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Association of redox and inflammation-related biomarkers with prognosis in IgA nephropathy: A prospective observational study

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ABSTRACT

Background: IgA nephropathy (IGAN) has a variable prognosis. Risk stratification tools are usually based on clinical parameters combined with histologic Oxford-MEST-C score. Circulating redox- and inflammation-related biomarkers may be related to histological changes in IGAN. Therefore, we studied the performance of these biomarkers in predicting the rate of GFR-loss in IGAN.

Methods: This was an observational prospective study. Fifty-seven stable patients with IGAN were examined at baseline and after a mean observational time of 5.9 ± 1.1 years. The main outcome measure was eGFR-loss per year with predefined groups, stable (<1.5 ml/min/1,73 m^2/year, intermediate (between 1.5 and 2.5), and progressive (>2.5).

Results: Fifteen patients were in the progressive, 11 in the intermediate, and 31 in the stable groups. Positive relationships were detected between eGFR-loss per year and baseline nitrate, oxidized free cysteine, parathyroid hormone, APRIL, TNFR1, CD30, chitinase 3, and LIF-5. The progressive group had elevated concentrations of these markers plus AOPP and osteopontin. Through ROC analysis, it was observed that AOPP, oxidized free cysteine, TNFR1, osteopontin, and LIF-5 had the best ability to identify progressive *vs.* non-progressive diseases. The combination of urinary albumin/creatinine ratio with AOPP and TNFR1 significantly improved the ability to identify progressive eGFR decline with ROC AUC 95% (adjusted 85%).

Conclusions: We found prognostic biomarkers related to the rate of eGFR-loss in IGAN. These biomarkers may help identify patients at risk of progressive disease. AOPP, oxidized free cysteine, TNFR1, and osteopontin are promising prognostic biomarkers in IGAN, however, further validation studies are needed.

1. Introduction

IgA nephropathy (IGAN) has been described as the most common glomerular disease (22%) in Europe [1]. Prognosis is highly variable, and risk stratification is of great interest in clinical practice. Risk stratification tools in disease progression have been developed based on findings from large international cohorts of patients with IGAN [2]. The statistical models have been based on a set of predictor variables consisting of the Oxford-MEST-C score and clinical data at the time of biopsy and two years later, such as eGFR, blood pressure, proteinuria, age, and race. The Oxford-MEST-C score contributes importantly to the reliability of 5–10 years risk prediction [3].

However, in clinical practice, kidney biopsy with enough tissue allowing the unequivocal determination of the MEST-C score is occasionally unavailable, and it has to be done without histological scoring. In that case, new prognostic serum- and plasma-based markers may improve risk calculation beyond clinical parameters. Reports have shown that patients with IGAN have disturbed redox status with elevated levels of several inflammation-related factors [4,5]. Furthermore, it appears safe to assume that there is a relationship between histological changes and circulating redox and inflammation-related biomarkers. We aim to explore the prognostic value of these non-traditional biomarkers and shed light on their possible role in

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Abbreviations

ACEI	angiotensin-converting enzyme inhibitor
A/C ratio	albumin/creatinine ratio in urine
ADPKD	autosomal dominant polycystic kidney disease
AOPP	advanced oxidation protein products
APRIL	a proliferation-inducing ligand;
ARB	angiotensin receptor blocker
AUC	area under the curve
BP	blood pressure
CD	cluster of differentiation
CKD	chronic kidney disease
Cys	cysteine;
eGFR	estimated glomerular filtration rate
EFA	exploratory factor analysis
IGAN	IgA nephropathy
IL6R	interleukin 6 receptor
LIF	Latent Inflammatory Factor
MDA	malondialdehyde
MMP-3	matrix metalloproteinase-3
NO	nitric oxide;
NO3	nitrate
PTH	parathyroid hormone
ROC	(receiver operating characteristic)
ROS	reactive oxygen species
TNFR1	tumour necrosis factor receptor-1
TNFR2	tumour necrosis factor receptor-2
TWEAK	tumour necrosis factor–like weak inducer of apoptosis

identifying IGAN patients with unfavorable prognoses and those in need of additional clinical care.

2. Materials and methods

2.1. Subjects

Stable patients with biopsy-verified IGAN, CKD stages 1–4, were invited from our outpatient clinic. Patients with eGFR <15 ml/min/1,73 m^2/year or significant coexistent diseases like diabetes mellitus and cardiovascular ischemic disease, were excluded. Fifty-eight patients gave informed consent and entered the *initial case-control study* between May 2014 and October 2015. Kidney biopsy had been done 10.8 ± 7.3 years (mean ± SD) before inclusion in baseline study [5]. Oxford-MEST-C scores were not always available or too old to be relevant. Previously, we reported that patients with IGAN have evidence of oxidative stress with elevated plasma levels of oxidized free cysteine, nitrate AOPP, and PTH. Oxidative stress was present from an early stage of IGAN and polycystic kidney disease (ADPKD) but was more pronounced in IGAN, despite being similar to eGFR. The underlying mechanisms are probably different. While oxidative stress in IGAN appeared to be related to inflammatory activities, patients with ADPKD have disturbed mitochondrial function with reduced antioxidant activity. Following this, we found that patients with IGAN had elevated concentrations of several inflammation-related factors that were correlated with systemic redox-markers [5–9].

Five to seven years later, after the baseline study, the same IGAN patients were contacted again. Unfortunately, one patient was lost to follow-up; the other 57 gave new written informed consent to participate in a *study of their IGAN outcome*. The results were obtained between February 2021 and November 2021.

