Biomarkers of inflammation, fibrosis, cardiac stretch and injury predict death but not renal replacement therapy at 1 year in a Canadian chronic kidney disease cohort

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ABSTRACT

Background. Newer biomarkers, reflective of biological processes, such as inflammation and fibrosis, cardiac stretch or damage and vascular health may be useful in understanding clinical events in chronic kidney disease (CKD). We assessed whether these newer biomarkers, alone or as a panel, improve risk prediction for renal replacement therapy or death, over and above conventional clinical, demographic and laboratory variables.

Methods. We conducted a prospective observational Canadian cohort study in 2544 CKD patients with estimated glomerular filtration rate (eGFR) of 15–45 mL/min/1.73 m², under nephrology care, in urban and rural centers. We measured traditional clinical and laboratory risk factors, as well as newer biomarkers: cystatin C, high sensitivity c-reactive protein (hsCRP), interleukin 6 (IL6), transforming growth factor β1 (TGFβ1), fibroblast growth factor 23 (FGF23), N-terminal probrain natriuretic peptide (NT-proBNP), troponin I and asymmetric dimethylarginine (ADMA). Key outcomes were renal replacement therapy (RRT, dialysis or transplantation) and death, during the first year follow-up after enrollment: a time point important for clinical decision-making for patients and providers.

Results. Newer biomarkers do not improve the prediction of RRT, when added to conventional risk factors such as eGFR, urine albumin to creatinine ratio, hemoglobin, phosphate and albumin. However, in predicting death within 1 year, cystatin C, NT-proBNP, hsCRP and FGF23 values significantly improved model discrimination and reclassification: c statistic increased by absolute 4.3% and Net Reclassification Improvement for categories of low, intermediate and high risk at 11.2%.

Conclusions. Our findings suggest that the addition of newer biomarkers may be useful in predicting death in patients with established CKD within a 1-year timeframe. This information may be useful in informing prognosis and redirect resources to serve patients at higher risk to improve outcomes and sustainability of the nephrology care system.

Keywords: biomarkers, CKD, outcomes, prediction, cohort study

INTRODUCTION

Chronic kidney disease (CKD), defined as a persistent reduction in kidney function [e.g. glomerular filtration rate (GFR) <60 mL/min for >3 months] or evidence of chronic kidney damage (e.g. proteinuria), affects 10–13% of adults in North America and prevalence is similar in Europe, Australia and China [1–6]. CKD is a risk factor for the development of end-stage renal disease (ESRD), which is associated with significant patient morbidity, excess mortality and high societal costs related to dialysis. Moreover, CKD of any severity is associated with excess cardiovascular risk and death [7, 8]. Appropriate identification, risk stratification and treatment of patients with
CKD represent major challenges for health systems worldwide.

CKD is a heterogeneous disorder with a highly variable clinical course [9, 10]. Some patients progress rapidly to ESRD, others remain stable indefinitely. CKD is linked to accelerated cardiovascular disease, thus many patients will die of cardiovascular causes before needing dialysis. Decision-making for short- and long-term outcomes is problematic for all stakeholders, given this variability, but is of particular interest to nephrologists caring for these patients at this latter stage.

Planning of treatment options usually commences ~12 months in advance of expected transitions. Thus, determining optimal timing for procedures such as arteriovenous fistula (AVF) creation or pre-emptive transplantation or planning and implementing conservative care requires the clinician to anticipate the risk of either progression to ESRD or death. From the health system’s perspective, CKD care is expensive [11], requiring specialized resources and clinics which should be directed to patients at true risk of progression. From a research perspective, testing of novel treatment strategies to prevent renal decline requires accurate identification of patients with sufficient risk of progression to make clinical trials viable; ideally using relatively short-time horizons of 12–24 months.

Conventional clinical and laboratory variables have been demonstrated to be helpful in predicting risk for progression to ESRD [12]; however, they are less helpful in predicting death. Newer biomarkers, which reflect underlying biological processes known to be associated with adverse outcomes, could potentially improve the prediction of death outcomes over the shorter term, and provide useful information to patients, clinicians and policy-makers.

We undertook this analysis to assess whether the addition of several newer biomarkers associated with vascular health, inflammation, fibrosis, heart failure and ischemia, either alone or in combination, could improve the prediction of important 1-year outcomes in a referred cohort of patients with advanced CKD. Specifically, we were interested in whether adding some newer biomarkers to clinical models, which use conventional demographic, clinical and laboratory variables, would aid in predicting important outcomes. The Canadian Study of Prediction of Dialysis, Death and Interim Cardiovascular events over Time (CanPREDDICT) is a Canadian cohort study designed to improve the understanding of variations in short- and long-term outcomes of patients followed by nephrologists using biomarkers assayed at enrollment into the study [13].

