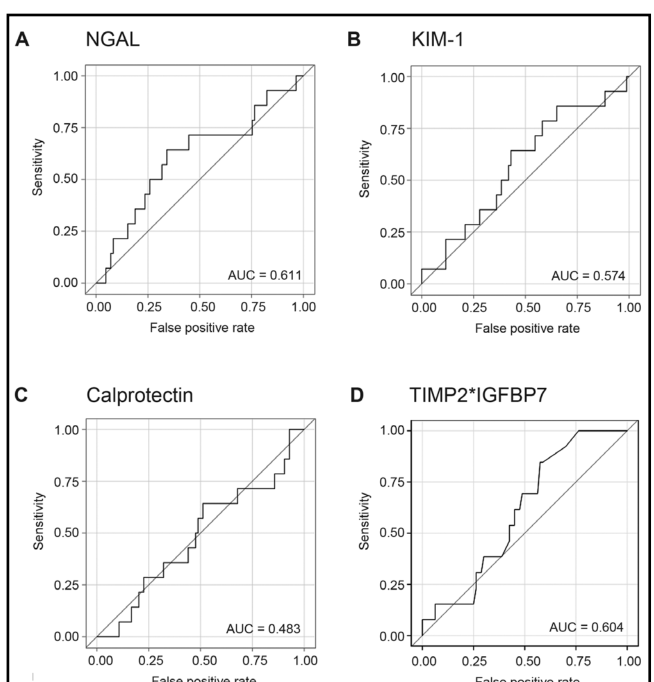

Fig. 1. Receiver operating characteristic curves of (A) neutrophil-gelatinase associated lipocalin (NGAL), (B) kidney injury molecule-1 (KIM-1), (C) calprotectin, and (D) the product of tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 (TIMP2•IGFBP7) for the prediction of reaching full clinical remission in the overall study population; p-values for all AUCs were >0.05.

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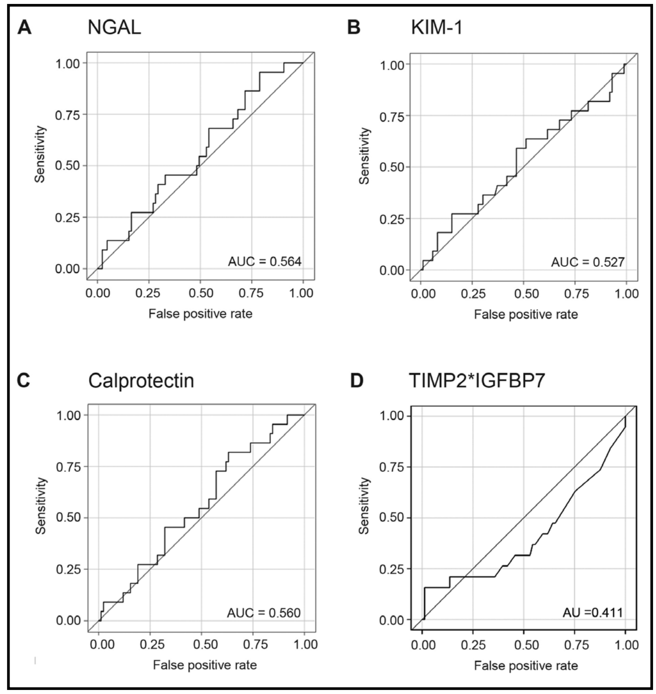

Fig. 2. Receiver operating characteristic curves of (A) neutrophil-gelatinase associated lipocalin (NGAL), (B) kidney injury molecule-1 (KIM-1), (C) calprotectin, and (D) the product of tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 (TIMP2•IGFBP7) for the prediction of an eGFR loss ≥ 15 ml/min/1.73 m^2 in the overall study population; p-values for all AUCs were >0.05.