2.2. Outcome measures

The primary outcome was the observed change in eGFR per year from the initial case-control to the outcome study, about six years later. Previous studies on the general population have revealed that healthy adults lose about 1.0 ml/min/1,73 m^2/year in eGFR. Thus, eGFR-loss below 1.5 ml/min/1,73 m^2/year was predefined as "stable." [10] In IGAN, a rate of kidney function declined above 2.5 ml/min/1,73 m^2/year may be considered high risk of progression with a significant reduction in eGFR [2]. Thus, eGFR-loss above 2.5 ml/min/1,73 m^2/year was predefined as "progressive." eGFR-loss between 1.5 and 2.5 ml/min/1,73 m^2/year was predefined as "intermediate". The eGFR data were censored for the new occurrence of other significant diseases, start of dialysis treatment, kidney transplantation or death. The eGFR was calculated by CKD-EPI creatinine equation [11]. If possible, we applied the mean of two creatinine measurements for eGFR calculation at baseline and outcome evaluation.

Otherwise, blood pressure, antihypertensive medication, and albuminuria were recorded.

2.3. Laboratory methods

In the initial cross-sectional study, blood and urine samples were obtained in the morning after an overnight fast. Serum calcium, phosphate, albumin, natrium, potassium, creatinine, uric acid, and urea were analyzed using Abbot Architect c16000. Plasma parathyroid hormone (PTH) was analyzed with an intact-PTH assay from Abbott Diagnostics on Architect i2000SR (Abbott Diagnostics, Illinois, USA). Urine albumin/creatine ratio (AC-ratio) was obtained from Abbot Architect c16000 analyzer. Where the albumin/creatinine ratio was unavailable, it was calculated from the urine protein/creatinine ratio [12].

At baseline, total plasma cysteine with its free reduced and oxidized forms was analyzed using HPLC. Advanced oxidation products (AOPP) were measured by spectrometry. Plasma malonedialdehyde (MDA) was analyzed by HPLC after extraction of thiobarbituric reactive substances (TBARS) [5]. Serum nitrate was analyzed by HPLC with spectrophotometry [6].

Thirty-seven inflammation-related proteins were analyzed in serum using a magnetic bead-based multiplex immunoassay (Bio-Plex Pro™ Human Inflammation Panel 1, Bio-Rad, California, USA). The inflammation-related factors were analyzed in a subset of 40 patients with the highest concentrations of oxidized free cysteine [6]. The serum concentrations of 20 inflammation-related factors were within the laboratory detection range and of these, 18 factors had a complete data set that could be included in further statistical analysis. These 18 factors were; APRIL/TNFSF13, BAFF/TNFSF13B, chitinase-3/CHI3L1, gp130/LRPPRC, CD30/TNFRSF8, CD163, IL-6R, IL-20, MMP-1, MMP-2, MMP-3, Osteocalcin/BGLAP, osteopontin/SPP1, Pentraxin-3/PTX3, TNFR1, TNRFSF1A, TNFR2/TNFRSF1B, and TWEAK/TNFRSF12. Through multivariate analysis, the IGAN group was characterized by five important circulating factors; APRIL, MMP-3, osteopontin, TNFR1, and TWEAK [6]. Through exploratory factor analysis (EFA), the five important factors could be reduced into a unifying latent inflammatory factor, LIF-5. Their factor loadings (weights) in EFA were 0.53, 0.18, 0.43, 0.82, and 0.14, respectively. LIF-5 could account for 53% of the variance [6].

2.4. Statistics

Continuous data were presented as their means with standard deviation (SD). All tests were two-tailed with a significance level $p < 0.05$. The descriptive data of eGFR-loss groups were analyzed by ANOVA with Bonferroni/Dunn post-hoc tests. The categorical variables were analyzed by chi-square tests.

Cysteine (Cys) redox species, nitrate, PTH, and inflammation-related factors had a skewed distribution that became close to normal distribution after log-transformation. Therefore, these factors were log-transformed before ANOVA or regression statistics.

The different potential prognostic biomarkers were evaluated by simple regression with the rate of eGFR-loss as the dependent variable. Furthermore, the biomarkers were evaluated using multiple logistic regressions with the three groups of eGFR-loss as dependent variables (stable, intermediate, and progressive).

The regression analyses were adjusted for sex and age; however not for baseline factors that might be influenced by IGAN diseases, including blood pressure, eGFR, and albuminuria [13].

For further evaluation of the biomarkers, ROC (receiver operating characteristic)

curves were calculated for each biomarker and their ability to correctly identify progressive *vs.* non-progressive patients. Their ROC AUCs (area under the curve) with optimal prediction cut-off thresholds as calculated using the Youden index, are presented together with associated sensitivity and specificity. Due to the small data set, ROC AUC might be optimism adjusted using the leave-1-out cross-validation method [14].

Statistical calculations were conducted by StatView v.5.0 (SAS Institute Inc., North Carolina, USA) and IBM SPSS Statistics v.28.0.1 (IBM Corporation, NY, USA).

2.5. Ethics

All subjects gave written informed consent before participating in the follow-up study. The Regional Ethics Committee approved the study (2021/216392/REK sør-øst C).

