

Sub study 2 – Urine/Renal biomarkers

The longitudinal profiles of biomarkers NGAL and ACR (albumin creatinine ratio) were considered in subgroups defined by tertiles (for NGAL) and thresholds (for ACR) respectively at baseline. For analysis of ACR, the sample set was divided into three subsets based on KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease⁶¹ (ACR<3 mg/mmol, Normal; ACR 3-30mg/mmol, microalbuminuria; and ACR>30mg/mmol, macroalbuminuria). Due to lower number of dropout in each subgroup (< 20), linear mixed effect models were used rather than joint models to analyse the longitudinal variations. The models were adjusted for ethnicity, and also for age and gender.

The tertile subgroups for NGAL included 106, 106 and 105 patients, and NGAL scores were fitted in log transformed scale for normality. The changes in weight from baseline were not significant and the final models were adjusted for age and gender, and information from dropped arms. The estimated treatment effects (Arm D (80mg) compared to Arm A (control)) on NGAL were not significant in either tertile subgroup: -0.215 (95% CI: -0.627 to 0.196, p value 0.3), -0.065 (95% CI: -0.512 to 0.382, p value 0.8) and -0.347 (95% CI: -0.893 to 0.198), p value 0.3) respectively.

The subgroup below 3 mg/mmol included 70 patients; 21 were included in the subgroups 3-30 mg/mmol and just 1 patient had ACR above 30 mg/mmol; hence subgroups of ACR 3-30 and above 30 were combined. ACR scores were fitted in log transformed scale for normality. The changes in weight from baseline or gender were not significant, and the final models for the subgroups ACR below 3 and above 3 were adjusted for age and information from dropped arms. The treatment effect on the longitudinal ACR for the subgroup with an ACR above 3mg/mmol was -0.665 (95% CI: -1.310 to -0.019, p value 0.04) suggesting a significant but marginal treatment effect in arm D compared to the control arm.

There were no evidence of significant correlations between NGAL and ACR at baseline or at any other follow-up time points (p values < 0.05).

Urinary biomarkers

NGAL

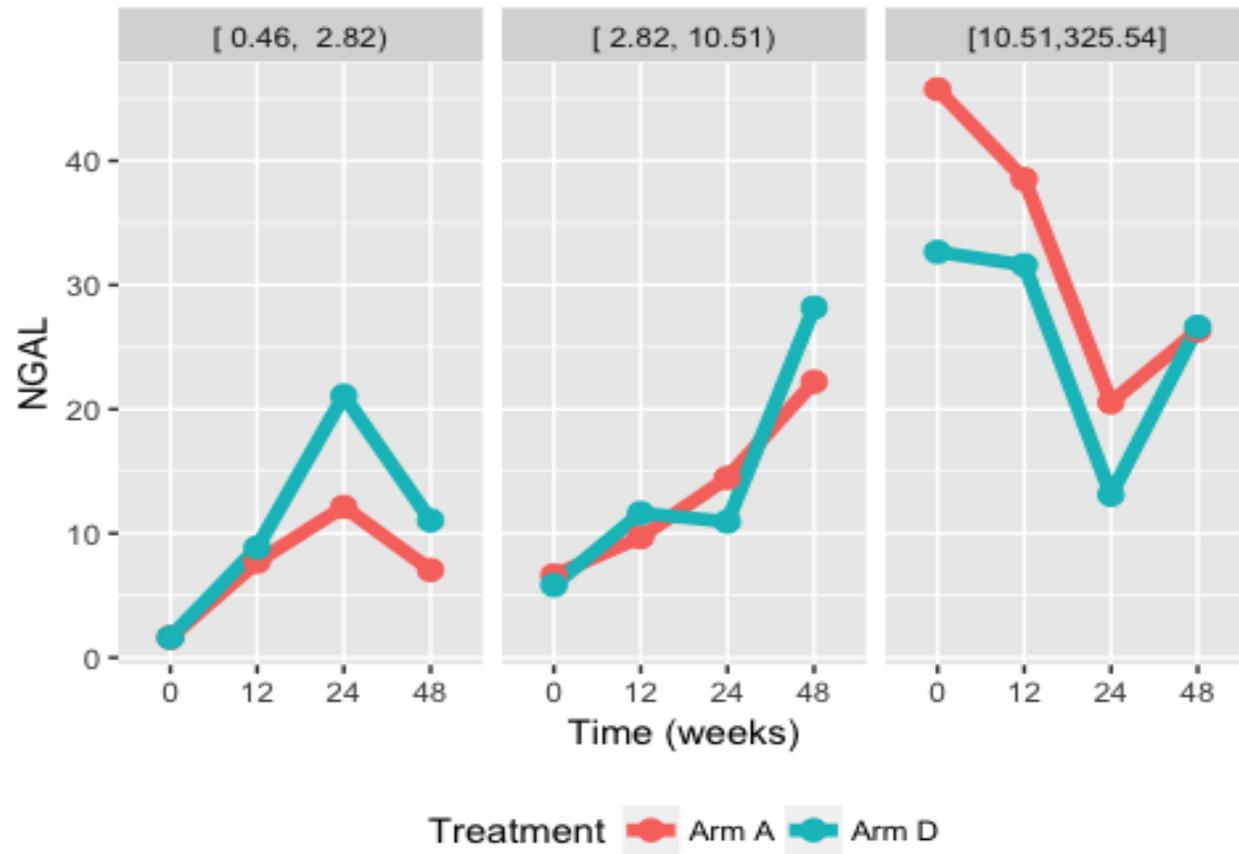


Figure 1 Longitudinal NGAL mean profiles for Arm A (control) and Arm D (80mg) over tertile subgroups

