an 8-h observation period identified all major biopsy complications allowing patients to be safely discharged on the day of renal biopsy. Given the benefits of day-case strategies in terms of patient and healthcare costs, we advocate an approach already widely adopted by our paediatric colleagues with increased utilization of this technique in adults.

Conflict of interest statement. None declared.

References


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Fractional excretions of albumin and IgG are the best predictors of progression in primary glomerulonephritis

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Abstract

Background. Proteinuria is the most sensitive predictor of development of progressive renal insufficiency, with increasing focus on the composition of proteinuria, particularly high molecular weight proteins such as immunoglobulin G (IgG) (molecular weight 150 kDa). Differing methods of assessing excretion of proteinuria molecules have limited interpretation of results. We aimed to assess the utility of available indices of IgG, total proteinuria and albumin excretions as predictors of chronic kidney disease (CKD) progression in patients with primary glomerulonephritis.

Methods. We recruited 97 patients with primary glomerulonephritis and measured 24-h urinary protein excretion, 24-h urinary albumin excretion, selectivity index, albumin:creatinine ratio, urinary IgG:creatinine ratio, fractional excretion of albumin (FE Alb) and fractional excretion of IgG (FE IgG) at baseline. The composite endpoint was developing stage 5 CKD, requiring RRT or death. Receiver operating characteristics curve analysis was used to assess the value of each measure in predicting outcome. From this analysis, high- and low-risk patient groups according to each measure were established. These were then tested using Kaplan–Meier and Cox survival analysis.
**Introduction**

Following the introduction of estimated glomerular filtration rate (eGFR) reporting and the subsequent increase in recognition of chronic kidney disease (CKD), it is more important than ever that we are able to determine the risk of progression to end-stage renal disease in individual patients. Established risk factors for the progression of CKD include proteinuria, hypertension, more advanced CKD and fibrosis in the interstitium on renal biopsy. There is increasing interest in the nature of proteinuria rather than its magnitude alone, in the attempt to identify biomarkers associated with the risk of progression and to elucidate the pathogenetic mechanisms leading to progression.

In both primary and secondary renal diseases, the magnitude of proteinuria has been shown to be a major determinant of progression [1,2], with reduction in proteinuria associated with renoprotection [3,4]. The measurements usually employed are 24-h urinary total protein, albumin or ratios of urinary total protein or albumin to creatinine. However, this approach does not completely quantify the risk of developing progressive CKD in an individual patient. Attention has, therefore, focussed on alternative methods, such as the composition of proteinuria, particularly the proportion of high molecular weight proteins, which are thought to reflect the degree of glomerular barrier dysfunction with loss of size selectivity, as well as being potentially toxic to tubular cells.

We have previously shown that high molecular weight proteinuria (>100 kDa) is associated with decline in renal function in patients with primary renal diseases [5]. We, therefore, decided to focus on the relationship between the excretion of one particular high molecular weight protein, immunoglobulin G (IgG) (molecular weight 150 kDa), and progression of CKD in primary glomerulonephritis. Previous studies have investigated the utility of urinary IgG in predicting outcome or treatment response in idiopathic membranous nephropathy (IMN) [6,7], crescentic IgA nephropathy [8] and focal segmental glomerulosclerosis (FSGS) [9]. These studies used different means of quantifying urinary IgG including fractional excretion and selectivity index (SI) as well as a variety of standard measures of proteinuria including total proteinuria and albumin:creatinine ratio. Results were variable and comparisons were difficult because of the variety of methods used.

The current study aimed to investigate the utility of all available indices of IgG and albumin excretions as predictors of CKD progression in a cohort of patients with primary glomerulonephritis and close follow-up. Fractional excretions of individual proteins are potentially the best indicators of the loss of glomerular permselectivity [10] at the molecular sizes of the molecules concerned. Therefore, baseline fractional excretions of both IgG (FE IgG) and albumin (FE Alb) were calculated from routinely available laboratory values. Ratios of urine IgG and albumin to urine creatinine (IgG:Cr and ACR, respectively), SI and traditional assays of proteinuria and albuminuria [24-h urinary protein excretion (24-h Uprot), 24-h urinary albumin excretion (24-h Ualb)] were also measured. Analyses were then undertaken to determine which measures best predicted renal outcome.

