Novel circulating biomarkers predict rapidly declining renal function in type 2 diabetes: The Fremantle Diabetes Study

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Background

- There is a need for earlier detection of individuals at risk of diabetic kidney disease (DKD) to optimise timely intervention and monitoring of disease progression
- Current usual-care tests urinary albumin:creatinine ratio (ACR) and estimated glomerular filtration rate (eGFR) are limited diagnostically and fail to predict onset and progression of DKD
- We recently identified a panel of novel plasma protein biomarkers "PromarkerD" (APOA4, APOC3, CD5L, C1QB, CFHR2, and IBP3) with significant diagnostic utility in DKD[1]

Aim

- To investigate the role of PromarkerD in predicting rapid eGFR decline over a 4 year follow-up period in type 2 diabetes (T2D)

Patients and Methods

- A mass spectrometry platform was used to measure baseline plasma biomarkers in 792 participants with T2D from the longitudinal observational Fremantle Diabetes Study Phase II (FDS2)
- **In the absence of a single clinically-accepted surrogate for ESRD, we assessed four commonly used definitions of rapid eGFR decline** i) ≥30% eGFR decline[2] (over 4 years), ii) incident CKD (eGFR <60 mL/min/1.73m^2 at Yr 4 in individuals above this at baseline), iii) eGFR declining trajectories (defined by latent class analysis)[3] and iv) eGFR decline ≥5 mL/min/1.73m^2/year[4]
- Prediction models were developed for each definition using forward conditional multiple logistic regression and clinical predictors (most parsimonious model) before biomarkers were considered for entry; the incremental predictive value of the biomarkers was assessed using indices of model fit (likelihood ratio test (LRT)), calibration (Hosmer-Lemeshow test (H-L test)), discrimination (ROC-AUC) and reclassification (NRI and IDI)
- All prediction models were derived using the development cohort (n=345) and internally validated using bootstrap resampling of the same individuals (1,000 resamples) before validation in an independent cohort (n=447)
- A simple consensus model comprising key clinical predictors and biomarkers was also assessed that was applied across all definitions of rapid eGFR decline

Results

- The development and validation cohorts had similar baseline ACR and eGFR, but differed in age, diabetes duration, HbA_{1c} and SBP (Table 1)

Table 1. Characteristics of participants in the development and validation cohorts.

Baseline Characteristics	Development (n=345)	Validation (n=447)	*P*-value
Age (years)	67.0±9.4	64.4±10.9	<0.001
Male gender (%)	51.9	56.2	0.25
Diabetes duration (years)	9.0 [3.0-15.2]	6.0 [1.3-14.0]	<0.001
HbA_{1c} (%)	7.0±1.0	7.1±1.4	0.037
Supine SBP (mm Hg)	147±20	143±20	0.003
Urinary ACR (mg/mmol)	2.9 (0.9-8.8)	2.5 (0.9-7.1)	0.050
eGFR (ml/min/1.73m^2)	80.6±18.8	82.7±16.9	0.091

Results

Table 2. Prognostic performance of PromarkerD in most parsimonious models.

Rapid eGFR decline:	≥30% decline	Incident CKD	eGFR trajectory	≥5mL/yr decline
Development Cohort Clinical + Biomarker Models [Adj OR (95%CI)]*				
Number of Outcomes (%)	30 (8.7)	**37 (12.3)**	35 (10.1)	28 (8.1)
Ln(APOA4)	4.85 (2.04-11.50)	**2.16 (1.04-4.47)**	2.40 (1.24-4.61)	NI
Ln(IBP3)	0.32 (0.13-0.81)	**NI**	NI	0.38 (0.15-0.95)
Ln(CD5L)	NI	**NI**	0.52 (0.29-0.93)	NI
Ln(C1QB)	NI	**NI**	2.41 (1.14-5.11)	NI
Development Cohort Prognostic Performance				
ΔLRT χ^2 test, *P*[δ]	17.1, <0.001	**4.7, 0.03**	19.2, <0.001	4.7, 0.03
H-L χ^2 test, *P*	3.4, 0.91	**4.0, 0.85**	13.0, 0.11	9.5, 0.31
AUC (95%CI), ΔAUC, *P*[δ]	0.88 (0.82-0.93), 0.04, 0.14	**0.92 (0.88-0.95), 0.01, 0.18**	0.82 (0.76-0.88), 0.07, 0.039	0.78 (0.69-0.87), 0.02, 0.42
Sn/Sp (%)	87/79	**92/82**	88/69	86/64
Optimism-corrected AUC†	0.85	**0.90**	0.78	0.75
Calibration intercept/slope†	-0.24/0.84	**-0.07/0.91**	-0.28/0.82	-0.26/0.86
NRI (>0) (95%CI)[δ]	0.81 (0.68-0.94)	**0.30 (0.25-0.35)**	0.76 (0.63-0.89)	0.18 (0.16-0.21)
IDI (%) (95%CI)[δ]	8.1 (1.0-15.1)	**1.8 (-1.3-5.1)**	6.3 (2.1-10.4)	1.8 (-0.1-3.8)
Validation Cohort Prognostic Performance				
Number of Outcomes (%)	24 (5.4)	**39 (8.7)**	NI§	32 (7.2)
AUC (95%CI)	0.74 (0.65-0.84)	**0.83 (0.77-0.88)**	NI	0.65 (0.54-0.75)
Sn/Sp (%)	54/88	**89/63**	NI	55/72
Accuracy (Brier Score)	0.05	**0.10**	NI	0.07

