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Development of a multiple urinary biomarker model to predict the tubulointerstitial fibrosis area in patients with primary IgA Nephropathy

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Abstract

Background Previous studies highlighted the utility of individual urinary biomarkers in the prediction of interstitial fibrosis in IgA Nephropathy patients. However, it´s uncertain which biomarker or combination of biomarkers provides a more accurate estimation of renal interstitial fibrosis Surface. Herein, we measured the urinary excretion of a set of seven tubular injury biomarkers in a group of patients with primary IgA Nephropathy and analyzed their utility as non-invasive estimators of interstitial fibrosis area found on kidney biopsy.

Methods Two hundred forty-seven adults with primary IgA Nephropathy diagnosed by kidney biopsy and a control group of 50 healthy control were included. The urinary excretion of EGF, MCP-1, NGAL, KIM-1, L-FABP, β2-microglobulin and DKK-3 was measured in urine samples collected at the day of the renal biopsy. Estimated glomerular filtration rate was measured by the CKD-EPI formula. Interstitial fibrosis area was quantified using a quantitative morphometric procedure and graded according to Oxford Classification. Predictive multivariate models were developed to predict the interstitial fibrosis surface.

Results Patients with primary IgA Nephropathy showed significantly higher urinary levels of DKK-3, L-FABP and β2-microglobulin, and lower EGF levels than healthy controls. Interstitial fibrosis was negatively correlated with urinary EGF levels and positively with age, proteinuria, eGFR and urinary DKK-3, L-FABP and β2-microglobulin. The best model to predict interstitial fibrosis area accounted for > 60% of its variability and included age, eGFR, proteinuria, DKK-3, EGF, L-FABP and β2-microglobulin.

Conclusions Our study provides a model to estimate the IFS in IgA Nephropathy which could be useful to monitor the progression of chronic kidney injury.

Keywords Renal fibrosis, Urinary biomarkers, IgA nephropathy, DKK-3, EGF, L-FABP, β2m

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Background

Immunoglobulin A Nephropathy (IgAN) is the most prevalent primary glomerulonephritis worldwide [1]. Its clinical course varies from indolent forms with asymptomatic microscopic hematuria to those with rapidly progressive glomerulonephritis. Despite treatment, about 20–40% of patients will progress to end-stage renal disease (ESRD). Poor prognostic factors are: the presence of kidney failure at diagnosis, proteinuria above 1 g/day despite renin–angiotensin–aldosterone system (RAAS) blockade, arterial hypertension [2–5], mesangial C4d deposits [6, 7, 11] and CD68 + tubulointerstitial infiltration of monocytes-macrophages, which correlates with tubulo-interstitial fibrosis (IF) and chronic damage [8]. These glomerular and interstitial lesions have prognostic value in untreated patients, but not in those who have received prior treatment with glucocorticoids or immunomodulators [9, 10].

The Working Group of the International IgA Nephropathy Network and the Renal Pathology Society (RPS) reported that tubulointerstitial injury on kidney biopsy plays an important role in the IgAN progression. Several studies have focused on certain tubular biomarkers as predictors of renal progression in patients with IgAN; thus, interstitial fibrosis (IF) is a powerful predictor of future progression in IgAN [13].

The Oxford Classification of IgAN [12] is the most widely accepted system for evaluating histological features in biopsy specimens with a minimum of 8 glomeruli. The MEST-C score, including mesangial hypercellularity (M), endocapillary cellularity (E), segmental sclerosis (S), interstitial fibrosis/tubular atrophy (T), and crescent (C) lesions, is predictive of clinical renal outcomes [14, 15].

IgAN may progress to end-stage renal disease (ESRD) despite absence of any previous clinical risk factors, therefore, it is not unusual to find severe chronic tubulointerstitial damage in patients with clinically mild IgAN [16]. Furthermore, when renal biopsy it´s performed at the early stages, with an estimated glomerular filtration rate (eGFR) above 60 ml/min, pathological findings might be inconclusive and renal prognosis could be difficult to stablish, therefore, a non-invasive method to follow these patients is needed [17].