**Materials and methods**

**Patients**

Ninety-seven adult patients with biopsy-proven primary glomerulonephritis were consecutively recruited from renal clinics at Glasgow Royal Infirmary between November 2001 and April 2002. Written informed consent was obtained, and the study was approved by the local ethics committee. Patients were excluded if they were currently receiving immunosuppression or received immunosuppression during follow-up, had nephrotic range proteinuria, active inflammation or infection (CRP >10), current neoplasia or secondary renal disease. To exclude secondary forms of membranous nephropathy, patients are routinely screened for hepatitis and anti-nuclear factor levels are analysed at presentation. They also undergo detailed history, examination and chest radiography to exclude underlying malignancy. To exclude secondary FSGS, consideration is given to previous nephrectomy, obesity, reflux nephropathy and family history of FSGS. Serology for CMV and HIV is also performed.

**Measurements**

A 24-h urine sample was collected for measurement of total proteinuria (24-h Uprot), urinary albumin (24-h Ualb), sodium and creatinine excretion. Spot urine was also routinely measured for albumin using nephelometry and creatinine using flame photometry, and from this the ACR ratio was calculated. Urine from the same spot sample was analysed by ELISA for IgG (R&D Biosystems, UK) and the IgG:Cr ratio was calculated. Blood sampling was performed for baseline serum creatinine to calculate eGFR, measured using the MDRD4 formula, bone biochemistry, C-reactive protein, serum albumin and serum IgG.

As well as calculating the spot IgG:creatinine ratio, we calculated:
- **Fractional excretion of IgG:**
  \[
  \text{FE IgG} = \left( \frac{\text{Serum Cr} \times \text{Urine IgG}}{\text{Serum IgG} \times \text{UCr}} \right) \%
  \]
- **Fractional excretion of albumin**
  \[
  \text{FE Alb} = \left( \frac{\text{Serum Cr} \times \text{Urine Alb}}{\text{Serum Alb} \times \text{UCr}} \right) \%
  \]
- **Selectivity index**:
  \[
  \left( \frac{\text{Urine IgG}}{\text{Serum IgG}} \right) \times \left( \frac{\text{Serum albumin}}{\text{Urine albumin}} \right)
  \]

**Outcomes**

Patients underwent routine outpatient review until 1 May 2009. The primary composite outcome measure was eGFR falling below 15 mL/min as measured by the MDRD4 formula (i.e. progression to stage 5 CKD), need for chronic renal replacement therapy (dialysis or transplantation) or death. Patients were censored at the time of first event. The slope of decline in eGFR was also calculated.
Statistical analysis
Statistical analyses were performed using SPSS version 15.0 (Illinois). Data were plotted to assess normality, and correlation between parameters was assessed using Spearman or Pearson correlation coefficients as appropriate. Receiver operating characteristic (ROC) curves were constructed to evaluate and compare different tests in terms of predictive value using the area under the curve (AUC). From these curves, cut-offs were calculated to maximize sensitivity and specificity, which were then applied to the patients to create two groups for each test—high risk and low risk. Kaplan–Meier analysis was then used to evaluate the independent effect of predictive cut-offs on progression to the primary endpoint, and Cox regression was used to calculate hazard ratios (HR) for the risk of developing the primary endpoint.

Results
Baseline demographics
Ninety-seven patients were recruited, 66% of patients were male with a median age of 56.3 (IQR 42.0–69.4) years. Mean eGFR at inclusion was 51.3 (SD 22.8) mL/min/1.73 m², median 24-h Uprot 0.8 (IQR 0.2–1.4) mg/24 h, median 24-h Ualb 662 (IQR 52.5–1213.5) mg/24 h and median ACR 48.0 (6.0–110.5) mg/mmol. At the time of inclusion, 69 patients (71.1%) were receiving inhibitors of the renin-angiotensin system. Patients who were receiving angiotensin-converting enzyme (ACE) inhibitors had significantly higher levels of proteinuria (P = 0.001).