**MATERIALS AND METHODS**

A full description of the study design and baseline characteristics has been previously published [13]. In brief, CanPREDDICT is a prospective, multicentre cohort study of 2544 adult CKD patients enrolled across Canada from 2008 to 2009 over 18 months, and followed for 3 years or until death. Inclusion criteria were simple: patients with an estimated glomerular filtration rate (eGFR) of 15–45 mL/min/1.73 m² and under the care of nephrologists. Those excluded had life expectancy <12 months, organ transplant(s) and active vasculitis (Supplementary data, Figure S1A). Blood pressure, height and weight, routine laboratory testing were collected 6 monthly, as were serum, plasma and urine samples.

**Rationale for selection and specifics of measurement of newer biomarkers**

Prespecified biomarkers were selected for analysis on the basis of biological relevance, commercial availability of assays, and published data suggesting prognostic value for heart disease or kidney disease progression. Asymmetric dimethylarginine (ADMA), a potent inhibitor of endothelial nitric oxide production, impacts vascular relaxation, contributes to hypertension, and is correlated with cardiovascular events and renal decline [14, 15]. N-terminal probrain natriuretic peptide (NT-proBNP) and troponin I are non-invasive measures of cardiac stretch and myocyte injury, respectively, and are known to predict cardiovascular events [16–20]. Serum interleukin 6 (IL6) and high sensitivity c-reactive protein (hsCRP) are key markers of chronic inflammation, an important contributor to vascular and renal disease progression [21–28]. Transforming growth factor β1 (TGFβ1) is a key mediator of fibrosis within the kidney and other organs [26, 27, 29]. Cystatin C is a well-known alternative marker of kidney function, with some advantages over serum creatinine, and is also associated with cardiovascular outcomes [30–32]. Fibroblast growth factor 23 (FGF23) is an endocrine hormone important in phosphate homeostasis, but has itself been shown to mediate direct, end-organ toxicity in the heart. A number of recent studies have demonstrated the importance of elevated levels in predicting adverse outcomes in CKD, dialysis and kidney transplant patients [33–36]. This selected panel is concordant with recent reviews on the subject [37]. Details of the measurement of each of the selected newer biomarkers are presented in the Supplementary data, Measurement of Biomarkers. A random 10% sample was run in duplicate to ensure reproducibility.

**Outcomes and adjudication**

Renal replacement therapy was defined as need for chronic dialysis initiation or transplantation. All deaths were confirmed either by documentation or verbally with family or clinic staff.

**Sample size**

The primary considerations for the sample size estimation was to ensure adequate power to demonstrate that the addition of newer biomarkers in predictive models enhanced discrimination between subjects who will or will not experience outcomes; and a high level of precision when assessing the discriminatory value of the new predictive models that include biomarkers. For sample size estimation details, see our previous publication [13].

**Statistical analysis**

Summary statistics were expressed as mean (standard deviation), median (interquartile range) or n (percent) as appropriate. Univariate comparisons were performed using ANOVA.
or the Kruskal–Wallis test for continuous variables, and $\chi^2$-tests for categorical variables.

We transformed parathyroid hormone (PTH), urine albumin to creatinine (uACR) ratio and biomarkers with highly skewed distributions (NT-proBNP, hsCRP, IL6, TGFb1 and FGF23) to the natural logarithmic scale. Troponin I was analyzed as a categorical variable due to the large percentage of values below the lower limit of detection (LLD).

The additional predictive value of newer biomarkers was evaluated using the following steps for each of the outcomes—progression to RRT or death:

(i) Base models: we first developed base proportional hazard models incorporating traditional clinical risk factors.

(ii) Adjusted hazard ratios (HRs) of individual biomarkers: we then added newer biomarkers individually to the base models and calculated adjusted HRs per one standard deviation (1SD) of each biomarker.

(iii) Biomarker models versus base models: we compared the base models versus models based on the addition of the newer biomarkers.

**Base models.** In order to determine the additional predictive value of newer biomarkers, we first developed base proportional hazard models for each outcome of interest (progression to RRT or death) by incorporating traditional clinical risk factors. Age, sex and baseline eGFR were included a priori in all initial models. Traditional risk factors were included into multivariate ‘base models’ using backward elimination. The overall fit of models was validated using the Akaike Information Criterion (AIC) [38]. The calibration of each model was determined using the Nam and D’Agostino $\chi^2$ statistic [39] and Hosmer–Lemeshow statistic (compares observed and predicted risk within categories) [40].