Traditional studies have measured urinary levels of various molecules whose excretion varies depending of the tubular injury (ischemic, toxic or inflammatory). Among them, β2-microglobulin is associated with lesion of the proximal convoluted tubule [18]. Other molecules, like kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL) and liver-type fatty acid-binding protein (L-FABP) are secreted physiologically by tubular cells with a very low urinary excretion and are increased when a tubulo-interstitial injury takes place, reflecting IF, which is correlated with renal prognosis [12, 18–21]. Molecules, such as monocyte chemoattractant protein-1 (MCP-1) or dickkopf-3 protein (DKK-3), are recognized IF promoters. Recent findings show how an increase in their urinary excretion may predict the IF surface (IFS) and eGFR decline in the short term in IgAN patients [22, 23]. On the other hand, urinary excretion of epidermal growth factor (EGF), which is produced by the ascending portion of Henle's loop and the distal convoluted tubule, decreases at the same time the interstitial fibrosis and tubular atrophy takes place in these patients [10, 24–26].

Previous studies have highlighted the utility of urinary biomarkers excretion in the prediction of IF in IgAN, but these biomarkers have been analyzed individually. It´s not clear wherever which one alone or in combination provides a more accurate prediction of renal interstitial fibrosis surface (IFS) on biopsies.

In this study, we measured the urinary excretion of a set of seven biomarkers representative of tubular injury (in all the segments of the tubule) in a group of patients with primary IgAN, and analyze their utility to predict IFS observed on kidney biopsy at the time of measurement.

Patients and methods

This study included consecutive patients from 2004 to 2019 attended in two Hospitals: Vall d'Hebron University Hospital (Barcelona, Catalonia, Spain) and Arnau de Vilanova University Hospital (Lleida, Catalonia, Spain), with biopsy proven IgAN. The inclusion criteria were: 1- adult patients over 18 years of age; 2- eGFR > 30 ml/min (using CKD-EPI equation [27]) and; 3- written informed consent. The exclusion criteria were as follows: 1.-secondary forms of IgAN (chronic alcoholism, celiac disease, thyroid disease, inflammatory bowel disease, acute or chronic infections, lymphomas, solid and/or skin tumors); 2.- unrepresentative kidney biopsy (with < 8 glomeruli); 3. eGFR < 30 mL/min/1.73 m^2 at the time of kidney biopsy; 4.- exposure to nephrotoxic drugs and; 5.- lack of informed written consent for participation in the study. At the day of kidney biopsy, we recorded clinical-demographic characteristics of the patients (age, gender, serum creatinine, urine protein excretion measured by protein/creatinine ratio and eGFR). GFR was estimated using the CKD-EPI Eq. (2009).

Urine samples were collected at the day of the kidney biopsy. First-morning spot urine samples were divided into aliquots and each of them was sent to perform the measurement of creatinine, proteinuria and urine sediment, some of them were centrifuged at 1500 g for 10 min within the first 10 min after collection and preserved

at -80 °C for L-FABP, β2-microglobulin, EGF, DKK-3, KIM-1, MCP-1 and NGAL measurement.

Serum creatinine was measured using a compensated IDMS-traceable method (Hitachi Modular P-800 Roche Diagnostics, Berlin, Germany). L-FABP was measured by ELISA from Bio-Rad (Hercules, CA, USA). KIM-1 and DKK-3 were quantified using ELISA kits from R&D Systems (Minneapolis, USA), MCP-1 and NGAL were measured by an ELISA from Abcam (Cambridge, UK); β2-microglobulin was measured by immunoturbidimetry (Pacific Biomarkers, Seattle, USA); EGF urine excretion was measured by an ELISA assay (Human EGF Quantikine ELISA Kit, R&D Systems, Minneapolis, MN, USA). All assays were calibrated with purified standards and reference serums obtained from the manufacturers and duplicated. The concentrations of the biomarkers analyzed were adjusted for urinary creatinine excretion and expressed in mg/g, µg/g, ng/mg or pg/mg. The reproducibility of urinary measurements was assessed by analyzing the coefficients of variation of the studied molecules in a random sample of 10 patients in three different urine samples the same day the biopsy was performed. The variation coefficient was less than 6.8% for all the biomarkers studied.