The primary renal diseases were IgA nephropathy (IgAN n = 34), membranous nephropathy (MGN n = 25), focal segmental glomerulosclerosis (FSGS n = 16), mesangiocapillary glomerulonephritis (MCGN n = 9), chronic glomerulonephritis (n = 6) and others (n = 7). Table 1 demonstrates baseline proteinuria results stratified by primary renal disease. There was no significant difference in measures of proteinuria between groups (Kruskal–Wallis test).

Outcomes
The median follow-up time was 7.07 years, with an average of 35 eGFR measurements per patient. Fourteen patients were discharged to other health boards and censored at time of discharge. Twenty-three patients reached the primary

<table>
<thead>
<tr>
<th>Primary Renal Disease</th>
<th>24-h Uprot (g/24 h)</th>
<th>24-h Ualb (mg/24 h)</th>
<th>ACR (mg/mmol)</th>
<th>FE IgG</th>
<th>IgG:Cr (mg/mmol)</th>
<th>FE Alb</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>0.85 (0.2–1.1)</td>
<td>735.0 (77.5–926.5)</td>
<td>47.5 (7.5–85.0)</td>
<td>0.011 (0.005–0.048)</td>
<td>1.05 (0.36–2.24)</td>
<td>13.9 (2.4–37.9)</td>
</tr>
<tr>
<td>MGN</td>
<td>0.92 (0.1–2.0)</td>
<td>717.0 (31.0–1824.0)</td>
<td>79.0 (3.5–193.5)</td>
<td>0.024 (0.008–0.100)</td>
<td>1.91 (0.65–6.26)</td>
<td>21.1 (1.1–52.4)</td>
</tr>
<tr>
<td>FSGS</td>
<td>0.47 (0.2–1.2)</td>
<td>210.0 (38.3–932.0)</td>
<td>26.5 (5.0–85.0)</td>
<td>0.019 (0.009–0.077)</td>
<td>1.51 (0.62–5.77)</td>
<td>7.1 (1.0–23.9)</td>
</tr>
<tr>
<td>Other</td>
<td>1.0 (0.2–2.1)</td>
<td>771.0 (110.0–1745.5)</td>
<td>85.0 (9.5–138.8)</td>
<td>0.017 (0.009–0.153)</td>
<td>1.35 (0.70–6.48)</td>
<td>20.2 (2.7–62.9)</td>
</tr>
</tbody>
</table>

Values = Median (IQR). No significant difference in measures of proteinuria between groups.
IgG:Cr < versus ≥ FE IgG < versus ≥ FE Alb < versus ACR < versus ≥ 24-h Ualb < versus ≥ 24-h Uprot < versus

Variable primary outcome regression analysis for the power of the individual predictors to predict the primary outcome

<table>
<thead>
<tr>
<th>Disease</th>
<th>n</th>
<th>Number reaching primary endpoint</th>
<th>Test</th>
<th>AUC</th>
<th>Cut-off</th>
<th>Sens (%)</th>
<th>Spec (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgAN</td>
<td>34</td>
<td>9 (26.5%)</td>
<td>FE Alb</td>
<td>0.99</td>
<td>32.0</td>
<td>100</td>
<td>96.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FE IgG</td>
<td>0.96</td>
<td>0.029</td>
<td>88.9</td>
<td>88.0</td>
<td>85.7</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>3 (12%)</td>
<td>FE Alb</td>
<td>0.91</td>
<td>52.4</td>
<td>100</td>
<td>85.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FE IgG</td>
<td>0.91</td>
<td>0.119</td>
<td>100</td>
<td>90.5</td>
<td></td>
</tr>
<tr>
<td>FSGS</td>
<td>16</td>
<td>3 (18.8%)</td>
<td>FE Alb</td>
<td>0.95</td>
<td>20.0</td>
<td>100</td>
<td>84.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FE IgG</td>
<td>0.97</td>
<td>0.065</td>
<td>100</td>
<td>92.3</td>
<td></td>
</tr>
</tbody>
</table>

