Stratifying risk in chronic kidney disease: an observational study of UK guidelines for measuring total proteinuria and albuminuria*

S. METHVEN1, J.P. TRAYNOR2, M.D. HAIR3, D. ST J. O’REILLY4, C.J. DEIGHAN5 and M.S. MacGREGOR1

From the 1John Stevenson Lynch Renal Unit, Crosshouse Hospital, Kilmarnock, KA2 0BE UK, 2Renal Unit, Monklands Hospital, Airdrie, ML6 0JS UK, 3Division of Physical Sciences, University of the West of Scotland, Paisley, PA1 2BE UK, 4Department of Biochemistry, Glasgow Royal Infirmary, Glasgow, G4 0SF UK and 5Renal Unit, Glasgow Royal Infirmary, Glasgow, G4 0SF UK

Address correspondence to S. Methven, Specialty Registrar in Nephrology and Clinical Teaching Fellow, John Stevenson Lynch Renal Unit, Crosshouse Hospital, Kilmarnock, KA2 0BE UK. email: shona.methven@nhs.net

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Summary

Background: Proteinuria predicts poor renal and cardiovascular outcomes. Some guidelines recommend measuring proteinuria using albumin:creatinine ratio (ACR), while others recommend total protein:creatinine ratio (TPCR).

Aim: To compare renal outcomes and mortality in the populations identified by these different recommendations.

Design: Retrospective longitudinal cohort study.

Methods: Baseline ACR and TPCR measurements were obtained from 5586 patients with chronic kidney disease (CKD) attending a Scottish hospital nephrology clinic. The cohort was divided into three groups with concordant results by ACR and TPCR (no proteinuria; low proteinuria; significant proteinuria) and one group with discordant results (significant proteinuria with TPCR, but not ACR). Outcomes were assessed using Kaplan–Meier plots and Cox proportional hazards models.

Results: Median follow-up was 3.5 years (interquartile range (IQR) 2.1–6.0); 844 (15%) died at 3.0 years (IQR 1.8–4.7) and 468 (8%) started renal replacement therapy (RRT) at 1.7 years (IQR 0.6–3.4). Proteinuria was associated with a substantially increased risk of RRT and death. Patients with significant proteinuria by TPCR, but not ACR (n = 231) had high renal risk, and the highest all-cause mortality (log-rank \( P < 0.001 \)). With multivariate analysis the risk fell below those with significant proteinuria with concordant results by ACR and TPCR but remained considerably higher than those without significant proteinuria.

Conclusions: Proteinuria screening with TPCR identifies an additional 16% of patients with significant proteinuria, not identified using ACR. This subgroup has high renal risk, and high risk of all-cause mortality and therefore warrant identification. Guideline recommendations on proteinuria screening in CKD should be reconsidered.
Introduction

Proteinuria is common with 1.3–8.2% of the population affected, depending on the definition.\(^1\) Proteinuria is associated with adverse outcomes, but the optimal method to measure proteinuria remains uncertain. Total proteinuria is the single strongest predictor of renal risk, predicting progressive kidney disease and end-stage kidney disease.\(^2–4\) Albuminuria predicts progressive kidney disease in patients with diabetes mellitus,\(^5\) progression to end-stage renal disease in those with reduced estimated glomerular filtration rate (eGFR),\(^6\) de novo renal impairment and cardiovascular mortality in the general population.\(^7,8\) Intervention studies in diabetic kidney disease have traditionally measured albuminuria,\(^9–11\) while those in non-diabetic kidney disease have used total proteinuria.\(^12\)

Two key thresholds have been identified in the management of proteinuria; 1 g/day of total proteinuria, above which aggressive blood pressure control has been demonstrated to reduce progression to end-stage kidney disease\(^13\) and 0.5 g/day of total proteinuria above which the use of angiotensin converting enzyme inhibitors have been found to be specifically beneficial, over and above their blood pressure lowering effects, to retard progression of kidney disease.\(^2\)

The National Institute for Health and Clinical Excellence (NICE) in England and Wales and Kidney Disease Outcomes Quality Initiative (K/DOQI) recommend quantifying proteinuria using albumin:creatinine ratio (ACR) in all patients with chronic kidney disease (CKD), whereas the Scottish Intercollegiate Guidelines Network (SIGN) and Caring for Australasians with Renal Impairment (CARI) guidelines recommend total protein:creatinine ratio (TPCR) in non-diabetic patients.\(^14–17\) The relationship between total protein and albumin in the urine is non-linear, but equivalent levels have been proposed: >1 g/day proteinuria [equivalent to ACR >70 mg/mmol (>619 mg/g) or TPCR >100 mg/mmol, (885 mg/g)] and >0.5 g/day proteinuria [equivalent to ACR >30 mg/mmol (265 mg/g) or TPCR >50 mg/mmol (442 mg/g)].\(^15\)

The biochemistry laboratory in Glasgow Royal Infirmary routinely analyses urine samples for both albumin and total protein. Therefore, the aim of this study was to compare the outcomes of patients identified as having significant proteinuria, according to the thresholds described above, by ACR and TPCR.