3. Results

3.1. Rate of eGFR-loss and group characteristics

The observation time from the initial cross-sectional to the outcome study was 5.9 ± 1.1 years (mean ± SD), range 2.5–7.3 years. For all 57 patients, the mean loss of eGFR was 1.8 ml/min/1,73 m^2/year. Thirty-one patients had eGFR-loss of <1.5 ml/min/1,73 m^2/year and belonged to a stable group, as predefined. Eleven patients had eGFR-loss between 1.5 and 2.5 ml/min/1,73 m^2/year and were in the intermediate group, as predefined. Fifteen patients had eGFR-loss above 2.5 ml/min/1,73 m^2/year and were in the predefined progressive group (Table 1).

The mean eGFR-loss was 5.5 ml/min/1,73 m2/year in the progressive group. Of the 15 patients, ten lost more than 50% of their initial eGFR during the observation period. Two patients received a kidney transplant, and three started treating with peritoneal dialysis.

Two patients had to be treated for new malignant disease and were out of the study. Unfortunately, one of them died.

No one lost 50% of their initial eGFR or started renal replacement therapy in the stable and intermediate groups. One patient got a new malignant disease and went out of the study, and no one died.

In the progressive group, all patients were men, and all of them were on treatment with angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB). There were nine women in stable and intermediate groups (21%). Ten patients in stable and intermediate groups were not treated with ACEI/ARB (24%) (Table 1).

Patients in the progressive group had a higher urine AC-ratio than both stable and intermediate groups. There was a linear relationship between eGFR-loss per year and AC- ratio. The regression coefficient was 0.04 (0.03–0.05; 95% CI), $p < 0.0001$, R^2 46%. Adjustment for age and sex did not change this finding (Table 1).

3.2. Redox biomarkers

There were positive relationships between eGFR-loss per year and baseline serum nitrate, plasma oxidized free cysteine, and serum PTH, however, not AOPP (Fig. 1). When adjusting for age and sex, nitrate was able to predict 20% of the variability of eGFR-loss per year ($R^2 = 0.20$). The adjusted R^2 for oxidized free cysteine and PTH were 0.14 and 0.12, respectively (Fig. 1).

The progressive group of eGFR-loss had significantly higher nitrate concentrations, oxidized free cysteine, PTH, and AOPP at baseline compared with stable and intermediate groups. These findings persisted after adjusting for age and sex (Fig. 2).

Table 1
Characteristics of patients according to GFR-loss rate.

	All patients n = 57	Stable group n = 31	Intermediate group n = 11	Progressive group n = 15	
Male/ Female ratio (%)	48/9 (84%)	26/5 (84%)	7/4 (64%)	15/0 (100%)	p = 0.04
Age at end study (years)	55.8 ± 12	56.6 ± 12	55.8 ± 13	54.1 ± 12	n.s.
On treatment with ACEI or ARB (n (%))	47 (82%)	22 (71%)	10 (91%)	15 (100%)	p = 0.04
GFR baseline (ml/min/ 1.73m^2)	68.2 ± 24	70.6 ± 23	75.8 ± 18	57.8 ± 27	n.s.
GFR loss per year (ml/ min/1.73 m^2/year)	1.8 ± 2.6	- 0.1 ± 0.7	2.0 ± 0.3	5.5 ± 2.0	p < 0.0001
Urine AC ratio baseline (mg/ mmol)	31.9 ± 46	12.0 ± 18	31.2 ± 36	73.4 ± 60	p = 0.001
BP baseline (mmHg)	127/84	125/84	125/85	134/86	n.s.*

Stable group, predefined as GFR-loss per year <1.5 ml/min/1.73 m^2/year; Intermediate group, GFR-loss per year 1.5–2.5 ml/min/1.73 m^2/year; Progressive group, GFR-loss per year >2.5 ml/min/1.73 m^2/year.
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; AC ratio, albumin/creatinine ratio; BP, blood pressure; GFR, estimated glomerular filtration rate. Data presented as means ± SD.
P-values identify the significant differences between groups. N.s. i.e., not significant. * ANOVA of mean BP.

3.3. Inflammation-related factors

There were positive relationships between five inflammation-related factors and the rate of eGFR-loss. These inflammation-related factors were LIF-5, APRIL, TNRF1, CD30, and chitinase 3 (Fig. 3). After adjusting for age and sex, LIF-5 predicted 31% of the variability of eGFR-loss per year ($R^2 = 0.31$). The adjusted R^2 for APRIL, TNFR1, CD30, and chitinase 3 were 0.26, 0.34, 0.36, and 0.23, respectively. The other inflammation-related factors did not have linear relationships with the rate of eGFR-loss (data not shown).

In the progressive group, there were higher levels of four different inflammation-related factors at baseline compared with stable and intermediate groups; LIF-5, APRIL, TNFR1, and osteopontin, as shown in Fig. 4. Moreover, the progressive group also had higher levels of the following two markers: (1) CD30 (Cluster of differentiation 30–TNFRSF8) (progressive *vs.* stable group and progressive *vs.* intermediate group, $p = 0.01$ for both) and (2) chitinase 3 (progressive *vs.* stable group and progressive *vs.* intermediate group, $p = 0.02$ for both). These findings persisted after adjusting for age and sex.