ROC curve analysis for predicting the primary endpoint: n = number of patients in the group; AUC = area under the curve AUC; 95% CI = 95% confidence interval; Cut-off = level above which a test result was deemed significant; Sens = sensitivity of defined cut-off; Spec = specificity of defined cut-off.

endpoint—18 reached stage 5 CKD, of whom 15 required renal replacement therapy. Nine patients died, of whom four had already reached stage 5 CKD or started RRT. The median time to the primary endpoint was 2575 (1200–2701) days. There was no significant difference in patients reaching the primary endpoint according to ACE inhibition (P = 0.279)—17.9% of those who did not receive ACE inhibition versus 26.1% in those who did; however, the groups were not equal at baseline with patients prescribed ACE inhibition having higher baseline levels of proteinuria. Overall mean slope of decline in eGFR was −1.1 (3.7) mL/min/year. Forty-one patients had a decline in eGFR ≥ −1 mL/min/year.

Correlations

Higher levels of the urinary IgG:Cr ratio (r = 0.58), FE IgG (r = 0.64), FE Alb (r = 0.64), 24-h Ualb excretion (r = 0.527), 24-h Uprot excretion (r = 0.503) and ACR (r = 0.534) were all associated with a greater chance of developing the primary endpoint (P < 0.001) and all correlated significantly (P < 0.001) with slope of decline in eGFR. The selectivity index was not significantly associated with the primary outcome or slope of decline in eGFR.

ROC curves

ROC curves were constructed to evaluate and compare different tests (Figure 1). The AUC for the IgG:Cr ratio to predict the primary endpoint was 0.894 (95% CI 0.824–0.964). AUC for 24-h UProt excretion was 0.86 (95% CI 0.784–0.935), for 24-h Ualb excretion 0.842 (95% CI 0.762–0.923) and for ACR 0.865 (95% CI 0.79–0.94). The AUC for FE IgG in predicting the primary outcome was 0.935 (95% CI 0.882–0.988) and for FE Alb, 0.935 (95% CI 0.883–0.988). The selectivity index was poor at predicting the primary outcome (AUC 0.472, 95% CI 0.352–0.592). Looking at each of the three largest groups of primary renal diseases individually (IgA nephropathy, membranous nephropathy and FSGS) each test, except selectivity index, was predictive with no significant difference in results between groups.

Using the ROC analysis, a cut-off was chosen to maximize the sensitivity and specificity for predicting the development of the primary endpoint for the whole cohort (Figure 1). It can be seen that the fractional excretion tests had the highest sensitivity and specificity. The cut-offs with the highest sensitivity and specificity for progression for IgA nephropathy, membranous nephropathy and FSGS were also determined (Table 2).

Kaplan–Meier analysis—survival by ROC-determined cut-off

Kaplan–Meier survival analysis was performed to assess outcome according to the high- or low-risk groupings for each measure of proteinuria, determined from the ROC curve analysis. FE IgG had the highest log-rank chi-square value (Table 3). Figure 2 shows the survival plot for patients in the two groups of fractional excretion of IgG (Figure 2A) and fractional excretion of albumin (Figure 2B), with 50% of patients in the higher groups having developed the primary endpoint by 1000 days of follow-up.

Cox regression analysis—HR for developing the primary endpoint

Each predictor was entered separately into a Cox regression model for predicting the primary endpoint, and from this HR were calculated. Patients in the higher group of FE Alb had a 35.2-fold and FE IgG had a 37.1-fold increased risk of developing the primary endpoint than those in the lower group. These HR were at least double compared with that seen for the other predictors (Table 3). Table 4 demonstrates the predictive ability of FE IgG and FE...
Fig. 2. (A) Kaplan–Meier survival plot of patient outcome dependent on grouping of fractional excretion of IgG (high or low) as a predictor of developing the primary endpoint. (B) Kaplan–Meier survival plot of patient outcome dependent on grouping of fractional excretion of albumin (high or low) as a predictor of developing the primary endpoint.
Alb cut-offs for the individual glomerulonephritides as well as the whole cohort cut-offs applied to each individual disease. It can be seen that there are not large differences in the predictive capacity of individual cut-offs compared with whole cohort cut-offs. Low event rates in individual groups limit the application of the cut-offs.