**Adjusted HRs of individual biomarkers.** The impact of individual biomarkers, adjusted for the traditional risk factors, was tested using adjusted HRs. Individual biomarkers were added to the respective base models for each outcome of interest with all conventional variables retained. Multivariable adjusted HRs were calculated per 1SD of each biomarker.

**Biomarker models versus base models.** Biomarker models were then created by the addition of biomarkers to the base models with all of the base model variables retained. All biomarkers except Troponin I (categorical variable, >LLD versus < LLD) were included in the models as continuous variables (NT-proBNP, hsCRP, IL6, TGFb1 and FGF23 transformed to the natural logarithmic scale).

We then examined the ability of biomarker models, containing the newer biomarkers alone and as a panel, in combination with the base models, to improve prediction of outcomes in comparison with the optimized base model. We compared the biomarker models versus the base models using the metrics of discrimination and reclassification. The metrics were assessed for each outcome of interest separately.

**Discrimination.** The concordance statistic (c-statistic) and integrated discrimination improvement (IDI) [38, 41–43] were calculated as measures of discrimination. The c-statistic was used as measure of improvement in models’ discrimination, the ability of models to correctly distinguish between two types of outcomes: RRT versus no RRT and death versus survival. The differences in c-statistic between each newer biomarker model and the base model were calculated using 50 bootstrap repetitions to generate the corresponding confidence intervals (CI) for this method [38, 44]. Sensitivity, specificity, positive-predictive value (PPV) and negative-predictive value (NPV) are calculated using the approach proposed by Chambless et al. [44] that accounts for censoring in survival models. We also measured discrimination improvement using the IDI as suggested by Pencina et al. [38, 44]. IDI measures the difference in the discrimination slope (i.e. the difference in the mean predicted probabilities of events and non-events) between base and biomarker models, and is considered more sensitive to improvements in discrimination than the c-statistic.

**Reclassification.** Reclassification improvement was quantified using the net reclassification improvement (NRI) statistic for survival data [42, 45]. The NRI reflects the net proportion of patients reclassified to a more appropriate risk category by the new model. The NRI estimates were based on the reclassification tables classifying patients in three risk categories for each outcome within a 1-year time window. For the purposes of this analysis, we defined low risk as a probability less than the average 1-year probability of the outcome in the cohort, moderate risk as a probability up to twice the overall probability, and high risk as greater than double the average population risk. For progression to RRT or death, this translated to risk categories of <5, 5–10 and >10%. The category-free, continuous NRI was calculated using the approach proposed by Pencina et al. [46].

**Sensitivity analysis.** To evaluate the effect of the competing risk of RRT versus death, we used the regression analysis approach proposed by Fine and Gray [46] for the direct regression modelling of the effect of covariates on the cumulative incidence function for competing risks.

All analyses were performed using SAS software, version 9.2 (SAS Institute Inc., Cary, NC, USA).

**RESULTS**

The study enrolled 2544 CKD patients recruited from nephrology clinics across Canada (rural, urban, academic and non-academic) between June 2008 and October 2009. Of these, 2402 patients (96%) had complete baseline data, on which this analysis and report are based (see Supplementary data, Figure SA1).

Table 1 describes the baseline characteristics, stratified by eGFR, for 2402 patients with complete data. The mean age of the cohort was 68 years, 90% were Caucasian, 62% were male and 48% had diabetes. Forty percent of the cohort had eGFR
between 30 and 45 mL/min/1.73 m², while the remainder (60%) had eGFR values <30 mL/min/1.73 m² at baseline; 22% had eGFR values <20 mL/min/1.73 m². The majority of patients had lower levels of albuminuria (61%). Patients with lower eGFR were younger and female compared to those with higher eGFR. Diabetes was significantly more prevalent in those with eGFR values between 20 and 29 mL/min/1.73 m², while PTH levels increased.

Serum albumin, hemoglobin (Hgb), calcium and 25-hydroxyvitamin D levels were progressively lower in the lower eGFR strata, whereas TGFβ1 values were lower at lower GFR.

