PromarkerD: A Novel Test for Predicting Rapid Decline in Renal Function in Type 2 Diabetes

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www.PromarkerD.com

Background

- Chronic kidney disease (CKD) affects one in three adults with diabetes, accounting for 40,000 deaths and $100 billion (USD) in healthcare spending annually.
- Current usual-care tests urinary albumin:creatinine ratio (ACR) and estimated glomerular filtration rate (eGFR) and has limited accuracy to predict progression of diabetic kidney disease (DKD).
- PromarkerD is a blood test that measures three plasma protein biomarkers (CD5L, ApoA4, and IBP3) combined with three clinical factors (age, HDL-cholesterol and eGFR) to predict risk of renal decline in the next 4 years in patients with type 2 diabetes (T2D).
- PromarkerD was developed using a proteomics workflow[1] in patients with T2D drawn from the longitudinal observational Fremantle Diabetes Study Phase II (FDS2)[2,3].
- The PromarkerD plasma biomarkers add significant incremental benefit to conventional clinical risk factors for predicting rapid decline in renal function in T2D[2,3].
- PromarkerD, a novel predictive test for predicting diabetic kidney disease (DKD) was evaluated.

Methods – Patients & Outcome

- PromarkerD score was assessed at baseline in 857 patients with T2D from FDS2.
- All patients were followed for 4 years, with a detailed clinical assessment performed at the year 2 and year 4 visits.
- Renal decline was defined as:
  - i) Incident DKD (eGFR <60 mL/min/1.73m^2 at year 4 in patients above this at baseline),
  - ii) ≥30% eGFR decline (over 4 years).
- The baseline clinical characteristics of all T2D patients (n=857), and those with preserved kidney function (n=750) [eGFR≥60 mL/min/1.73m^2] or decreased kidney function (n=107) [eGFR<60 mL/min/1.73m^2] at baseline are shown in Table 1.

Table 1. Baseline clinical characteristics of patients with T2D.

	All T2D (n=857)	eGFR ≥60 (n=750)	eGFR <60 (n=107)
Age (years)	65.4±10.4	64.2±10.3	73.4±7.3
Male gender (%)	54.0	54.0	54.2
Diabetes duration (years)†	7.4 [2.0-15.0]	6.0 [2.0-14.0]	14.7 [8.0-19.9]
HbA_{ic} (%)	7.1±1.3	7.1±1.3	7.1±1.2
Urinary ACR (mg/g)*	29.2 (8.8-97.2)	26.7 (8.4-84.5)	55.1 (14.3-212.2)
eGFR (mL/min/1.73m^2)	81.7±18.6	86.9±12.7	45.4±11.5
ACE-I/ARB use (%)	65.3	62.9	82.2

All values are proportions (%) or mean±standard deviation unless labelled otherwise;
* Geometric Mean (standard deviation range); † Median [interquartile range].

Methods – The PromarkerD Test

- The PromarkerD plasma biomarkers were measured using a targeted mass spectrometry (MS) platform[1] (suitable as a laboratory developed test) and an immunoassay (suitable as a kit version of the test).
- The MS method utilises bead-based antibody binding for the specific PromarkerD protein biomarkers in a single multiplex capture step and is known as Immunoaffinity-MS. The captured protein biomarkers are reduced, alkylated and digested in situ on the beads with injection onto a microflow LCMS system for targeted MS.
- The immunoassay has been designed using advanced Capt*Sure*™ ELISA technology (TGR BioSciences, Australia), whereby chemically tagged antibodies bind to the target biomarker in solution, and are then immobilised on the surface through the peptide tag. This technology allows a simple, rapid, one-wash ELISA platform in which a single generic Capt*Sure* ELISA plate underpins all assays.
- Age, serum HDL-cholesterol and eGFR (calculated using the CKDEPI equation) were measured using standard procedures.
- All data were uploaded to the PromarkerD Hub at www.PromarkerD.com for risk prediction which was performed using algorithms developed by logistic regression[2,3].
- Risk predictions were compared between the two platforms using Bland and Altman plot analysis.
- Prognostic performance of prediction models was assessed using the area under the receiver operating characteristic curve (AUC, with sensitivity (Sn), specificity (Sp), and positive and negative predictive values (PPV and NPV) given at the optimal cut-off (maximum Youden Index = Sn+Sp-1).

Results – Test Performance

- The prognostic performance of PromarkerD for predicting incident DKD and a drop in eGFR ≥30% over a mean±SD follow-up of 4.2±0.3 years is shown in Table 2.

Table 2. Prognostic performance of PromarkerD for predicting renal decline.

	Incident DKD	eGFR Decline ≥30%
N with Outcome (%)	84/750 (11.2%)	69/857 (8.1%)
AUC (95% CI)	0.90 (0.87-0.93)	0.80 (0.70-0.85)
Sn/Sp (%)	85/81	94/53
PPV/NPV (%)	36/98	15/99

Results – Test Performance

- The PromarkerD protein biomarkers outperform both eGFR and ACR in predicting renal decline.