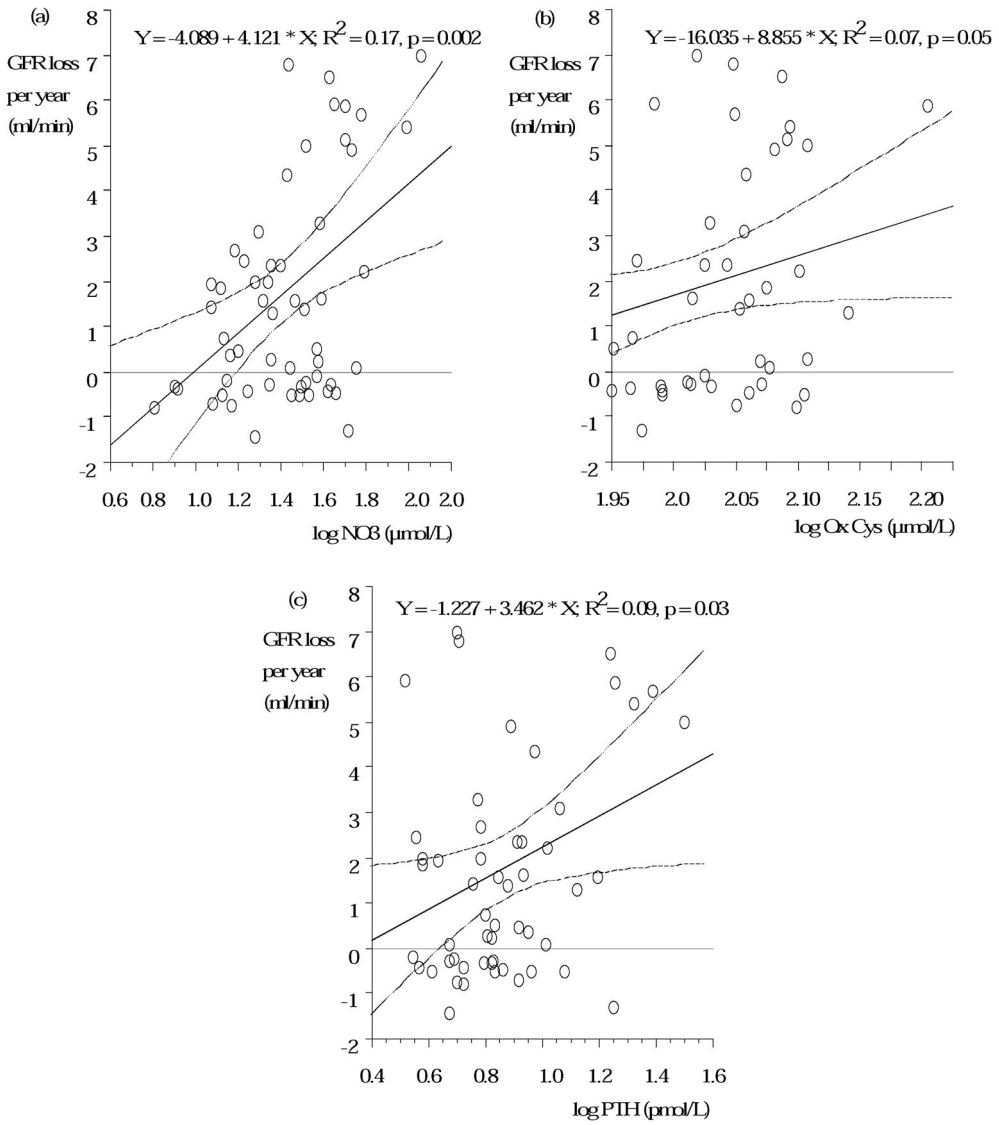

Fig. 1. Linear regression relationships between rate of eGFR-loss and redox-related markers
95% confidence bands are shown. NO3, nitrate; Ox Cys, oxidized free Cysteine; PTH, parathyroid hormone. R^2 adjusted for age and sex was: (a) 0.20; (b) 0.14; (c) 0.12.

3.4. Biomarkers' ability at baseline to identify patients with subsequent progressive eGFR-loss

ROC curve can help to classify patients into two groups; progressive group *vs.* non-progressive group (stable and intermediate patients together). The area under the curve (AUC) measures the general ability of biomarkers to classify correctly. AUC above 80% was considered acceptable [15].

AC-ratio in urine, a measure of proteinuria, is an established prognostic factor in IGAN [2]. In logistic regression analysis with the dichotomous variable–progressive group vs. non-progressive group–as the dependent variable and AC-ratio as the independent variable, ROC AUC was 0.84. Three inflammation-related factors, LIF-5, CD30, and TNFR1, performed equally well with AUCs of 81%, 83%, and 83%, respectively. Of the redox biomarkers, nitrate and AOPP appeared to be the most interesting prognostic factors, with AUCs of 77% and 74%, respectively (Table 2).

The prognostic factors may be further characterized by their optimal cut-off concentrations (Youden) with associated sensitivity and specificity (Table 2). Urine AC-ratio with a cut-off concentration of 32,9 had a sensitivity of 87% and specificity of 85%, which may be considered good. Of the inflammation-related factors, LIF-5, and TNFR1 and osteopontin performed comparably well with optimal sensitivity and specificity of 77%/85%, and 77%/93%, and 85%/74%, respectively (Table 2). Of the redox-markers, AOPP and oxidized free cysteine were the best prognostic factors with sensitivity and specificity of 83%/62% and 80%/60%, respectively (Table 2).

At baseline, if AC-ratio was combined with AOPP and TNFR1 as independent variables, ROC AUC rose to 95%, i.e., the combined factors were better to predict the dichotomous outcome correctly; progressive *vs.* non-progressive eGFR-loss. The observed probability of correct prediction may be too optimistic in small data sets. To compensate for this, we calculated optimism adjusted ROC AUC, which was 84%. This was still a good result.