### Table 1. Baseline characteristics stratified by eGFR level

<table>
<thead>
<tr>
<th>Variables</th>
<th>All</th>
<th>eGFR Level</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;20 mL/min</td>
<td>20–29 mL/min</td>
</tr>
<tr>
<td>N</td>
<td>2402</td>
<td>533 (22%)</td>
<td>933 (39%)</td>
</tr>
<tr>
<td>Age (in years)</td>
<td>68.1 (12.7)</td>
<td>66.9 (13.5)</td>
<td>68.7 (12.6)</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>1502 (63%)</td>
<td>311 (58%)</td>
<td>569 (61%)</td>
</tr>
<tr>
<td>Race (% Caucasian)</td>
<td>2142 (89%)</td>
<td>471 (88%)</td>
<td>834 (89%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1160 (48%)</td>
<td>251 (47%)</td>
<td>480 (51%)</td>
</tr>
</tbody>
</table>

### Primary kidney disease

- Diabetic nephropathy: 699 (29%)
- Hypertensive nephropathy: 637 (27%)
- Glomerulonephritis: 273 (11%)
- Polycystic kidney disease: 103 (4%)
- Other: 690 (29%)

### Other
- Primary kidney disease: 690 (29%)
- Other: 690 (29%)

### One-year outcome data

After 12 months, a total of 142 patients (5.9%) had progressed to RRT and 137 patients (5.7%) had died [15 (0.6%) after starting RRT]. A small proportion (1.5%) missed the 12-month follow-up (only 6-month follow-up data available), and was censored with the last available follow-up date. During 12 months, 10% progressed rapidly (defined as decline of renal function of more than 30%), 13% had slower decline of renal function (20–30%), and 69% were stable, as defined by the change within 20% of baseline, while 9% improved (defined as increase in eGFR >20%).

### Adjusted HRs of individual biomarkers

Figures 1 and 2 illustrate adjusted HRs for the newer biomarkers for RRT and death, respectively, as presented in Table 1. These models are presented as Model 1 in Tables 2 and 3, respectively.

### Evaluation of newer biomarkers in predicting events

#### Adjusted HRs of individual biomarkers

Figures 1 and 2 illustrate adjusted HRs for the newer biomarkers for RRT and death, respectively, as presented in Table 1. These models are presented as Model 1 in Tables 2 and 3, respectively.
Table 2. Predictive models for traditional and newer biomarker parameters associated with 12-month progression to RRT

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>Model 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio</td>
<td>95% CI</td>
<td>Hazard ratio</td>
<td>95% CI</td>
<td>Hazard ratio</td>
</tr>
<tr>
<td>Age per 5 years</td>
<td>0.981</td>
<td>0.921–1.046</td>
<td>0.925</td>
<td>0.861–0.993</td>
<td>0.921</td>
</tr>
<tr>
<td>GFR per 1 mL/min/1.73 m²</td>
<td>0.863</td>
<td>0.834–0.892</td>
<td>0.866</td>
<td>0.837–0.897</td>
<td>0.868</td>
</tr>
<tr>
<td>Log urine ACR mg/mmol per 1SD</td>
<td>1.971</td>
<td>1.598–2.431</td>
<td>1.714</td>
<td>1.385–2.120</td>
<td>1.722</td>
</tr>
<tr>
<td>Serum hemoglobin per 5 g/L</td>
<td>0.919</td>
<td>0.871–0.969</td>
<td>0.948</td>
<td>0.898–1.001</td>
<td>0.950</td>
</tr>
<tr>
<td>Serum bicarbonate per 1 mmol/L</td>
<td>1.069</td>
<td>1.021–1.119</td>
<td>1.063</td>
<td>1.015–1.113</td>
<td>1.069</td>
</tr>
<tr>
<td>Serum phosphate per 0.1 mmol/L</td>
<td>1.070</td>
<td>1.007–1.137</td>
<td>1.057</td>
<td>0.993–1.125</td>
<td>1.061</td>
</tr>
<tr>
<td>Serum calcium per 0.1 mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.007</td>
</tr>
<tr>
<td>Serum albuminper 1 g/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.960</td>
</tr>
<tr>
<td>Log NT-proBNP pg/mL per 1SD</td>
<td>1.458</td>
<td>1.210–1.756</td>
<td>1.441</td>
<td>1.198–1.733</td>
<td>1.491</td>
</tr>
<tr>
<td>Log TGFβ1 pg/mL per 1SD</td>
<td>0.825</td>
<td>0.692–0.983</td>
<td>0.821</td>
<td>0.690–0.978</td>
<td></td>
</tr>
<tr>
<td>Akaike information criterion c-statistic</td>
<td>0.865 (0.835–0.891)</td>
<td>0.869 (0.845–0.894)</td>
<td>0.871 (0.846–0.898)</td>
<td>0.863 (0.836–0.889)</td>
<td>0.871 (0.848–0.897)</td>
</tr>
</tbody>
</table>

Model 1: Base model, based traditional risk factors.
Model 2: Base model + best individual newer biomarker.
Model 3: Base model + best combination of newer biomarkers.
Model 4: Reparameterized Model 6 from the analysis by Tangri et al. [12].
Model 5: Reparameterized Model 6 from the analysis by Tangri et al. [12] + best combination of newer biomarkers.

significant association with death, after adjusting for traditional risk factors (Figure 2).