Reference values for the urinary excretion of each one of the biomarkers were obtained from a control group comprising of 50, age and gender matched, healthy volunteers, mainly hospital staff and blood donors. In this group, we only analyzed the urinary biomarkers studied, none of them were biopsied. All volunteers had a CKD-EPI eGFR > 90 mL/min/1.73m^2, and in all cases, we performed urine sediment and urinary protein excretion. Urine samples with > 3 red blood cells /µL or > 10 leukocytes per high power field or proteinuria/creatinine ratio > 10 mg/g, were excluded. All volunteers signed a questionnaire declaring not suffering from chronic comorbidities and have not received any nephrotoxic drug or any other drug capable to modify urinary creatinine excretion 12 weeks prior to samples collection. A questionnaire with these drugs was included. In case of women at childbearing age, a negative pregnancy test was required. This study adhered to the parameters established by the Declaration of Helsinki and all patients gave their informed consent in writing.

Histopathological analysis of kidney biopsies and quantification of interstitial fibrosis

Kidney tissue samples were fixed in paraffin, stained with different techniques such as hematoxylin–eosin, Masson's trichrome, periodic acid Schiff, and Jones methenamine. Immunofluorescence studies were performed on frozen tissue samples, using antibodies against immunoglobulins A, G and M, fibrinogen, C3 and C1q.

All kidney biopsies were classified according to the Renal Pathological Society (RPS) classification criteria [28]. The quantification of the IFS was carried out using 4 µm sections stained with Masson's trichrome. The image was digitalized and captured using a Pixera Professional VSC 1.2 camera, connected to a Ziess Axioskop 2 microscope and a Macintosh powerbook G3 computer. Histopathologic morphometric analysis was performed with the "QuPath" software [29]. This software incorporates a machine learning interactive system to classify pixels and combines a collection of machine learning algorithms with a set of selected image features to produce pixel-based segmentations. It also contains a collection of visualization tools and algorithms for data analysis and predictive modeling. For detecting interstitial fibrosis, the software applied a technique of separation of interstitial fibrosis areas of the tissue slide based on certain features such as texture, color and density. Once these areas have been classified as "healthy" or fibrous tissue, the software allows for visualization and quantification of fibrosis in each image section of the slide by the pathologist. Results of the IFS, were expressed in percentage and classified in accordance with the Oxford international consensus document (2009) [15].

Statistics

Quantitative variables, were expressed by mean ± standard deviation or median and percentiles 25 and 75. Qualitative parameters were expressed as percentages. Comparison between quantitative and qualitative variables was done by using Student's T test. Correlation analysis between quantitative variables was performed using Pearson's correlation coefficient. With the aim to analyze the relationship between each of the biomarkers studied and the extent of the IFS, an univariate regression analysis was performed, followed by a multiple regression analysis in which IFS was included as a quantitative variable after logarithmic transformation and verification of normality of its distribution. $P < 0.05$ was considered statistically significant and Statistical analysis was performed using the SPSS version 20.0.

Results

Two-hundred and forty-seven patients (127 males and 120 females) with IgA Nephropathy were included. Clinical, demographic and biochemical characteristics are shown in Table 1. Mean eGFR was 65 ml/min, mean age was 38 years and mean proteinuria was 1400 mg/g. The mean of IFS found was 25%.