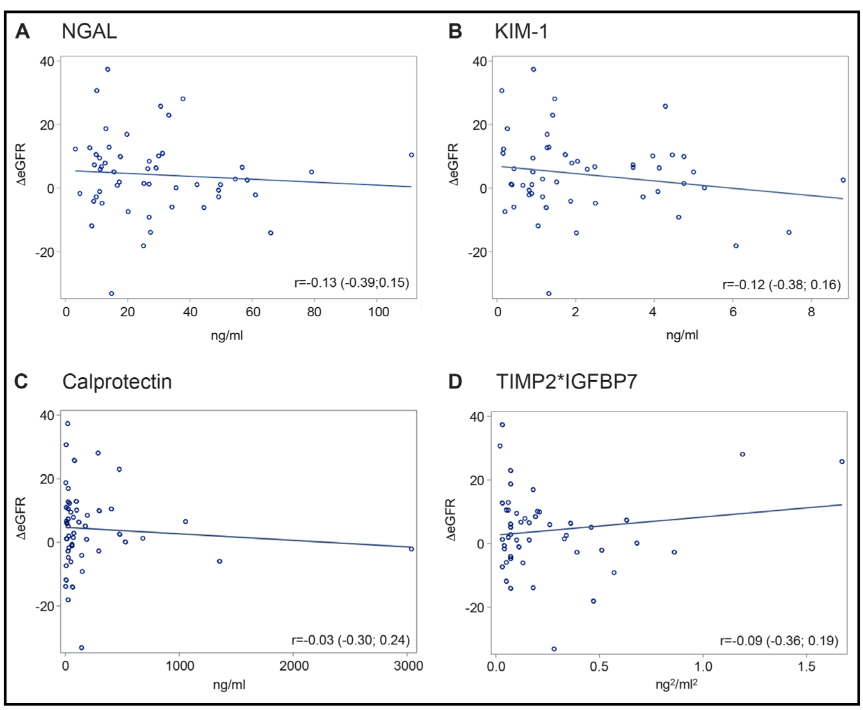

Fig. 3. Association of loss of estimated glomerular filtration rate (eGFR) and urinary biomarker concentrations in patients with sole supportive care during the 3-year study period for (A) neutrophil-gelatinase associated lipocalin (NGAL), (B) kidney injury molecule-1 (KIM-1), (C) calprotectin, and (D) the product of tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 (TIMP2•IGFBP7). Spearman correlation coefficient r and 95% confidence intervals (in brackets) are provided, *p<0.05 was regarded significant.

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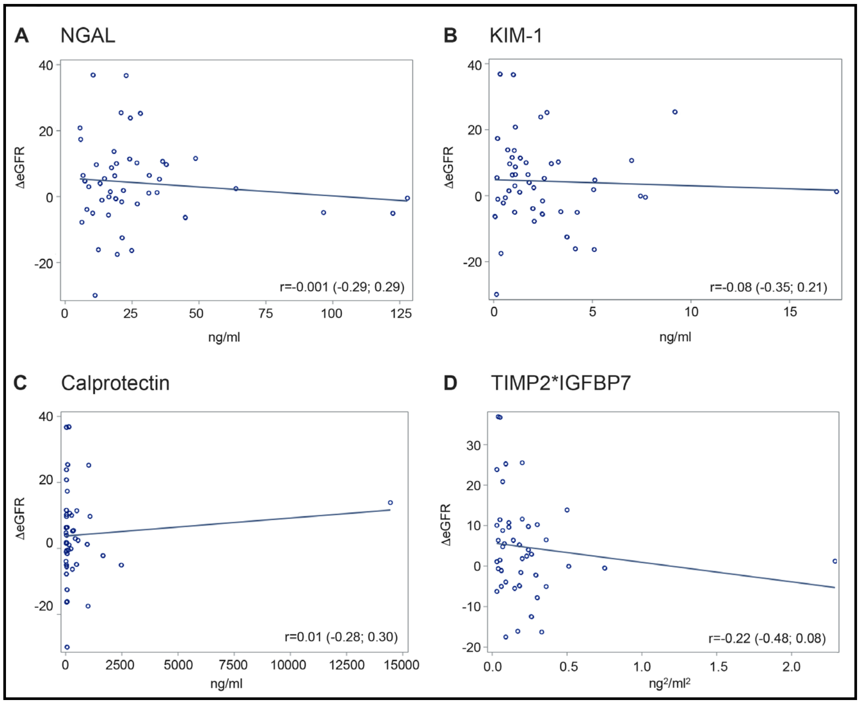

Fig. 4. Association of loss of estimated glomerular filtration rate (eGFR) and urinary biomarker concentrations in patients with supportive care plus immunosuppression during the 3-year study period for (A) neutrophil-gelatinase associated lipocalin (NGAL), (B) kidney injury molecule-1 (KIM-1), (C) calprotectin, and (D) the product of tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 (TIMP2•IGFBP7). Spearman correlation coefficient r and 95% confidence intervals (in brackets) are provided, *p<0.05 was regarded significant.

Discussion

NGAL, KIM-1, and [TIMP-2]•[IGFBP7] were chosen as candidate biomarkers for an assessment of the tubulointerstitial damage associated with IgAN, calprotectin was selected as a candidate molecule to assess the extent of the inflammatory activity of the disease. In urine samples obtained at enrollment into the STOP-IgAN study, i.e. before supportive care optimization was initiated, however, all of the chosen biomarkers failed to predict the progression of the disease or to identify subjects who benefit from immunosuppressive therapy added on top of supportive treatment.