NT-proBNP [HR = 1.90 (95% CI: 1.54–2.34)], cystatin C [HR = 1.52 (95% CI: 1.25–1.84)] and FGF23 [HR = 1.46 (95% CI: 1.24–1.71)] were biomarkers with the highest HRs (per 1SD) for death.

Biomarker models versus base models. We compared the ability of biomarker models, using biomarkers alone and in combination, to improve prediction of outcomes in comparison with the optimized base models for each outcome of interest separately.

Renal replacement therapy. Table 2 summarizes the different models (1–5) predicting progression to RRT within a year. The base model (Model 1) included age, sex, eGFR, uACR, serum hemoglobin, bicarbonate and phosphate, and exhibited very good discrimination [c-statistic 0.865 (0.835–0.891)]. When biomarkers were added singly, only NT-proBNP and TGFβ1 were statistically significant. Addition of NT-proBNP to the base model provided the best improvement in c-statistic of any of the biomarkers considered alone (Table 2, Model 2). The addition of both NT-proBNP and TGFβ1 resulted in further small improvements in c-statistic (Table 2, Model 3). In all cases, improvements in discrimination were marginal, and no biomarker alone or in combination improved classification performance (NRI) of any model. The categorical and continuous NRIs and IDIs are presented in Supplementary data, Table SA1. The performance of one contemporary published model, also using Canadian data (Tangri et al. analysis [12]), was not significantly different from the base model (Table 2, Model 4 versus Model 1). Similarly, the addition of newer biomarkers with the highest discrimination/classification improvement (NT-proBNP and TGFβ1) to the published model did not significantly improve discrimination and classification (Table 2, Model 5).

Death. Table 3 describes models for death within 12 months of baseline. The base model includes age, sex, eGFR, history of cardiovascular disease, serum phosphate and albumin; it exhibited moderate discrimination [Table 3, Model 1; c-statistic 0.750 (0.724–0.817)]. Note that uACR was not a significant predictor of mortality in this cohort.

NT-proBNP had the largest impact on model prediction, improving discrimination by an absolute 3% over the base model (Table 2, Model 2 and Supplementary data, Table SA2). When all biomarkers were placed in the model, NT-proBNP, hsCRP, FGF23 and ADMA together remain statistically significant after adjusting for the traditional risk factors in the base model. This panel further improved c-statistic to 0.796 (Table 3, Model 3). Model 3, with a panel of biomarkers on top of traditional markers, significantly improved patient mortality risk stratification: the categorical (low/intermediate/
high) NRI was 14.4%, while the continuous NRI was 51.3%. Model 4 shows the ‘best’ model wherein all variables were allowed to compete for inclusion in the final model; this model included only age, albumin and newer biomarkers (cystatin C, NT-proBNP, FGF23 and hsCRP) and performed similarly to ‘fully loaded’ Model 3 (NRI = 11.2% over Model 1). Importantly, neither eGFR nor uACR were maintained in the best model to predict death at 12 months. The discrimination improvement metrics (c-statistics change and IDI) and reclassification metrics (categorical and continuous NRI) for all models are presented in Supplementary data, Table SA2. Supplementary data, Table SA3 presents sensitivity, specificity, PPV and NPV of the newer-biomarkers’ model for a range of cut-off points. The newer-biomarkers’ model for death attains higher sensitivity than specificity across cut-off points, as well as high NPV. However, the PPV appears lower due to 6% rate of events. The ROC curves for models based on traditional markers versus newer markers are presented in Supplementary data, Figure SA2.

Table 3. Predictive models for demographic, traditional and novel biomarker parameters associated with 12-month progression to death