Correlations between IF and tubular biomarkers are shown in Table 2. Among all analyzed variables, IF showed a significant negative association with eGFR and urinary EGF levels, and a positive correlation with

Table 1 Clinical, demographic and biochemical characteristics of patients

	Patients	Healthy Volunteers	*p*
Gender n (%)[b]	247	50	0.26
Male	127 (51.4)	29 (58)	
Female	120 (48.6)	21 (42)	
Age (years)	38.8 ± 16.06	41.2 ± 19.8	0.44
eGFR (ml/min)	65.09 ± 20.43	109 ± 6.8	< 0.001
Hematuria (red blood cells/μL)[a]	25 (0—48)	0	< 0.001
Urine-spot proteinuria (mg/g creatinine)	1400 ± 1040	6 ± 11	< 0.001
Hypertension (%)[b]	111 (45)	0	< 0.001
SBP (%)	129 (13.2)		
DBP (%)	83 (4.1)		
ACEI (%)	197 (79.7)		
Interstitial fibrosis (%)	25.35 ± 14.84	NA	-
Oxford classification[b]:		NA	-
M1 (%)	125 (50.6)		
E1 (%)	43 (17.4)		
S1 (%)	87 (35.2)		
T0 (%)-T1 (%)-T2 (%)	149 (60.3)-77 (31.2)- 21 (8.5)		
C2 (%)	15 (6.07)		
EGF (ng/mg of creatinine)	34.4 ± 17.2	78.9 ± 13.4	< 0.001
MCP-1 (ng/mg of creatinine)	0.98 ± 0.5	0.15 ± 0.09	< 0.001
ß2-microglobulin (mg/gr of creatinine)	1.98 ± 1.5	0.18 ± 0.1	< 0.001
NGAL (ug/gr of creatinine)	56.82 ± 21.1	14.2 ± 8.9	< 0.001
L-FABP (ng/mg of creatinine)[a]	49.9 (28.3—8252.8)	16.8 (2.5 – 29.6)	< 0.001
KIM-1 (ng/mg of creatinine)[a]	243.3 (146.7- 9342.1)	110 (15.3—132)	< 0.001
DKK-3 (pg/mg of creatinine)	209 ± 22	32 ± 16.1	< 0.001

Continuous variables are expressed as mean ± standard deviation (SD)

eGFR estimated glomerular filtration rate, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *ACEI* number of patients under treatment with angiotensin converting enzyme inhibitors, *M1* mesangial hypercellularity, *E1* endocapillary hypercellularity, *S1* segmental glomerulosclerosis, *T0* tubular atrophy < 25% tubules, *T1* tubular atrophy 26–50% tubules, *T2* tubular atrophy > 50% tubules, *C2* crescents in > 25% glomeruli, *EGF* Epidermal Growth Factor, *MCP-1* Monocyte chemoattractant protein-1, *β2-microglobulin* Beta2-microglobulin, *L-FABP* Liver fatty acid-binding protein, *KIM-1* Kidney Injury Molecule-1, *DKK-3* Dickkopf-3

[a] Continuous variables are expressed as median (interquartile range)

[b] Qualitative data are expressed as an absolute frecuency (percentage)

age, proteinuria and urinary levels of DKK-3 (being the most relevant), MCP-1, L-FABP, β2m, NGAL and KIM-1.

Table 3 shows the best predictive model for IF in multiple regression analysis. This model included age, proteinuria, eGFR, DKK-3, EGF, L-FABP and β2m and accounted for more than 60% of variability of IFS. Among them, DKK-3, EGF and, in a lesser degree, L-FABP, were the urinary biomarkers who showed the highest values of standardized coefficients. DKK-3 was the biomarker accounting for the greatest percentage (42%) of IFS variability. The addition of the urinary excretion of EGF, L-FABP and β2-microglobulin to age and proteinuria improved the strength of the model, increasing the percentage of the explained variability nearly twenty points (Table 4).