4. Discussion

These findings demonstrate the variable prognosis in IGAN. Fifteen patients (26.3%) had progressive disease with significant loss of kidney function during the observation period of about six years. Thirty-one patients (54.4%) had stable kidney function, while 11 patients (19.3%) had an intermediate rate of eGFR decline.

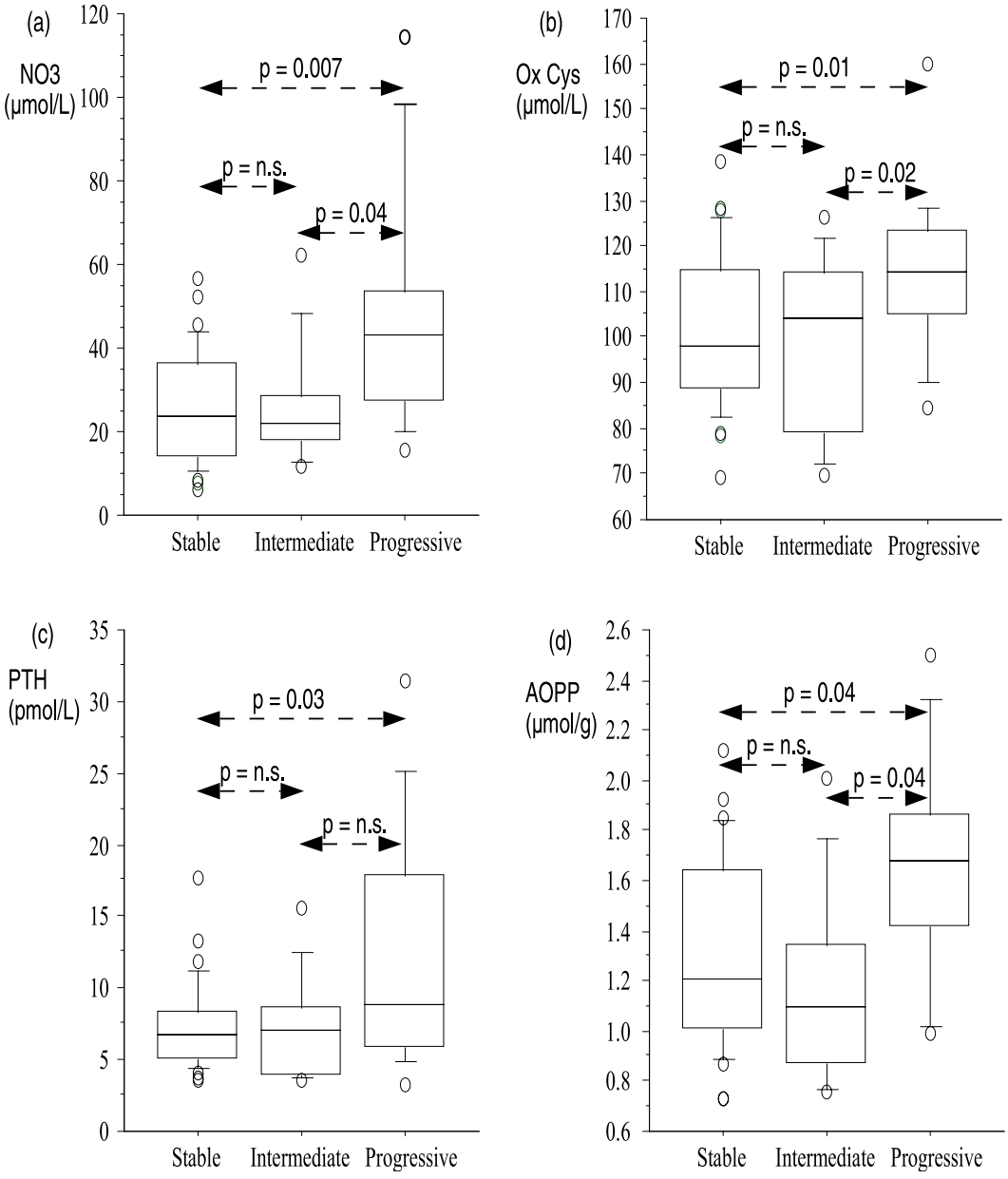

Fig. 2. Groups of GFR-loss and redox-related markers
Stable group, that is GFR loss per year <1.5 ml/min/1.73 m^2; Intermediate group, that is GFR loss per year 1.5–2.5; Progressive group, that is GFR loss per year ≥2.5 The box plots display the groups' 10th, 25th, 50th,75th, and 90th percentiles. NO3, nitrate; Ox Cys, oxidized free Cysteine; PTH, parathyroid hormone; AOPP, advanced oxidation protein products. P-values identify the significant differences between groups. N.s. i.e., not significant. The polytomous logistic regression tests were adjusted for age and sex.

There were positive relationships between the subsequent rate of eGFR-loss and the baseline levels of specific redox-markers; nitrate, oxidized free cysteine, and PTH. Moreover, patients in the progressive group had high nitrate concentrations, oxidized free cysteine, PTH and AOPP compared with non-progressive groups. Thus, redox-markers may help identify patients with progressive loss of eGFR.

In this study, oxidized free cysteine and AOPP were the best prognostic redox-markers with 83% and 80% sensitivity, respectively. This was comparable to AC-ratio at baseline–an established prognostic biomarker in kidney disease.

Plasma oxidized free cysteine is a new prognostic marker. Cysteine is the most abundant aminothiol (sulfur-containing amino acid) in plasma. Cysteine is redox-sensitive, and the dynamic balance between its reduced and oxidized species in plasma reflects the systemic redox status [6].