Materials and methods

Participants and setting

The clinical details of all patients attending the renal clinic at Glasgow Royal Infirmary are entered into an electronic patient record (Proton, Clinical Computing UK Ltd, Brentford, UK, www.ccl.com), which also receives laboratory data electronically, and captures date of death from the central hospital database. Random spot urine samples are routinely sent from all patients. We retrospectively searched our database for all patients who had total protein, albumin and creatinine measured on a spot urine sample on the same date. The earliest available paired results for ACR and TPCR were used from 1999 to 2009, as details of laboratory assays prior to this date were not available. Patients were excluded from analysis if they were under the age of 18 years, on renal replacement therapy (RRT) including renal transplantation, or had <1-year follow-up (on the basis of insufficient exposure to the variable of interest). The following baseline data were also obtained: gender, age at time of urine sample, primary renal disease, use of angiotensin-converting enzyme inhibitor (ACEi) and/or angiotensin receptor blocker (ARB), weight, height, blood pressure, serum creatinine and estimated glomerular filtration rate (eGFR). Subsequent measurements of serum creatinine and eGFR were obtained. Date of commencing RRT for established renal failure (RRT for acute kidney injury was excluded from this analysis), and date of death were collected.

For the past decade, written consent for use of the electronic patient record has been requested from patients, which states that the data will be used for audit and research, in addition to routine clinical care. Data were downloaded with patient identifiers removed prior to analysis. The National Health Service National Research Ethics Service confirmed that ethical approval was not required.

Laboratory assays

Before August 2006, urine albumin was measured on a Bayer Advia 1650 analyser (Siemens, formerly Bayer Diagnostics, Wittelsbäckerplatz 2, Munich, Germany, www.siemens.com) using an immunoturbidimetric method with anti-human albumin antiserum [mean between batch co-efficient of variation (CV) 4.4% at a concentration of 54 mg/l]. The urine total protein assay was performed on the same analyser using the pyrogallol red colorimetric method (mean between batch CV 8.32% at a concentration of 0.56 g/l). In August 2006, the analyser
was replaced by an Abbott Architect 2000 (Abbott Laboratories, Abbott Park, Illinois, USA, www.abbott.com). Subsequently urinary albumin was measured using an immunoturbidimetric method using anti-human albumin antiserum (mean between batch CV 5.1% at a concentration of 111 mg/l). The urinary total protein was analysed using a turbidimetric method with benzethonium precipitation (mean between batch CV 1.8% at a concentration of 0.58 g/l). The urine creatinine was assayed by a reaction rate Jaffe method using Abbott reagents (mean between batch CV 3.4% at a concentration of 5.9 mmol/l; 3.0% at 13.2 mmol/l). The analyses were performed by the staff of the biochemistry laboratory, and they were not blinded to the results. In-house comparison was made between the results obtained on the Bayer Advia 1650 and the Abbott Architect 2000, and no significant differences were found in precision and accuracy, between the results obtained before and after the change in instrumentation for these analytes. Returns to the United Kingdom External Quality Assurance Scheme showed no change in accuracy, precision or bias in the laboratory’s results during this period. The laboratory is fully accredited by Clinical Pathology Accreditation (UK) Ltd.

Statistical analyses
Data were analysed using SPSS 16.0 for Windows (SPSS Inc, Chicago, IL, USA, www.spss.com). All data were assessed for normality, and appropriate summary statistics are presented. TPCR and ACR data were log transformed given the skewed distribution of values. Comparison of the groups was performed using two-sample \(t\)-test, analysis of variance, Mann–Whitney U-test and the Kruskall–Wallis test as appropriate.

Proteinuria was defined as significant using the two thresholds of 0.5 g/day or 1 g/day (0.5 g/day being equivalent to ACR \(\geq 30\) mg/mmol, and TPCR \(\geq 50\) mg/mmol and 1 g/day being equivalent to ACR \(\geq 70\) mg/mmol and TPCR \(\geq 100\) mg/mmol). Mild proteinuria was defined as below the thresholds described, and above the laboratory reference range [ACR 3–29 mg/mmol and TPCR 15–49 mg/mmol (i.e. microalbuminuria) for the 0.5 g/day threshold and ACR 3–69 mg/mmol and TPCR 15–99 mg/mmol for the 1 g/day threshold].