Cox regression analysis

One measure of total proteinuria (24-h Uprot group) and one measure of high molecular weight proteinuria (fractional excretion of IgG group) were entered alone as categorical variables into a Cox regression survival model to assess which factor was superior in predicting the primary outcome. Using this analysis, FE IgG remained a statistically significant predictor (HR 42.3, 95% CI 7.8–228.9, P < 0.001) whereas 24-h Uprot did not (HR 0.84, 95% CI 0.27–2.65, P = 0.763). When entering FE Alb and 24-h Uprot into a Cox regression model together, again fractional excretion was a statistically significant predictor (HR 77.8, 95% CI 13.1–461.0, P < 0.001) and 24-h Uprot was not. Including histological diagnosis in the model did not significantly impact upon the model. When entering both FE IgG and FE Alb into a Cox regression survival model with 24-h Uprot, only FE Alb group remains a significant predictor (= 0.044).

Discussion

In this relatively large cohort of patients with mixed primary renal diseases and extensive close follow-up, we have confirmed that urinary IgG and albuminuria are significant predictors of the composite outcome of death or progression to stage 5 CKD. Urine IgG, whether expressed as a fractional excretion or ratio to urinary creatinine, is not superior to urinary albumin in predicting progression to stage 5 CKD. This is out of keeping with previous evidence suggesting that IgG may be particularly tubulotoxic. Interestingly, fractional excretions of IgG and albumin appear to be better predictors of outcome than urinary IgG:creatinine or ACR. Finally, in the population studied, the SI of IgG versus albumin is not predictive of death or progression to CKD 5 and is not correlated with the rate of CKD progression.

The detrimental effects of proteinuria appear to be mediated at both the glomerulus and in the proximal tubule, where the protein overload is toxic. In vitro, proximal tubular cells stimulated with serum proteins (albumin, IgG, transferrin) produce a number of pro-fibrotic and pro-inflammatory markers at the basolateral membrane including endothelin and IL-8, signalling for the recruitment of local macrophages. High molecular weight proteinuria has also been associated with proximal tubular cell apoptosis [11]. When primary and HK-2 proximal tubular cells are exposed to human plasma-derived high molecular weight fraction (100–440 kDa), they exhibit features consistent with a pro-apoptotic phenotype, namely Fas and FasL expression. This was not seen when the cells were exposed to low molecular weight proteins [12]. It is, therefore, reasonable to extrapolate that long-term exposure of proximal tubular cells in vivo to high molecular weight proteins would be toxic and promote interstitial fibrosis and glomerulosclerosis.

Previous studies looking at the predictive value of high molecular weight proteinuria have been limited by utilizing various methods and differing populations, with IMN being the most widely studied condition. Wetzels and colleagues showed that total excretion of urinary IgG in a 24-h urine sample predicted decline in renal function in patients with IMN and preserved renal function [13]. They went on to show that in patients with preserved renal function and nephrosis, 24-h urine IgG normalized to creatinine predicted outcome, although urinary β2M microglobulin (Mol Wt = 11.8 kDa) was more predictive [6]. Like us, they found selectivity index to be of limited prognostic value. In a diverse Italian population with membranous nephropathy [7], a morning spot urinary IgG normalized to creatinine predicted remission in patients with IMN better than total proteinuria at baseline. It did not, however, predict the development of end-stage renal failure.

Bazzi et al. [8] looked at fractional excretion of IgG and α1 microglobulin (Mol Wt = 26–33 kDa) in 37 patients with crescentic IgA nephropathy as predictors for developing end-stage renal failure or doubling serum creatinine. They normalized these values to the percentage of non-globally sclerosed glomeruli in biopsy specimens to calculate the effective tubular load of surviving nephrons. FE IgG corrected for sclerosed glomeruli was the most sensitive and specific test for predicting progression and the only independent predictor in a Cox survival analysis. In patients with primary FSGS, a similar picture was seen in that FE IgG predicts remission, progression and response to therapy [14], although this has not been borne out in all studies [15].