AOPP is a stable end-product of protein oxidation that previously identified as a strong prognostic marker in IGAN. AOPP may even have toxic effects on the podocytes [16]. Patients with ADPKD and similar eGFR had lower concentrations of AOPP than IGAN. Therefore, AOPP may be a useful prognostic factor in IGAN, but perhaps not in ADPKD or other kidney diseases [5].

Nitrate and PTH were identified as prognostic biomarkers and illustrated the close relationship between oxidative stress and disease progression in IGAN. Nitrate is an oxidative degradation product of NO and may also be a NO precursor. Therefore, nitrate may be considered a dynamic measure of systemic redox status [5,6]. PTH is an established prognostic marker in chronic kidney disease that appears to be influenced by oxidative stress [5,7,17].

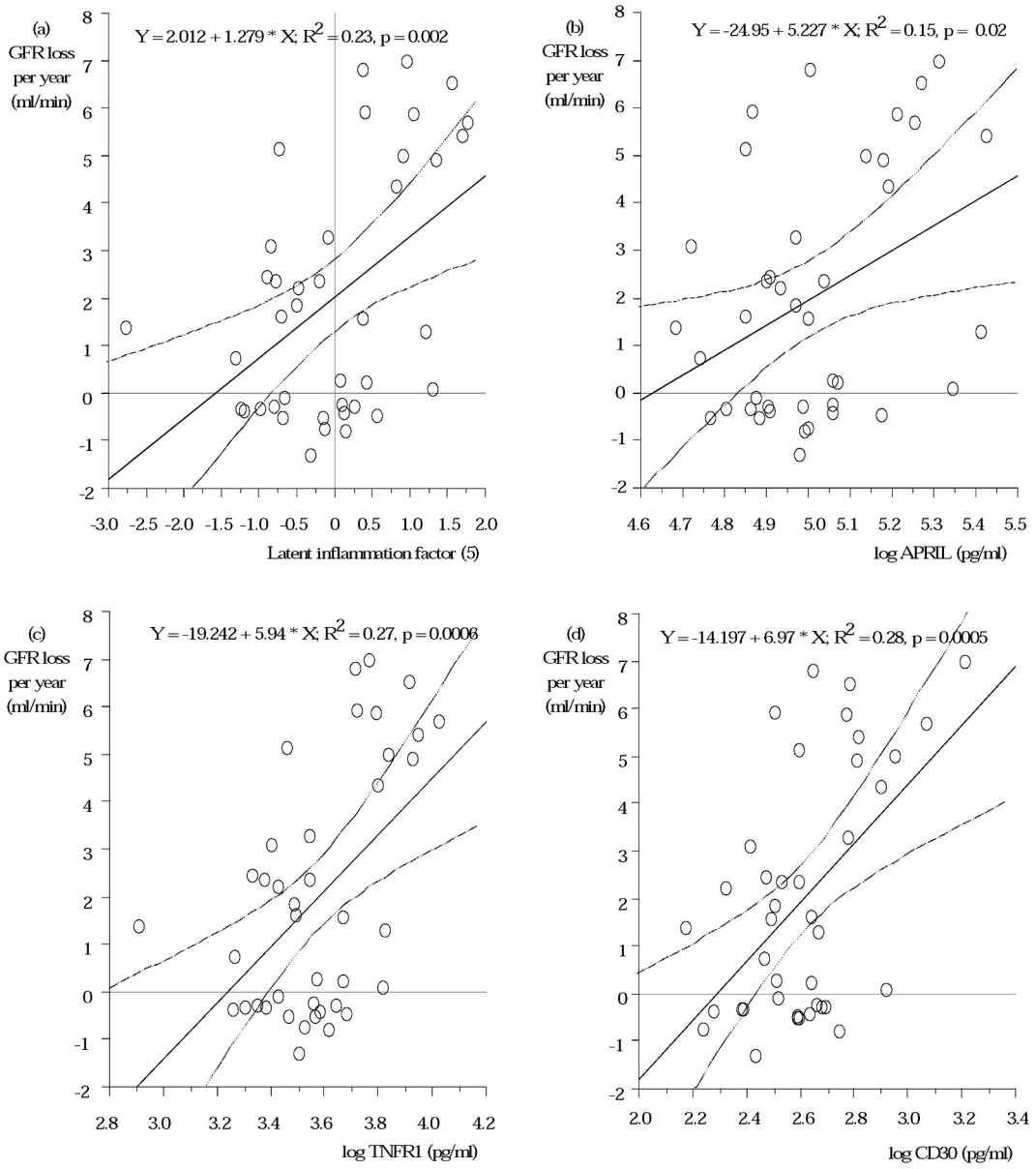

Fig. 3. Linear regression relationships between rate of GFR-loss and some inflammation-related factors
95% confidence bands are shown. Latent inflammation factor (5), i.e., calculated LIF-5; APRIL, a proliferation-inducing ligand (TNFSF13); TNFR1, tumor necrosis factor receptor-1 (TNRFSF1A); CD30, cluster of differentiation 30 (TNFRSF8). R^2 adjusted for age and sex was: (a) 0.31; (b) 0.26; (c) 0.34; (d) 0.36.

These findings extend and corroborate previous reports on the close relationship between oxidative stress and poor prognosis in IGAN [4,18]. Furthermore, it appears that oxidative stress may result from inflammation, and conversely, oxidative stress may modulate and enhance inflammatory processes [19,20]. Previously, we have reported positive correlations between redox-markers and inflammation-related markers in IGAN, partially independent of eGFR [6].