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
<th>Model 3</th>
<th></th>
<th>Model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio</td>
<td>95% CI</td>
<td>Hazard ratio</td>
<td>95% CI</td>
<td>Hazard ratio</td>
<td>95% CI</td>
<td>Hazard ratio</td>
</tr>
<tr>
<td>Age per 5 years</td>
<td>1.275</td>
<td>1.163–1.397</td>
<td>1.225</td>
<td>1.116–1.345</td>
<td>1.249</td>
<td>1.136–1.373</td>
<td>1.241</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.269</td>
<td>0.888–1.814</td>
<td>1.213</td>
<td>0.848–1.734</td>
<td>1.223</td>
<td>0.854–1.752</td>
<td></td>
</tr>
<tr>
<td>History of cardiovascular disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ischemic heart disease</td>
<td>1.870</td>
<td>1.156–3.026</td>
<td>1.342</td>
<td>0.821–2.194</td>
<td>1.354</td>
<td>0.828–2.121</td>
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</tr>
<tr>
<td>Congestive heart failure</td>
<td>2.214</td>
<td>1.315–3.728</td>
<td>1.339</td>
<td>0.774–2.317</td>
<td>1.296</td>
<td>0.746–2.252</td>
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<tr>
<td>Both</td>
<td>2.993</td>
<td>1.941–4.615</td>
<td>1.600</td>
<td>0.995–2.572</td>
<td>1.583</td>
<td>0.985–2.542</td>
<td></td>
</tr>
<tr>
<td>eGFR per 1 mL/min/1.73 m²</td>
<td>0.972</td>
<td>0.951–0.993</td>
<td>0.987</td>
<td>0.965–1.008</td>
<td>0.994</td>
<td>0.972–1.017</td>
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<tr>
<td>Serum phosphate per 0.1 mmol/L</td>
<td>1.082</td>
<td>1.020–1.148</td>
<td>1.064</td>
<td>0.998–1.136</td>
<td>1.051</td>
<td>0.983–1.125</td>
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</tr>
<tr>
<td>Serum albumin per 1 g/L</td>
<td>0.925</td>
<td>0.891–0.961</td>
<td>0.950</td>
<td>0.914–0.988</td>
<td>0.958</td>
<td>0.921–0.996</td>
<td>0.956</td>
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<tr>
<td>ADMA μM per 1 SD</td>
<td>1.138</td>
<td></td>
<td>1.002–1.293</td>
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<td></td>
<td></td>
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<tr>
<td>Log NT-proBNP pg/mL per 1 SD</td>
<td>1.898</td>
<td>1.540–2.340</td>
<td>1.628</td>
<td>1.311–2.021</td>
<td>1.794</td>
<td>1.475–2.181</td>
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<tr>
<td>Log hsCRP mg/L per 1 SD</td>
<td>1.278</td>
<td>1.065–1.535</td>
<td>1.242</td>
<td>1.037–1.487</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log FGF-23 RU/mL per 1 SD</td>
<td>1.250</td>
<td>1.050–1.488</td>
<td>1.242</td>
<td>1.037–1.487</td>
<td></td>
<td></td>
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<tr>
<td>Cystatin C ng/mL per 1 SD</td>
<td>1.223</td>
<td>1.026–1.457</td>
<td></td>
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<td>Akaike information criterion</td>
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<td>1976</td>
<td>1963</td>
<td>1957</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-statistic</td>
<td>0.750 (0.724–0.717)</td>
<td>0.784 (0.742–0.836)</td>
<td>0.796 (0.770–0.847)</td>
<td>0.793 (0.763–0.836)</td>
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Model 1: Base model, based on traditional risk factors.
Model 2: Base model + best individual newer biomarker.
Model 3: Base model + best combination of newer biomarkers.
Model 4: ‘Best’ model based on significant traditional risk factors and newer biomarkers.

a1 standard deviation: ADMA = 0.11 μM; NT-proBNP = 1.38 log pg/mL; hsCRP = 1.29 log mg/L; FGF-23 = 0.77 log RU/mL; Cystatin C = 537 ng/mL.

Figure 1: Adjusted HRs for renal replacement therapy per 1SD of newer biomarkers.
Sensitivity analyses. Analyses using the competing risk approach showed no significant differences between the reported Cox proportional hazards method results and the results of regression modeling of the effect of covariates on the cumulative incidence function (data not shown).

DISCUSSION

Our study adds to the literature in a number of ways. First, we were able to determine that these specific newer biomarkers, some of which are commercially available, do not substantially improve prediction of RRT within 1 year, when added to conventionally available clinical and biochemical variables, in a cohort with advanced CKD. However, NT-proBNP, FGF23, hsCRP and cystatin C as a panel significantly do improve the prediction of death at 1 year in this cohort. Collectively, this panel reflects biological processes that have been linked to cardiovascular and inflammatory processes.

Although some improvements in RRT risk prediction were observed with the new biomarkers, these effects were marginal and did not lead to improvements in fit, discrimination or reclassification compared with the base model. Our results are congruent with other RRT prediction studies. Tangri et al. [12] and others [32, 47–49] have previously demonstrated that models based on clinical and conventional laboratory values alone can successfully predict RRT. Our results confirm that these models are robust, and there are limited opportunities to improve them.