Table 4. Kaplan–Meier log-rank analysis (X²) and HR derived from Cox regression analysis for the power FE IgG and FE Alb cut-offs to predict the differing primary renal diseases. Whole-group cut-offs have also been included, as applied to each individual disease.

<table>
<thead>
<tr>
<th>PRD</th>
<th>Test</th>
<th>Cut-off: group specific</th>
<th>X²</th>
<th>P</th>
<th>Cut-off: whole cohort</th>
<th>X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgAN</td>
<td>FE Alb</td>
<td>&lt; versus ≥ 32.0</td>
<td>38.2</td>
<td>&lt;0.001</td>
<td>&lt; versus ≥ 32.5</td>
<td>38.2</td>
<td>0.001</td>
</tr>
<tr>
<td>MGN</td>
<td>FE IgG</td>
<td>&lt; versus ≥ 0.029</td>
<td>21.3</td>
<td>&lt;0.001</td>
<td>&lt; versus ≥ 0.043</td>
<td>26.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FSGS</td>
<td>FE Alb</td>
<td>&lt; versus ≥ 0.119</td>
<td>14.0</td>
<td>&lt;0.001</td>
<td>&lt; versus ≥ 0.043</td>
<td>6.6</td>
<td>0.016</td>
</tr>
<tr>
<td>All patients</td>
<td>FE IgG</td>
<td>&lt; versus ≥ 0.065</td>
<td>7.78</td>
<td>0.005</td>
<td>&lt; versus ≥ 0.043</td>
<td>12.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

X² = Chi-squared value, calculated using Kaplan–Meier survival analysis and log-rank test. P = P-value test for significance.
Establishing the response of FE IgG to treatment, both supportive and immunosuppressive, would be interesting; however, there are limited data. An observational, retrospective study looked at fractional excretion of urinary IgG in 140 patients with IgA nephropathy [16] and found that FE IgG was a predictor of responsiveness to ACE inhibitors. In a small cohort of patients with MGN, reduction in tubular proteins in response to immunosuppressive treatment was not sufficiently predictive of relapse or renal impairment [17].

Our population differs from those published in previous literature, in that previously published patients had lower levels of proteinuria and only mildly impaired renal function at baseline. It is the first study to look at a more diverse composite population such as one meets in the general nephrology clinic, although all patients had subnephrotic proteinuria. The finding that fractional excretion of albumin, a marker of loss of glomerular barrier function, has comparable value as a predictor to measurement of high molecular weight proteinuria is of use, as it is a value which can be calculated from routinely available laboratory data. Our results differ from those of Deegens and Wetzels, who found fractional excretion of albumin to have low predictive power in primary FSGS [15].

Clearly, different renal diseases have differing rates of progression and therefore we looked more closely at the three most prevalent diseases in the cohort. Overall, despite differences in FE IgG and FE Alb cut-offs between diseases, the whole-cohort cut-offs still significantly predict outcome in individual diseases, although event rates in individual groups were low. There were no baseline differences in levels of FE IgG and FE Alb between the groups and therefore the association between renal outcome and urinary variables likely reflects a true finding, rather than being the result of confounding due to different primary renal diseases.

Conclusions

In summary, in a homogenous group of patients with CKD, we have found that fractional excretions of IgG and albumin are superior to conventional measures of proteinuria, and to the urinary IgG:creatinine ratio, in predicting outcome in patients with primary glomerulonephritis. This may be because they are more accurate indicators of impairment of glomerular permselectivity. The reliance of many studies on urinary protein or albumin measurements as predictors must be questioned, particularly as the FE Alb can easily be calculated from routine laboratory measurements. FE Alb should be used, in conjunction with other measures of proteinuria, in future studies of prediction of CKD progression.

Conflict of interest statement. None declared.

References


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