No proteinuria was defined as less than the laboratory reference range (ACR <3 mg/mmol and TPCR <15 mg/mmol). Kaplan–Meier survival plots were constructed. Hazard ratios were calculated for the main outcome measures (all-cause mortality, commencement of RRT and doubling of serum creatinine) using a hierarchical Cox regression survival analysis with age, gender, blood pressure and serum creatinine as co-variates entered in the first block, and either ACR or TPCR entered in the second block. The hazard ratios presented are for a 10-fold increase in the variable measured (due to the use of a logarithmic scale). Cases were excluded from the Cox regression survival analysis if any of the variables were missing (mostly blood pressure). The analyses were repeated with missing variables imputed using regression, to ensure there was no influence on the model.

The linearity of each continuous predictor was tested by calculating martingale residuals for the Cox regression model without the predictor and then plotting these against the predictor using lowess smoothing. The Proportional hazards assumption was tested by creating time dependent covariates for each predictor and including them in the model if the interaction was significant.

Results
We identified 8457 patients with both ACR and TPCR measured on the same day, between 24 November 1999 and 28 May 2008. A flow diagram of the population and exclusions is shown in Figure 1. Baseline data for the remaining 5586 patients are presented in Table 1. The primary renal disease (PRD) of the patients (defined according to the European Renal Association—European Dialysis and Transplantation Association codes) was available in 68% of the total cohort: primary glomerulonephritis 17%; interstitial disease 22.5%; multisystem disease 16.3%; diabetic nephropathy 11.1%; other 0.1% and CKD unknown cause 33.1%. Of the patients in Group 4 (discordant group), 72% had a PRD recorded, and the proportion of primary glomerulonephritis was lower (7.2%), interstitial disease was considerably higher (39.5%) multisystem disease 16.3%; diabetic nephropathy 11.1%; other 0.1% and CKD unknown cause 33.1%. Of the patients in Group 4 (discordant group), 72% had a PRD recorded, and the proportion of primary glomerulonephritis was lower (7.2%), interstitial disease was considerably higher (39.5%) multisystem disease and diabetic nephropathy lower (4.8 and 8.4%, respectively) and CKD cause unknown was higher (40.1). Of note, there were 26 patients in the total cohort with a PRD of myelomatosis and of these five patients were in Group 4 (discordant group). Patients were followed up for a total of 22 289 patient-years and a median of 3.5 years (interquartile range (IQR) 2.1–6.0 years).

Patient outcomes
There were 844 deaths during follow-up (15% of the population) at a median of 3.0 years (IQR 1.8–4.7 years). RRT was commenced in 468 patients
The serum creatinine of 999 patients (18%) doubled at a median of 2.2 years (1.1–3.8 years).

**Cohort subgroups: clinically important thresholds of proteinuria: 1 g/day**

The cohort was divided into three groups with concordant ACR and TPCR results: Group 1: no proteinuria (within laboratory reference range of ACR <3 mg/mmol and TPCR <15 mg/mmol) (n = 1001), Group 2: mild proteinuria (<1 g/day equivalent) (n = 3069) and Group 3: significant proteinuria (>1 g/day equivalent) (n = 1250). Two groups with discordant results by ACR and TPCR were also defined. Group 4: significant proteinuria by TPCR but not ACR (urine total protein over 1 g/day equivalent, but low urine albumin) (n = 231) and Group 5: significant proteinuria by ACR but not TPCR (urine total protein <1 g/day equivalent, but high urine albumin) (n = 35). The numbers in Group 5 are very small and have therefore been excluded from the results presented here. However when Group 5 were included, the results did not alter significantly.

The demographics of Groups 3 and 4 were compared using a two-sample t-test and Mann–Whitney U-test, as appropriate. Group 4 (discordant proteinuria) was significantly older with lower eGFRs (P<0.001), while Group 3 (significant proteinuria) had significantly higher blood pressures and proteinuria, measured by ACR and TPCR (P<0.001). There was no difference in gender between the groups (P=0.936). Kaplan–Meier survival plots were constructed for all-cause mortality (Figure 2a) and renal survival (Figure 2b). Patient survival of Group 4 (discordant proteinuria) was significantly worse than Groups 2 (mild proteinuria) and 3 (significant proteinuria) (log-rank test, P<0.001). Renal survival for Group 4 (discordant proteinuria) is similar to Group 3 (significant proteinuria), and significantly worse than Group 2 (mild proteinuria) (P<0.001). Kaplan–Meier plots were also constructed for doubling of serum creatinine, and demonstrated a similar pattern of outcomes to that of renal survival.