Discussion

In this cross-sectional study, we analyzed the ability of a set of seven biomarkers indicative of tubular injury to predict (in a non-invasive fashion) the extent of IFS in patients with primary IgAN. It´s noteworthy that, despite being patients with a relative preserved renal function, a quarter of the biopsies showed an IFS greater than 25% of the cortical area. We observed significant associations between the IFS and the urinary levels of DKK-3, EGF, L-FABP and β2-microglobulin. A multivariate model that included age, proteinuria and urinary excretion of DKK-3, EGF, L-FABP and β2-microglobulin as independent predictors of the IFS. DKK-3 was the biomarker accounting for the greatest percentage (42%) of IFS variability. The introduction of age, proteinuria and urinary excretion of EGF, L-FABP

Table 2 Pearson´s correlation matrix among clinical and biochemical variables

	GFR	Age	Prot	EGF	β-2M	NGAL	LFABP	DKK3	KIM1	MCP1
%IF	**-0.46**[1]	**0.28**[1]	**0.34**[1]	**-0.65**[1]	**0.21**[2]	**0.19**[3]	**0.35**[1]	**0.68**[1]	**0.16**[6]	**0.35**[1]
GFR		-0.04	-0.1	**0.37**[1]	**-0.25**[1]	**-0.18**[4]	**-0.26**[1]	**-0.43**[1]	**0.16**[2]	**-0.27**[1]
Age			**0.27**[1]	**-0.24**[1]	**-0.09**	-0.007	**0.01**	**0.17**[5]	-0.03	0.06
Prot				**-0.26**[1]	**-0.22**[1]	0.1	**0.32**[1]	**0.26**[1]	-0.04	**0.42**[1]
EGF					**-0.19**[3]	**-0.21**[2]	**-0.35**[1]	**-0.48**[1]	-0.05	**-0.36**[3]
β-2M						**0.23**[1]	**0.15**[7]	**0.25**[1]	**0.1**[2]	-0.04
NGAL							**0.23**[1]	**0.18**[4]	**0.4**[1]	**0.12**[9]
LFABP								**0.22**[1]	**0.4**[8]	**0.53**[1]
DKK3									**0.17**[5]	**0.35**[1]
KIM1										0.1

Pearson´s correlation coeficients

% IF interstitial fibrosis (in %), *GFR* estimated glomerular filtration rate, *Prot* urine-spot proteinuria, *β-2M* β2-microglobulin

The statistically significant correlations are shown in bold characters

[1] $p < 0.001$

[2] p: 0.001

[3] p: 0.002

[4] p: 0.004

[5] p: 0.006

[6] p: 0.008

[7] p: 0.013

[8] p: 0.032

[9] p: 0.049

and β2-microglobulin improved the strength of the model, increasing the percentage of the explained variability [30, 31].

DKK-3 is involved in signaling processes through the Wnt/β-catenin pathway, when faced with cellular stress, induces renal fibrosis in different experimental models. Previous studies [22], show that an elevated DKK3-to-creatinine urine ratio was significantly associated with a higher degree of IF in the biopsy specimens of both patients with glomerular and/or interstitial kidney diseases, but this association was independent of eGFR, proteinuria, or type of kidney disease. In IgAN patients, a rise in urinary DKK3-to-creatinine concentrations was associated with a significant eGFR decline, whereas stable or decreasing urinary DKK3-to-creatinine levels indicated a more favorable course, therefore predicting eGFR loss. The data observed in our study are in concordance with previous studies and clearly highlight the potential value of measuring urinary levels of DKK-3 to estimate the IFS in IgAN patients.

Urinary levels of L-FABP [21] and β2-microglobulin [18], are both mainly related to proximal convoluted tubule injury. In our study, the excretion of both molecules, were found to be significantly and positively correlated with each other. In the step-by-step selection model, both were identified as independent predictors of IFS, with similar weight, after adjusting for the rest of variables. These data highlight the role of proximal tubular injury in the process of interstitial fibrosis and indicates that each biomarker provides different and complementary information. Although both showing significant correlations with IFS in the univariate analyses, neither urinary KIM-1, related to proximal tubule injury, nor NGAL excretion, a biomarker indicative of both proximal and a distal tubular injury [16, 19, 20, 32–41], were identified as independent predictors of the IFS in the multivariate model.

EGF, produced by the ascending portion of Henle's loop and by the distal convoluted tubule, seems to modulate tissue responses to injury in kidneys with tubulointerstitial damage [40]. In previous publications [9, 10, 23–25], we and others, reported a decrease in its renal expression and a subsequent decrease in its excretion correlating with renal disease progression. The data of our current study concurs with the bulk of evidence provided so far, and identifies EGF as a marker for IFS, providing aditional value above eGFR measurement itself.