* Odds ratio (OR) adjusted for the most parsimonious clinical model for each definition of rapid decline.
δ Compared to the most parsimonious clinical model for each definition of rapid decline.
† Based on internal validation by 1,000 bootstrap resamples.
§ Data not shown as no rapidly declining trajectory was present in the validation cohort.
Ln = natural logarithm transformation, NI=not included, Sn/Sp=sensitivity/specificity at the optimal cut-off, AUC=area under the curve, NRI(>0)=category-free net reclassification index, IDI=integrated discrimination index

Model development (n=345):

- **Separate most parsimonious models were developed for each definition of rapid eGFR decline (Table 2):**
  - **≥30% eGFR fall:** older age, diuretic use, ischemic heart disease (IHD), higher DBP and lower total cholesterol (*P*≤0.033). After adjustment, APOA4 and IBP3 independently predicted outcome and improved model performance
  - **Incident CKD:** IHD, lower eGFR at baseline and lower total cholesterol (*P*≤0.022). After adjustment, APOA4 independently predicted outcome and improved model performance
  - **Rapid declining eGFR trajectory:** older age, diuretic use, longer diabetes duration and lower HDL-cholesterol (*P*≤0.033). After adjustment, APOA4, CD5L and C1QB independently predicted outcome and improved model performance
  - **eGFR decline ≥5mL/min/1.73m^2/year:** IHD, higher DBP and higher HbA_{1c} (*P*≤0.014). After adjustment, IBP3 independently predicted outcome and improved model performance

Results

Model validation (internal) (n=1,000 bootstrap resamples)

- Internal validation by bootstrap resampling showed good discrimination and acceptable calibration for each model (Table 2)

Best model: Incident CKD: AUC=0.90, calibration intercept/slope=-0.07/0.91

Model validation (external) (n=447)

- Applied to the validation cohort, the discrimination and accuracy of each model was good (Table 2), but calibration was poor (*P*<0.05) due to small numbers in lower deciles, which is not unusual in validation studies[5]

Best model: Incident CKD: AUC=0.83, sensitivity=89%, specificity=63%

Consensus model

- **One simple consensus model was developed across all definitions of rapid eGFR decline (Table 3)**
- Based on the combined data a simple consensus model was derived that comprised key clinical and biomarker predictors: baseline eGFR, HDL cholesterol, age, APOA4, CD5L and IBP3
- The consensus model performed well in both cohorts with good discrimination and calibration (Table 3)

Table 3. Prognostic performance of PromarkerD Consensus model.

Rapid eGFR decline:	≥30% decline	Incident CKD	eGFR trajectory	≥5mL/yr decline
Development Cohort				
AUC (95%CI)	0.81 (0.75-0.87)	**0.89 (0.85-0.94)**	0.86 (0.80-0.93)	0.70 (0.61-0.80)
Sn/Sp (%)	97/62	**95/68**	84/82	61/73
Calibration *P*	0.06	**0.39**	0.41	0.07
Validation Cohort				
AUC (95%CI)	0.72 (0.63-0.82)	**0.88 (0.84-0.93)**	NI	0.62 (0.53-0.72)
Sn/Sp (%)	65/71	**86/78**	NI	69/57
Calibration *P*	0.68	**0.77**	NI	0.61

Best model: Incident CKD: AUC=0.88, sensitivity=86%, specificity=78%

Conclusions

- The present study assessed and validated the prognostic utility of PromarkerD – a panel of plasma protein biomarkers including apolipoprotein A-IV (APOA4), insulin-like growth factor-binding protein (IBP3), CD5 antigen-like (CD5L), and complement C1q subcomponent subunit B (C1QB))
- PromarkerD predicts rapid eGFR decline in type 2 diabetes across clinically significant definitions of DKD independently of recognized clinical risk factors
- PromarkerD may be useful for risk stratification in future clinical trials
- PromarkerD would enable earlier intervention of at-risk individuals, monitoring of disease progression, and allow improvement in patient outcomes

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