A multivariate analysis was performed using Cox regression analyses for all-cause mortality, RRT and doubling serum creatinine, with age, sex, kidney function and blood pressure as co-variates (Table 2). The risk of all-cause mortality for Group 4 (discordant proteinuria) compared with Group 3 (significant proteinuria) is attenuated by the multivariate analysis, but the risk does not fall to that of Group 2 (mild proteinuria). The same pattern is seen.
for commencement of RRT and doubling of serum creatinine. Repeat analyses with imputed data (using regression) for any missing variables did not alter the results significantly.

Cohort subgroups: clinically important thresholds of proteinuria: 0.5 g/day

The same process was applied to the 0.5 g/day threshold, using the appropriate cut-points (see Figure 1 for a description of Groups 1–5). Kaplan–
Meier plots were also constructed for the three outcome measures using a proteinuria threshold of 0.5 g/day (see Supplementary Data Figure S1). For the outcome of all-cause mortality, Group 4 (discordant proteinuria) had a significantly worse outcome than Groups 2 (mild proteinuria) and 3 (significant proteinuria) \((P<0.001)\), and for renal survival and doubling of serum creatinine, Group 4 had a significantly worse outcome than Group 2 (mild proteinuria), but better than Group 3 (significant proteinuria) \((P<0.001)\).

A multivariate analysis was also performed for the 0.5 g/day proteinuria threshold using Cox regression analyses for all-cause mortality, RRT and doubling serum creatinine, with age, sex, kidney function and blood pressure as co-variates, (Table 3). The same pattern of results is seen as for the 1 g/day threshold. Repeat analyses with imputed data (using regression) for any missing variables did not alter the results significantly.

### Discussion

In this cohort of patients attending a hospital kidney clinic, ACR failed to identify 231 patients with significant proteinuria who were identified with TPCR. This represents 15% of patients with significant proteinuria (defined as TPCR and/or ACR >1 g/day equivalent of urine protein, \(n=1516\)) or 16% of patients who would have been identified by TPCR alone (231/1481). This subgroup of patients (with significant proteinuria by TPCR but not ACR) has a high risk of renal events and death, with comparable renal survival and poorer patient survival than those with significant proteinuria by both TPCR and ACR. This increased risk for the subgroup of patients with significant proteinuria by TPCR but not ACR remains when the lower threshold of 0.5 g/day of proteinuria was used.

With multivariate analysis, some of the excess risk is abolished, with the risk of all-cause mortality,

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**Table 2** Association of baseline urinary ACR and TPCR with subsequent patient outcomes in 4824 patients with CKD, (approximately equivalent to 1 g/day of proteinuria)

<table>
<thead>
<tr>
<th>Group</th>
<th>No proteinuria (ACR and TPCR within reference range)</th>
<th>Group 2 Mild proteinuria (low ACR, low TPCR)</th>
<th>Group 3 Significant proteinuria (High ACR, high TPCR)</th>
<th>Group 4 Discordant proteinuria (Low ACR, high TPCR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td>1.00</td>
<td>1.57 (1.18–2.09)</td>
<td>2.59 (1.91–3.50)</td>
<td>1.91 (1.29–2.83)</td>
</tr>
<tr>
<td>RRT</td>
<td>1.00</td>
<td>2.06 (1.07–3.97)</td>
<td>7.91 (4.15–15.08)</td>
<td>4.40 (2.17–8.91)</td>
</tr>
<tr>
<td>Doubled sCr</td>
<td>1.00</td>
<td>1.70 (1.28–2.25)</td>
<td>5.07 (3.82–6.74)</td>
<td>3.56 (2.43–5.20)</td>
</tr>
</tbody>
</table>

Adjusted hazard ratios (with 95% confidence intervals) from multivariate Cox regression analyses are presented, for a 10-fold increase in the variable measured. Age, gender, blood pressure and serum creatinine are co-variates in all models. Serum creatinine (sCr) is a time-dependent co-variate for RRT. Age is a time-dependent co-variate for doubling sCr.