MCP-1 has been identified as one of the molecules directly involved in the recruitment of inflammatory cells to the renal interstitium both in experimental models and patients with glomerular diseases, including IgA nephropathy [43, 44]. In our patients, MCP-1 correlated significantly with proteinuria, IFS and eGFR, in agreement with the data described in previous studies [42–46].

Table 3 Independent predictors of interstitial Fibrosis (constant) in multiple regression model

Variables		Unstandarized Coefficients		Standarized Coefficients	t	p	95% IC	
		B	Std. Error	Beta			Lower Bound	Upper Bound
(Constant)		27.94	3.622		7.714	0.000	20.8	35.07
DKK3		0.002	0.000	0.328	6.48	0.000	0.002	0.003
EGF		-0.267	0.042	-0.315	-6.335	0.000	-0.351	-0.184
LFABP		0.003	0.001	0.130	3.013	0.003	0.001	0.004
eGFR		-0.098	0.033	-0.138	-2.973	0.003	-0.163	-0.033
Age		0.090	0.038	0.099	2.349	0.020	0.15	0.166
β2m		1.205	0.126	0.126	2.638	0.009	0.305	2–105
Proteinuria		1.349	0.098	0.098	2.104	0.036	0.086	2.611
Model	R	R^2	Adjusted R^2	Std. Error	Change Statistics				
					R^2 change	F change	df1	df2	p
1	0.650[a]	0.422	0.420	11.133	0.42	173.32	1	237	0.000
2	0.750[b]	0.563	0.559	9.707	0.14	75.78	1	236	0.000
3	0.768[c]	0.590	0.585	9.415	0.028	15.86	1	235	0.000
4	0.779[d]	0.607	0.600	9.244	0.016	9.75	1	234	0.002
5	0.785[e]	0.617	0.608	9.149	0.10	5.90	1	233	0.016
6	0.789[f]	0.623	0.613	9.092	0.006	3.89	1	232	0.042
7	0.794[g]	0.630	0.619	9.026	0.007	4.42	1	231	0.036

MODELS:

[a] Predictors: DKK-3

[b] Predictors: DKK-3, EGF

[c] Predictors: DKK-3, EGF, L-FABP

[d] Predictors: DKK-3, EGF, L-FABP, eGFR

[e] Predictors: DKK-3, EGF, L-FABP, eGFR, Age

[f] Predictors: DKK-3, EGF, L-FABP, eGFR, Age, β2-microglobulin

[g] Predictors: DKK-3, EGF, L-FABP, eGFR, Age, β2-microglobulin, Proteinuria

Table 4 Best predictive model for interstitial fibrosis surface in our primary IgA nephropathy cohort

	ß	IC 95%	t	*p*
Age	0.09	0.015 – 0.16	2.34	0.02
eGFR	-0.13	-0.16 – -0.033	-2.97	0.003
Proteinuria	0.09	0.08 – 2.61	2.1	0.036
DKK3	0.32	0.002 – 0.003	6.48	< 0,001
EGF	-0.31	-0.35 – -0.18	-6.33	< 0.001
LFABP	0.13	0.001 – 0.004	3.03	0.003
ß2-microglobulin	0.12	0.30 – 2.1	2.63	0.009

However, MCP-1 showed significant associations with other biomarkers such as L-FABP, DKK3 and EGF, which in the univariate analysis, had a greater association with IFS than MCP-1. When analyzed in a multivariate context, MCP-1 excretion was not identified as an independent predictor for IFS.

Compared with previous studies, the most relevant feature of our study is, that an analysis of a set of biomarkers of tubular dysfunction measured at the time of kidney biopsy was performed, allowing for comparison of the predictive value of each biomarker and, through a multivariate model, their performance among clinical variables for a more accurate estimation of the IFS.