The lack of prognostic information on disease progression was consistent in the three statistical approaches that were used in this study: There were no significant differences in the biomarker concentrations of those subjects reaching vs. not reaching full clinical remission, nor between subjects reaching or not reaching adverse renal endpoints. It is very unlikely that the different treatment strategies (supportive alone versus additional immunosuppression) led to this finding, since analysis of biomarker concentrations in the individual groups revealed concordant results. This finding was confirmed by logistic regression analyses, yielding no association of biomarker concentrations and the above mentioned endpoints either. At this point, one might speculate that an association of baseline biomarker concentrations and loss of GFR might have been overlooked due to the sharp definition of the endpoint “full clinical remission” and the secondary endpoint “GFR loss ≥30 ml/min/1.73 m^2”. Therefore, we added the Spearman correlation analysis as a third statistical approach. This, however, was in line with the previous statistical approaches and also failed to show any significant association of biomarker concentrations and loss of renal function.

The most likely explanation for the lack of prognostic information of urinary NGAL, KIM-1, and [TIMP-2]•[IGFBP7] may be found in the nature of IgAN. As long as disease is limited to the glomerulus and has a low degree of accompanying tubulointerstitial inflammation, these biomarkers are unable to reflect the extent of tissue damage in the urine. NGAL is a 25-kDa protein of the lipocalin family that is widely expressed at very low and constant baseline levels in different cell types including neutrophils and epithelial cells. In the kidney, the primary production site is the distal tubule [17]. Circulating NGAL is filtered in the glomerulus and reabsorbed in the proximal tubule. Kidney injury leads to an upregulation with apical and basolateral secretion of NGAL by tubular epithelial cells [18, 19]. The molecular weight of NGAL is far below the cut-off of the slit membrane (69 kDa). Hence, an increase in glomerular permselectivity, e.g. in glomerular disease, will lead to an increase of NGAL filtration. This might explain the absent statistical association between the extent of glomerular damage and urinary NGAL concentration in the present IgAN cohort. Indeed, the median urinary NGAL concentration of 21.1 ng/ml was only mildly elevated compared to healthy adults [20]. Manifest tubular injury is usually associated with urinary NGAL concentrations >50 ng/ml, KIM-1 concentrations >5-10 ng/ml, and calprotectin concentrations >200 ng/ml [11, 12, 21, 22]. Hence, the collateral acute tubular damage in the present population has to be regarded as rather low.

KIM-1 is a 39-kDa type I transmembrane glycoprotein with an extracellular immunoglobulin-like domain and therefore passes the slit membrane as well [23]. Acute kidney injury is associated with a largely increased expression in proximal tubule cells [24, 25]. KIM-1 has been shown to independently predict disease progression and negative renal outcomes in IgAN patients [6]. TIMP-2 and IGFBP7 have a molecular weight of 21 and 29 kDa and are upregulated in the early phase of tubular injury caused by a wide variety of reasons (inflammation, ischemia, drugs, and toxins) [26]. These two biomarkers outperformed NGAL and KIM-1 in the prediction of acute kidney injury in some critical care populations [27, 28]. Of note, NGAL, KIM-1 and [TIMP-2]•[IGFBP7] are well-established markers for acute kidney injury, but not yet for an active glomerulonephritis.

The STOP-IgAN trial did not show a beneficial effect of immunosuppression on GFR loss. An individualized approach with a biomarker-based identification of those subjects who are indeed likely to benefit from immunosuppression is therefore highly desirable. All the four tested biomarkers, however, failed to do so in our cohort. They did neither identify subjects with favorable nor with adverse outcome after initiation of immunosuppression. This is somewhat disappointing, since calprotectin and NGAL had appeared as promising candidates in this context given that both are mediators of the innate immune system and are thereby highly increased after epithelial damage and an accompanying inflammation. The present data from our STOP-IgAN sub-cohort suggest that the tubulointerstitial damage and inflammation in IgAN are not indicative for the response to anti-inflammatory treatment. Based on these findings, the future search for alternative biomarkers should probably focus on indicators of glomerular rather than tubulointerstitial inflammation.

The strength of this work is the use of urine samples obtained from a controlled randomized trial with a very systematic follow-up of renal outcome data. It is nevertheless limited by the study size. Moreover, leukocyturia is a relevant bias for the diagnostic accuracy of calprotectin and NGAL, since both are produced by neutrophils. However, only 2.7% of the present population were positive for leukocytes in the dipstick examination at the time point of urine sample retrieval, thus we consider this phenomenon to be of minor relevance for the poor prognostic performance.

Conclusion

NGAL, KIM-1, calprotectin, and [TIMP-2]•[IGFBP7] had neither a prognostic value for the progression of IgA nephropathy, nor for the response to immunosuppression in the STOP-IgAN trial. The search for appropriate biomarkers for an individualized strategy for the treatment of IgA nephropathy needs to continue.

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J.N. performed the statistical analyses and wrote the manuscript. F.B., F.S. and A.D. performed the biomarker measurements, C.F. and R.D.H. supervised the statistical analyses. J.F. and F.E. designed and initiated the main STOP-IgAN trial. T.R. and T.H.W. designed and supervised the project and revised the manuscript.

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Disclosure Statement

Patent for "Assay method for intrinsic acute kidney injury (PCT/EP2012/056754)" is granted to T.H.W.

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