**Table 3** Association of baseline urinary ACR and TPCR with subsequent patient outcomes in 4824 patients with CKD (approximately equivalent to 0.5 g/day of proteinuria)

<table>
<thead>
<tr>
<th>Group</th>
<th>No proteinuria (ACR and TPCR within reference range)</th>
<th>Group 2 Mild proteinuria (low ACR, low TPCR)</th>
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<th>Group 4 Discordant proteinuria (Low ACR, high TPCR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td>1.00</td>
<td>1.49 (1.11–1.99)</td>
<td>2.48 (1.86–3.32)</td>
<td>2.34 (1.63–3.35)</td>
</tr>
<tr>
<td>RRT</td>
<td>1.00</td>
<td>2.28 (1.08–4.77)</td>
<td>8.46 (4.14–17.26)</td>
<td>2.90 (1.31–6.43)</td>
</tr>
<tr>
<td>Doubled sCr</td>
<td>1.00</td>
<td>1.47 (1.11–1.95)</td>
<td>4.11 (3.14–5.38)</td>
<td>2.35 (1.62–3.40)</td>
</tr>
</tbody>
</table>

Adjusted hazard ratios (with 95% CIs) from multivariate Cox regression analyses are presented, for a 10-fold increase in the variable measured. Age, gender, blood pressure and serum creatinine are co-variates in all models. Serum creatinine (sCr) is a time-dependent co-variate for RRT. Age is a time-dependent co-variate for doubling serum creatinine (sCr).
commencement of RRT and doubling of serum creatinine falling below that of the significant proteinuria group (Group 3), but remaining higher than the low proteinuria group (Group 2). This can be explained, in part, by the differences in the demographics of the groups, with Group 4 being significantly older and with a lower eGFR. However, Group 4 (discordant proteinuria) still represents a high-risk group that would be identified using an appropriate total proteinuria threshold, but not using an equivalent albuminuria threshold.

Our study has several limitations. It was retrospective, and we therefore cannot confirm the causation of the observed relationships. The number of patients in Group 4 was relatively small. There may have been drift in the assays over such a prolonged period, but this will affect all assays and we have no reason to expect a systematic bias. The relationships demonstrated may only apply to the assays used in our study. A wide variety of assays is used to measure total protein, and even albumin immunoassays have considerable inter-assay and inter-laboratory variation. However, the strengths of this study are the large numbers of patients, and the representative nature of the population, namely an unselected adult population attending a general nephrology clinic. However, the proportion of the cohort with interstitial disease was relatively high and these findings may not be applicable to populations with lower prevalence of interstitial disease. Although, our study population is based on a secondary care cohort of patients, there are clear lessons from this study for both primary and secondary care practitioners who adhere to a referral pattern based solely on level of ACR.

NICE currently recommends that ACR should be used to screen all patients with CKD annually, with thresholds for action of 30–70 mg/mmol. KDOQI similarly recommends the use of ACR, although allows the use of TPCR when ACR is >56 mg/mmol. However, SIGN recommends using TPCR for CKD patients without diabetic nephropathy using action thresholds of 50 and 100 mg/mol. CARI also recommends using TPCR. Our data illustrate the potential impact of these differing recommendations on an unselected adult population attending a general nephrology clinic. Simply reducing the albuminuria threshold to improve sensitivity is ineffective, as it leads to unacceptably low specificity. Microalbuminuria (30–300 mg of urine albumin per day) has an established role in detecting early diabetic nephropathy, and has been shown to predict cardiovascular mortality in the general population. However, we have recently shown that total proteinuria is also predictive at equivalent levels.

In urine, total protein is comprised predominantly of albumin, but also of physiological proteins (such as Tamms-Horsfall protein) and other non-albumin proteins of various molecular weights. Only TPCR takes account of the non-albumin protein component. The relative proportions of these proteins vary widely in pathological states, and the non-albumin proteins are a less well-defined group of proteins compared with albumin. High-molecular weight proteinuria has been shown to correlate more strongly with rate of progression of renal disease than intermediate molecular weight, low-molecular weight or even total proteinuria.

Furthermore, there is substantial variation in the amount of non-albumin proteinuria between individuals at clinically significant levels of albuminuria. There is less inter- and intra-laboratory variation in albumin assays than total protein assays and efforts are underway to standardize the albumin assay across laboratories. However, ACR is 2–10 times more costly than TPCR.

Prospective studies are required to clarify the roles of total proteinuria and albuminuria as predictors of patient outcomes. Interventional studies in CKD should also assess the impact on both ACR and TPCR. Further research should examine the importance of specific non-albumin proteins in the urine both for prognostication, and to shed light on underlying pathophysiology.

In conclusion, screening with ACR alone will fail to identify 16% of patients with significant levels of proteinuria who would be identified by TPCR. This subgroup is at higher risk of death and renal outcomes than those with low proteinuria (low ACR, low TPCR) and merit identification. The current approach to measuring proteinuria recommended by guidelines should be reconsidered, to take account of albumin and non-albumin proteinuria. The non-albumin component of proteinuria may have pathophysiological significance.

### Supplementary Data

Supplementary Data are available at *QJM* online.

### Conflict of interest

None declared.

### References