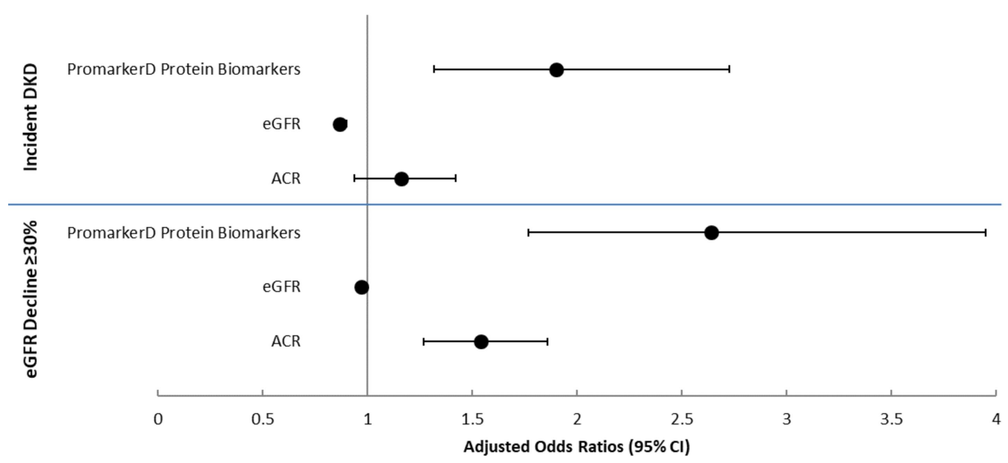

Figure 1. Adjusted odds ratio of biomarkers for predicting renal decline.
All odds ratios are adjusted for age and HDL-cholesterol at baseline. The PromarkerD plasma biomarkers and ACR were Ln-transformed prior to analysis due to a non-normal distribution.

Results – Platform Comparison

- Comparison of the different test formats in a random sample of 100 T2D patients by Bland Altman plot analysis shows acceptable agreement between mass spectrometry and ELISA.

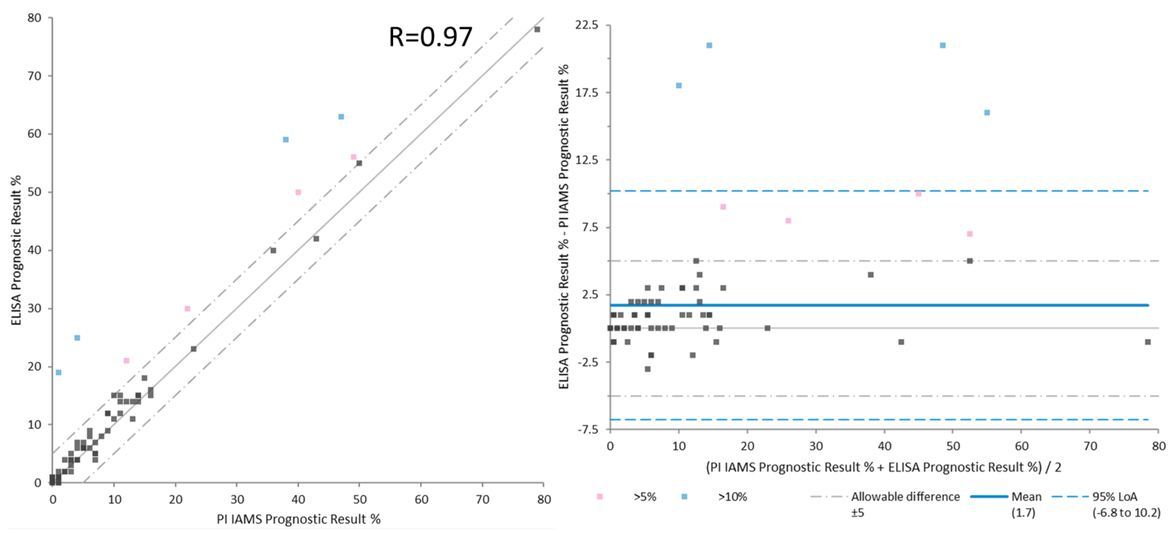

Figure 2. Scatter plot (left) and difference plot (right) comparing PromarkerD risk predictions based on mass spectrometry and ELISA platforms.

Conclusions

- **PromarkerD is a novel test for predicting rapid decline in renal function in type 2 diabetes.**
- PromarkerD can be used in T2D patients with preserved renal function (eGFR≥60 mL/min/1.73m^2) and also those with an eGFR<60 mL/min/1.73m^2 to predict further rapid eGFR decline.
- The PromarkerD biomarkers outperform both eGFR and ACR for predicting future renal decline.
- PromarkerD may be useful for **risk stratification in future clinical trials.**
- PromarkerD provides physicians with a more informed approach to manage DKD and patient care:
  - **Early detection** – identify at-risk individuals
  - **Intervention** – implement preventative measures before kidney damage occurs
  - Tighter **monitoring** and control of blood glucose, blood pressure and blood lipids, alongside follow-up PromarkerD testing
  - **Targeted treatments** and medications in those at-risk
  - **Management** of disease progression
- **PromarkerD could save the healthcare system up to $50 billion (USD) per year in direct costs** associated with treating end-stage renal disease, assuming intervention was successful in 50% of cases (detailed cost-benefit analysis - data not shown).

References: 1. EuPA Open Proteomics 2017;14:1-10; 2. Diabetes Care 2017;40(11):1548-1555; 3. Journal of Diabetes & its Complications 2019; In Press, Journal Pre-proof, Available online 27 August 2019 (doi:10.1016/j.jdiacomp.2019.07.003).

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