The addition of NT-ProBNP, hsCRP, FGF23 and cystatin C did significantly improve the prediction of death within 1 year, in this cohort of patients with established advanced CKD, cared for by nephrologists. This finding is novel given that previous publications have traditionally used only conventional biomarkers [50]. Our best model, which is based on these selected newer biomarkers, outperforms previous models for death prediction. Note that cystatin C remains in our model but not eGFR and uACR. This may signify the superiority of cystatin C over markers of kidney function to predict all-cause mortality in those with advanced CKD [8, 51]. The physiological-based biomarkers reflecting volume overload or myocardial stretch, inflammation and fibrosis, drawn in asymptomatic outpatients appear to be helpful in predicting death at 1 year.

Research and clinical implications

We need better prediction models in order to make decisions regarding planning for RRT (e.g. AVF creation, preemptive transplantation work-up) or initiating interventions for cardiovascular disease to reduce morbidity or delay progression of CKD. Thus, identifying high-risk populations with both conventional and easily accessible newer biomarkers is valuable information for research and clinical care. Risk stratification and enrollment into clinical trials may consider using these relatively accessible biomarkers, since they do appear to discriminate between outcomes in this cohort. From a clinical perspective, we would need to evaluate the utility and uptake of current prediction models in clinical practice to see if decision-making can be impacted using these models: can timing of fistula creation or referral for transplant assessment or institution of diuretic therapy be more confidently pursued if these equations using newer biomarkers are systematically applied in clinical practice?

Perhaps more importantly, can we design trials with clinical interventions based on specific processes deemed to be important in predicting outcomes? Since markers of cardiac stretch, inflammation and fibrosis appear to have discriminatory ability in those with relatively advanced CKD, it would
be important to determine whether these markers are responsive to therapeutic strategies aimed at these processes. Patients with advanced CKD are at higher risk of dying thus, we need to determine how best to use the prediction models in clinical settings. Recent reviews of predictive models [50] emphasize the lack of models to predict death in CKD populations, so that our finding that easily accessible newer biomarkers facilitate discrimination of key outcomes of interest in CKD, may be helpful. Selected newer biomarkers used in a mortality risk prediction equation could provide the basis for more precise estimation of death versus RRT risk within 12 months: thus helping to inform decisions about whether and when to initiate specific therapies or end of life discussions with patients.

Schell and colleagues [52] have demonstrated that uncertainty about outcomes leads to issues with communication for both patients and providers, which may impact timeliness of decision-making. Thus, use of prediction equations, which can discriminate these two events in individual patients, should be useful in clinical practice.

Of interest, and in contrast to other studies, we found that uACR as a variable, was not maintained in any of the models for predicting death [8, 53]. This is likely due to multiple factors: first, these are all CKD patients, referred and treated, so that the uACR may not add informative value in advanced CKD as it does in general population cohorts. Second, the majority of patients have uACR values in the lower ranges: reflecting a combination of either treatment effects, and/or, possibly lower eGFR. We suggest that uACR in this advanced CKD cohort, followed by nephrologists, has less discriminatory value for death, than in untreated general populations. However, the uACR values are retained in predicting RRT: this is important for the purposes of RRT planning.

The value of examining biomarkers reflective of biological processes is that we may foster additional research into mechanisms of disease. This information may in turn lead to therapeutic targeting in delaying or modifying disease processes or expression. For example, gene therapy for FGF23 has been recently entertained to regulate metabolic bone disease [54], and antibodies to FGF23 have been demonstrated to attenuate cardiac disease in animals [55]. There is also current interest in drugs with antagonistic properties to TGFβ to slow fibrogenesis [56].

**Strengths**

The main strengths of this study include a large sample size, multicenter design, inclusion of both academic and community clinics, detailed prospective data capture and independent adjudication of outcomes. The measurement of several newer biomarkers of vascular health, inflammation and kidney function is important. NT-proBNP, FGF23 and hsCRP were most consistently identified as potentially useful. NT-proBNP and hsCRP can be measured in most commercial laboratories currently.

We used contemporary and sophisticated statistical approaches to predict two important outcomes; RRT and death, within 1 year. We tested the predictive utility of a panel of newer biomarkers, singly and in combination. Our analyses permit comparison of the relative importance of these biomarkers as predictors and recognize the complexity of the biological state. The cohort is broadly representative of typical advanced CKD patients seen and followed by nephrologists across Canada.

The focus on 1-year outcomes has advantages and disadvantages. As a strength, it is this timeframe which is clinically relevant to clinical decision-making, which is required to optimally educate and prepare patients for RRT. Note that 1-year non-RRT event rates in this cohort were high: 1-year cumulative incidence of death was 6% (equal to 10 year event rates in an intermediate risk non-CKD population) [57]. Knowing which patients will likely die with a functioning kidney within the year may guide clinicians and patients to focus on other issues, including cardiovascular risk modification, or conservative care options. The 1-year rate of these outcomes is highly relevant to patients, nephrologists and other healthcare professionals caring for these patients. While there is a need to evaluate longer term outcomes, this information ~1 year outcomes is important, and is more tangible in clinical practice for many, especially the patients.