However, some redox imbalances may be partly related to reduced eGFR. It is possible that low baseline eGFR causes a more oxidative stress. Alternatively, it is also possible that high disease activity causes more oxidative stress with a higher rate of eGFR-loss and lower baseline eGFR.

In this study, we detected positive relationships between subsequent eGFR-loss and the level of specific inflammation-related factors, i.e., APRIL, chitinase 3, CD30, and TNFR1. Moreover, the progressive group had elevated concentrations of APRIL, chitinase 3, CD30, osteopontin, and TNFR1 compared with the non-progressive group.

It appears that TNFR1 and osteopontin are the best prognostic biomarkers for identifying patients with a progressive disease with a sensitivity of 77% and 85%, respectively. Previously, TNFR1 has been advocated as an important prognostic biomarker in chronic kidney disease [21,22]. Additionally, plasma TNFR1 has been associated with renal inflammation and fibrosis and may identify patients at risk of progressive eGFR decline [23].

Upregulated osteopontin has been found in chronic inflammatory states and mineral bone disorder. High osteopontin levels have been associated with elevated mortality and progressive eGFR decline in chronic kidney disease [24,25].

Previously, elevated serum levels of APRIL (TNFSF13) have been associated with progressive IGAN, possibly through increased production of galactose-deficient IgA [26]. High urine and plasma levels of chitinase 3 (YKL-40) have been linked to progressive eGFR decline and poor outcomes in chronic kidney disease [22,27]. High levels of soluble CD30 have been related to fibrosis and declining kidney function [28].

LIF-5 is an interesting new prognostic biomarker for progressive IGAN. It illustrates and confirms the significant relationship between

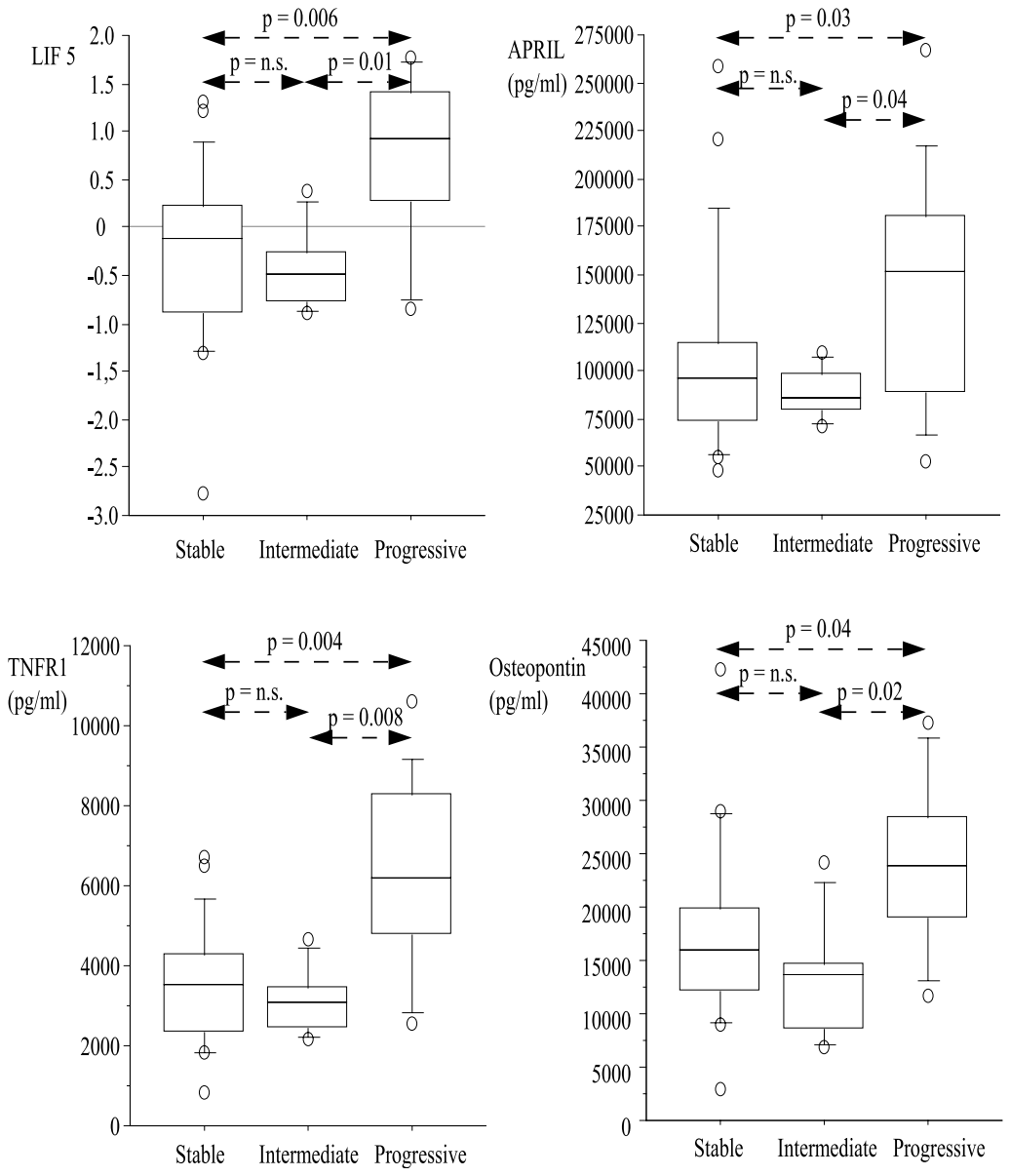

Fig. 4. Groups of GFR-loss and inflammation-related markers.
Stable group, predefined as GFR-loss per year <1.5 ml/min/1.73 m^{2}/year; Intermediate group, predefined as GFR-loss per year 1.5–2.5; Progressive group, predefined as GFR -loss per year >2.5.The box plots display the groups' 10th, 25th, 50th,75th, and 90th percentiles. P-values identify the significant differences between groups. N.s, i.e., not significant. The polytomous logistic regression tests were adjusted for age and sex. LIF 5, latent (calculated) inflammation factor; APRIL, a proliferation-inducing ligand (TNFSF13); TNFR1, tumor necrosis factor receptor-1 (TNRFSF1A).

