**DUPDN- Development of Diagnostic Urinary Panel for Diabetic Nephropathy: A model to predict progressive CKD in diabetic patients.**

**Introduction.** CKD in patients with diabetes is detected and monitored using eGFR derived from creatinine-based equations and albuminuria, which are limited in value particularly for identification of patients likely to develop progressive CKD. A significant proportion of patients with type2 diabetes have CKD without albuminuria. We recruited a large cohort of patients with diabetes with full spectrum of renal function and tested a panel of urinary biomarkers to reflect areas of injury (Glomeruli and tubulointerstitium) and pathogenic mechanisms (inflammation and fibrosis) to develop a model to predict progression of CKD.

**Methods.** 400 patients with diabetes (with all stages of CKD) were recruited in to this prospective single centre cohort study and 388 were included in analysis. A random urine sample was collected from all the patients, divided in to aliquots and stored at -80oC prior to analysis for constituents of the panel. and analysed for a panel of biomarkers to represent markers and mediators of renal injury/damage. The panel of biomarkers consisted of- ACR, NGAL, inflammatory cytokines-IL1β, IL6, MCP1, Markers of proximal tubular injury/damage- NAG and RBP, Pro-fibrotic growth factor TGFβ (1,2 and 3); and the matrix protein Fibronectin (Fn), all measured once at baseline and indexed to urinary creatinine. The outcome measure was eGFR measured by creatinine based MDRD equation. Logistic regression (Univariate and multivariate) analysis was done to test the association of individual and combination of biomarkers with presence of CKD stage 3 or 4 and whether progressor or not. ROC AUC results were obtained for each combination of biomarkers to measure their sensitivity and specificity to CKD stages (3 and 4) and progression. A mixed model was used for the association of biomarkers with the trajectory of eGFR over the five year period. Models with the same numbers of observations were compared using the AIC goodness-of-fit measure. Eight biomarkers (ACR, NGAL, IL1 β, IL6, MCP1, NAG, RBP, Fn) were included in the first model and those with non-significant (at the 5% level) fixed effects were removed one by one to reduce the model to one where each biomarker had a significant fixed effect on the gradient of eGFR over time in years. This final model also demonstrated the best AIC value.

 **Results.** N=388**.** Ethnicity: White-217 (55.9%), Black-70 (18%), Asian-93 (24%), Other-8 (2.1%). Approximately 50% of patients did not have albuminuria (ACR <3). Patients were followed for 5 years to identify progressors. Progression data was available in 357 patients of which 54 (15%) patients developed progressive CKD. Initial comparisons between CKD stages demonstrated significant differences in urinary RBP, NAG, MCP1, IL6 (higher urinary levels with advancing CKD, comparisons made with Kruskal-Wallis test) but not IL1 β. TNFα and TGFβ (1,2 and 3) were not detectable in majority of patients and therefore, were excluded from subsequent analysis. In multivariate analysis, at all levels of ACR, urinary RBP demonstrated better correlation with CKD stage 3 and 4 compared to ACR (AUC of 78% for RBP Vs 65% for ACR) but this effect was not seen after adjusting for age, sex and race. Adjusted ACR predicted CKD stage 3 as well as any other combination of markers tested with AUC of 83%. However, for progression of CKD, unadjusted and adjusted ACR was not a good predictor (With an AUC of 62-67%). Addition of RBP and MCP1 as covariates in addition to adjustment for age, sex and race in patients with ACR of <3 improves the ROC AUC to 75%. The logarithms of RBP/Cre and ACR are positively correlated (r=0.61) and can be substituted one for the other in most models. Interestingly, urinary Fn was raised in diabetic patients with early CKD (1,2) compared to 20 healthy controls and this merits further evaluation. Using 1,243 observations of eGFR over 5 years we have arrived at two models: 1. where the eGFR gradient against time in years is associated with baseline ACR and RBP/Cre: 2. where the eGFR gradient against time in years is associated with baseline MCP1/Cre and RBP/Cre. Using these models, plots were drawn showing predicted eGFR values year by year (with confidence bands) and actual eGFR.

**Conclusions.** Our study suggests that models that include proximal tubule functional marker RBP and inflammatory cytokine MCP1 in addition to or instead of ACR improve our ability to predict future eGFR and identify patients likely to develop progressive CKD. We are working on further analysis to improve the model, and measure its predictive value. The future plan is to test the models that we have developed in a prospective study. Increased urinary excretion of fibronectin in diabetic patients with no CKD or stage1/2 CKD needs further study.