The IFS is considered one of the variables with the greatest long-term prognostic value in IgAN [8–10]. Since the effective filtration area is usually reduced in parallel with the increase in the IFS, eGFR is currently considered to be the best surrogate measure of the IFS in clinical practice. The results of our study, however, clearly indicate that the measurement of the urinary excretion of DKK-3, EGF, L-FABP and β2-microglobulin, complements the information provided by the eGFR and allows for a significantly more precise estimate of IFS. Consequently, a longitudinal design on future studies is needed to show the true clinical relevance in clinical practice.

Our results have some limitations. First, all measurements were made in spot urine samples, and its consistency with total excretion in 24-h urine was not analyzed; therefore, results could differ depending on the urine sample used. Although both analytical and within-subject

variability in repeated measurements was low (< 6.8%) for all biomarkers, a more detailed knowledge of the biological variability of the urinary excretion of the different molecules studied is of crucial importance before considering their potential applications into clinical practice. Second, we focused on analyzing predictive models of IFS in patients with IgAN. We do not have data on the performance of this set of biomarkers to predict IFS in other types of glomerular disease. In future studies, we aim to analyze the behavior of the set of biomarkers studied in predicting the IFS in other types of kidney disease. The cross-sectional design of the study is also a limitation, and a more reliable assessment would be provided by a longitudinal study. Lastly, our results lack of external validation, hence, so far, our data must be considered valid only for the group of patients included in this study.

Conclusions

In summary, our data indicate that, in IgAN, the amount of IFS can be accurately estimated (non-invasively) by a multivariate model including age, proteinuria, eGFR and urinary concentrations of DKK-3, EGF, L-FABP and β2-microglobulin. Among the biomarkers studied, DKK-3 and EGF were the ones who provided a better estimation of the IFS in patients with mild IgAN. The results of this study could indicate that the measurement of these urinary biomarkers complements the information provided by the eGFR and allows for a significantly more precise estimation of IFS. Prospective studies analyzing the performance of this model as an early prognostic biomarker of renal progression of IgAN are needed.

Abbreviations

IgAN	IgA Nephropathy
IF/IFS	Interstitial Fibrosis/Interstitial Fibrosis Surface
GFR/eGFR	Glomerular Filtration Rate/estimated Glomerular Filtration Rate (by CKD-EPI)
ESRD	End-Stage Renal Disease
RPS	Renal Pathological Society
NGAL	Neutrophil Gelatinase-Associated Lipocalin
KIM-1	Kidney Injury Molecule-1
β2-m	βEta2-microglobulin
L-FABP	Liver-type Fatty Acid-Binding Protein
MCP-1	Monocyte Chemoattractant Protein-1
DKK-3	Dickkopf-3 protein
EGF	Epidermal Growth Factor

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Authors' contributions

Jorge González Rodríguez wrote the manuscript and did the statistical analysis, Jose Manuel Valdivielso Revilla revised the manuscript, Elías Jatem Escalante revised the manuscript, Mercè Borràs Sans revised the manuscript, Alicia García Carrasco did the data collection and measurements of each urinary biomarker, Jacqueline Del Carpio Salas revised the manuscript, Andrea Muijsemberg Alcalá revised the manuscript, Miquel Pinyol Ribas reviewed the renal biopsies, Elena Ostos-Roldán did the data collection and measurements of each urinary biomarker, Alfons Segarra Medrano designed the study and performed the statistical analysis and Maria Luisa Martín Conde co-designed the study and revised the manuscript.

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Data availability

Data will be available from the corresponding author after reasonable request.

Declarations

Ethics approval and consent to participate

Dr. José-Bruno Montoro Ronsano, Secretary from Vall d'Hebron's University Hospital Research Institute in Barcelona (Spain), and its Ethical Committee, CERTIFICATES the aprovement of the Clinical Study with clinical code number SEG-CUC-2003–01 titled: "Analysis of urine and serum biomarkers related to the interstitial and glomerular fibrosis surface on glomerular and interstitial nephropathies". This study adhered to the parameters established by the Declaration of Helsinki. All patients gave their informed consent in writing.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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