inflammatory activity and the risk of disease progression. LIF-5 is a latent inflammation factor, calculated based on the concentrations of APRIL, MMP-3, osteopontin, TNFR1, and TWEAK. Thus, the calculation of LIF-5 represents the multivariate analysis of inflammation-related factors [6].

We found that combining a clinical parameter (AC-ratio), a redox marker, and an inflammation-related marker improved the ability to identify patients at risk of progressive eGFR decline correctly. In the future, the combination of urinary albumin/creatinine ratio with AOPP and TNFR1 may become a new risk stratification test for identifying patients with progressive eGFR decline. Thus, some of the new biomarkers may be useful additions to traditional prognostic factors.

5. Conclusions

We have identified several promising prognostic markers related to redox disturbance and inflammation in patients with IGAN. Additionally, the concentrations of the biomarkers are higher in patients with the progressive disease already at the beginning of the six-year observation period, before further loss of kidney function, and partly independent of baseline eGFR. Thus, redox- and inflammation-related biomarkers may help identify patients at risk of progressive IGAN disease. Furthermore, this finding highlights the importance of inflammatory activity and oxidative stress for progressive eGFR decline in IGAN. There is a close relationship between inflammatory activity and oxidative stress. It appears that inflammation may generate oxidative stress and oxidative stress may modulate and enhance inflammatory processes.

Importantly, these findings cannot be transferred to other kidney

Table 2
Biomarkers at baseline and their ability to identify patients with progressive GFR-loss (>2.5 ml/min/1.73 m^2/year).

Biomarker	OR (95%CI)	P-value	AUC	Cut-off conc.	Sensitivity	Specificity
LIF-5	4.80 (1.87–16.4)	0.004	81%	0.38*	77%	85%
APRIL (pg/L)	1.01 (1.00–1.03)	0.039	70%	137249§	62%	89%
Chitinase3 (pg/L)	1.32 (1.08–1.70)	0.014	76%	9613§	62%	85%
CD30 (pg/L)	1543 (18.8–816097)	0.006	83%	593.4§	69%	96%
Osteopontin (pg/L)	1.12 (1.03–1.24)	0.017	77%	18439§	85%	74%
TNFR1 (pg/L)	2.26 (1.45–4.19)	0.002	83%	5185§	77%	93%
Urine AC-ratio (mg/mmol)	1.04 (1.02–1.07)	0.001	84%	32.9	87%	85%
PTH (pmol/L)	1.19 (1.06–1.38)	0.011	67%	9.4	50%	85%
AOPP (μmol/g)	9.82 (1.86–74.7)	0.013	74%	1.4	83%	62%
Oxidized free Cys (μmol/L)	1.05 (1.01–1.09)	0.019	70%	104.3	80%	60%
NO3 μmol/L (μmol/L)	1.06 (1.02–1.12)	0.006	77%	38.2	60%	83%

ROC AUC are presented with optimal cut-off concentration (Youden) and associated sensitivity and specificity.
§ Cut-off concentrations given in pg/mL. *Cut-off concentration is a dimensionless number.
LIF-5, latent (calculated) inflammation factor, APRIL, a proliferation-inducing ligand (TNFSF13); Chitinase 3, CHI3L1; CD30, cluster of differentiation 30 (TNFRSF8); Osteopontin, SPP1; TNFR1, tumor necrosis factor receptor-1 (TNRFSF1A); AC ratio, urine albumin/creatinine ratio; PTH, parathyroid hormone; AOPP, advanced oxidation protein products; Oxidized free Cys, Oxidized free Cysteine; NO3, nitrate.

diseases without further investigations. AOPP may be a prospective marker mostly for IGAN, while TNFR1 may be useful in several chronic kidney diseases.

Oxidized free cysteine, AOPP, TNFR1, and osteopontin have sufficient sensitivity to be of clinical interest. The prognostic value of the key IGAN prognostic markers must be validated in future prospective studies. Moreover, the relationship to Oxford-MEST-C score remains to be elucidated. The urinary albumin/creatinine ratio along with serum AOPP and TNFR1 may become the basis of a new risk prediction tool that is not dependent on kidney biopsy.

Limitations of the study

The present findings have not been validated in a new group of patients with IgA nephropathy.

Ethics approval and consent to participate

All subjects provided written informed consent. The Regional Ethics Committee approved the study (2021/216392/REK sør-øst C).

Authors' contributions

This study was designed by TA and HPM. The subjects were recruited by TA. Laboratory analyses were conducted by MAM, JF, and GJ. TA, HPM, and AU analyzed data and drafted the manuscript. All authors actively contributed to reviewing, revising, and approving the manuscript.

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Declarations of competing interest

None declared.

Data availability statement

The dataset used and analyzed during the current study are available from the corresponding author on reasonable request.

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