**Limitations**

All observational cohort studies have limitations. The models derived in the present study may not apply to non-referred patients with CKD, or those with higher eGFR values: by design, we studied only those with established advanced CKD, known to nephrologists. This is a relative limitation, since, as nephrologists, we need to improve our prediction of outcome in the patients who are directly under our care. The 1-year timeframe may also be considered a limitation, as patients are often followed for longer than 1 year by specialists, and are interested in longer term prognosis. Future analyses will address these longer term outcomes.

Similar to other epidemiological studies such as CRIC and Framingham, CanPREDDICT will use the tools of observational epidemiology to help further address questions regarding risk of longer term progression to dialysis or cardiovascular disease [57, 58]. The CanPREDDICT cohort is different in terms of race and case-mix from other national CKD cohorts such CRIC (USA), German CKD Cohort study and CRIB (UK) [58–60], it is similar in that it is a large national longitudinal observational cohort study with serial follow-up and biosample collection and storage. It remains unique in its sampling of patients at lower eGFR strata and its setting in the Canadian Health System, with universal access to care. These features make it complementary to other national cohorts, and provide the basis for international comparisons and validation.

Substantial variability occurs between different assays of the biomarkers which may introduce some bias into the analysis. All biomarkers examined were ascertained in a central laboratory, and a 10% random sample was run in duplicate. Subsequent analyses to ascertain the stability of these biomarkers over time within and between individuals are planned. In addition, we are planning comparative analyses with other cohorts (CRIC, German CKD).

In patients with advanced CKD, newer biomarkers do not improve the prediction of RRT within the 1-year time horizon,
SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

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ROLE OF SPONSOR

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CONFLICT OF INTEREST STATEMENT

None declared. Some of the authors have received speaking fees or other fees for topics not related to this manuscript of subject matter, by Jansse and other companies.

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Biomarkers predict death in CKD

but do significantly improve prediction of death, within that same timeframe. Whether application of these risk models in clinical practice results in improved and more cost-effective care needs to be tested in clinical trials. CanPREDDICT begins to fill a recognized void regarding the utility of biologically justified newer biomarkers. The ability of these biomarkers as a group to enhance prediction, above and beyond conventional biomarkers, in CKD needs to be validated and put into clinical context. As we increase our sophistication in understanding, so might we increase our ability to use this information to stratify patients into risk categories and then test appropriate interventions to improve outcomes.


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Glycemic load is associated with oxidative stress among prevalent maintenance hemodialysis patients

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ABSTRACT

Background. High glycemic index (GI) and glycemic load (GL) are associated with increased levels of oxidative stress and systemic inflammation in the general population. Maintenance hemodialysis (MHD) patients are known to have excessive oxidative stress burden and inflammation. In this study, we examined the relationship between dietary GI or GL and markers of oxidative stress or inflammation among prevalent MHD patients.

Methods. A registered dietitian obtained GI, GL and other dietary data from 58 MHD patients. Two separate 24-h diet recalls (a hemodialysis day and a non-hemodialysis day) were analyzed using the Nutrition Data System for Research (NDS-R) software. Plasma or serum concentrations of F2-isoprostanes, high sensitivity C-reactive protein (hsCRP), leptin and adiponectin (ADPN) were measured in fasting state. Fat mass was measured by dual-energy X-ray absorptiometry (DEXA).

Cross-sectional associations between GI, GL and markers of interest were examined by multiple regression analysis with adjustment for potential covariates.

Results. Mean (±SD) age, body mass index (BMI) and total trunk fat were 47 ± 12 years, 29.5 ± 6.8 kg/m² and 16.4 ± 8.8 kg, respectively. Dietary GI was associated with trunk fat (r = −0.182, P = 0.05) but not with F2-isoprostanes and hsCRP. In contrast, GL was significantly associated with F2-isoprostanes (P = 0.002), in unadjusted analysis, which remained in adjusted analyses, adjusting for age and sex (P = 0.005), and after adjusting for BMI, trunk fat and waist/hip ratio (P = 0.04). Addition of leptin or ADPN did not alter the significance of the association. GI also correlated with hsCRP (P = 0.03), but this association was modified by BMI and trunk fat.

Conclusions. Dietary GL is significantly associated with markers of oxidative stress and inflammation among prevalent MHD patients, independent of the body composition and adipocytokines. These data indicate the importance of the