Table 53 Estimates from linear mixed effect model for each subgroup for longitudinal NGAL

	Parameter	SE	95% CI lower limit	95% CI Upper limit	p-value	Parameter	SE	95% CI lower limit	95% CI Upper limit	p-value	Parameter	SE	95% CI lower limit	95% CI Upper limit	p-value
Marker subgroup	1st Tertile					2nd Tertile					3rd Tertile				
Intercept	2.271	0.469	1.345	3.197	<0.0001	0.180	0.691	-1.184	1.543	0.7952	1.192	0.759	-0.309	2.693	0.1186
TIME	0.005	0.004	-0.003	0.013	0.2545	0.006	0.004	-0.003	0.015	0.1854	0.003	0.004	-0.006	0.012	0.4670
Baseline NGAL	0.562	0.183	0.198	0.926	0.0028	0.480	0.227	0.031	0.930	0.0366	0.417	0.137	0.1460	0.688	0.0030
Age	-0.015	0.009	-0.032	0.002	0.0873	0.011	0.010	-0.008	0.030	0.2635	-0.002	0.010	-0.022	0.018	0.8741
Ethnicity	-0.057	0.249	-0.551	0.437	0.8194	0.094	0.275	-0.452	0.640	0.7336	-0.232	0.303	-0.833	0.368	0.4448
Gender - male	-0.500	0.250	-0.997	-0.004	0.0483	0.097	0.294	-0.486	0.681	0.7413	0.048	0.307	-0.562	0.657	0.8772
Treatment B vs A	-0.511	0.224	-0.955	-0.067	0.0247	0.075	0.234	-0.390	0.540	0.7490	-0.028	0.281	-0.585	0.528	0.9195
Treatment C vs A	-0.300	0.240	-0.776	0.175	0.2133	-0.563	0.262	-1.084	-0.043	0.0343	0.080	0.291	-0.498	0.658	0.7846
Treatment D vs A	-0.215	0.207	-0.627	0.196	0.3018	-0.065	0.225	-0.512	0.382	0.7733	-0.347	0.275	-0.893	0.198	0.2093

ACR

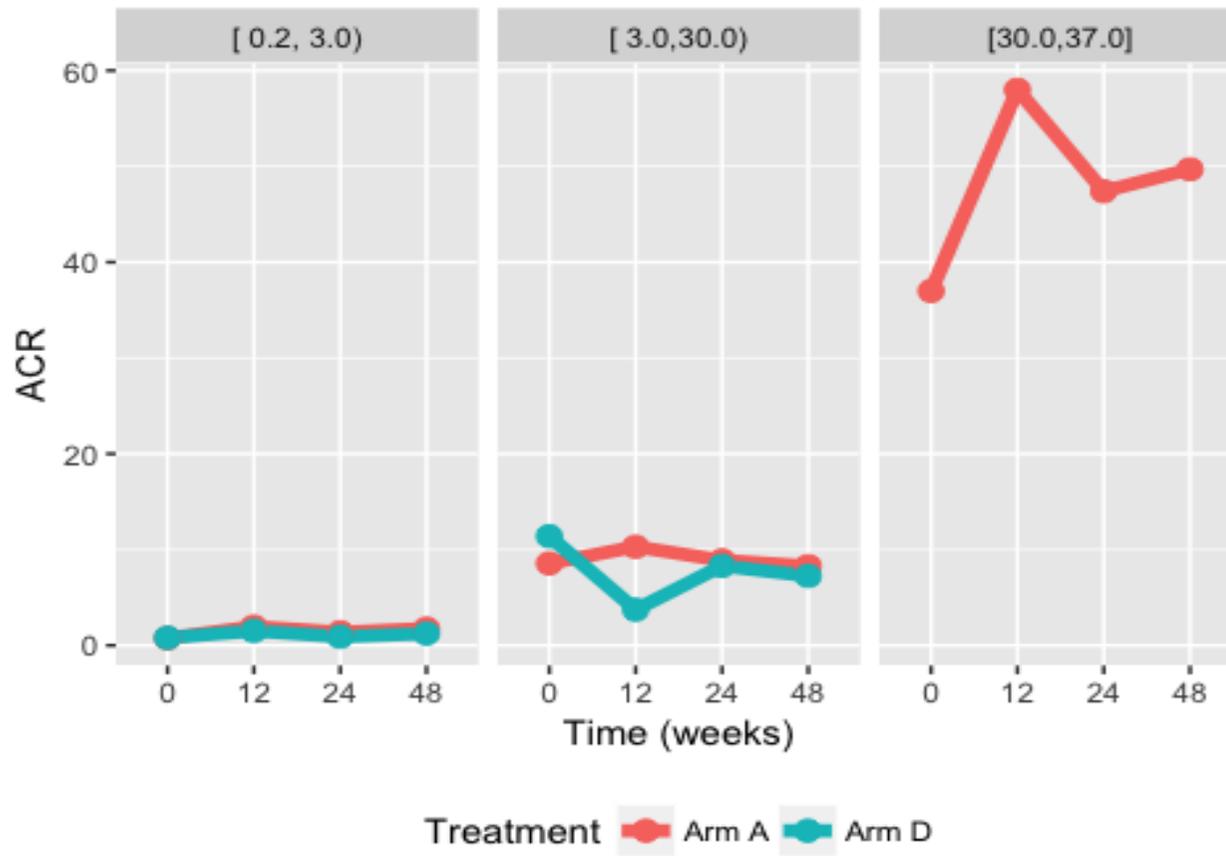
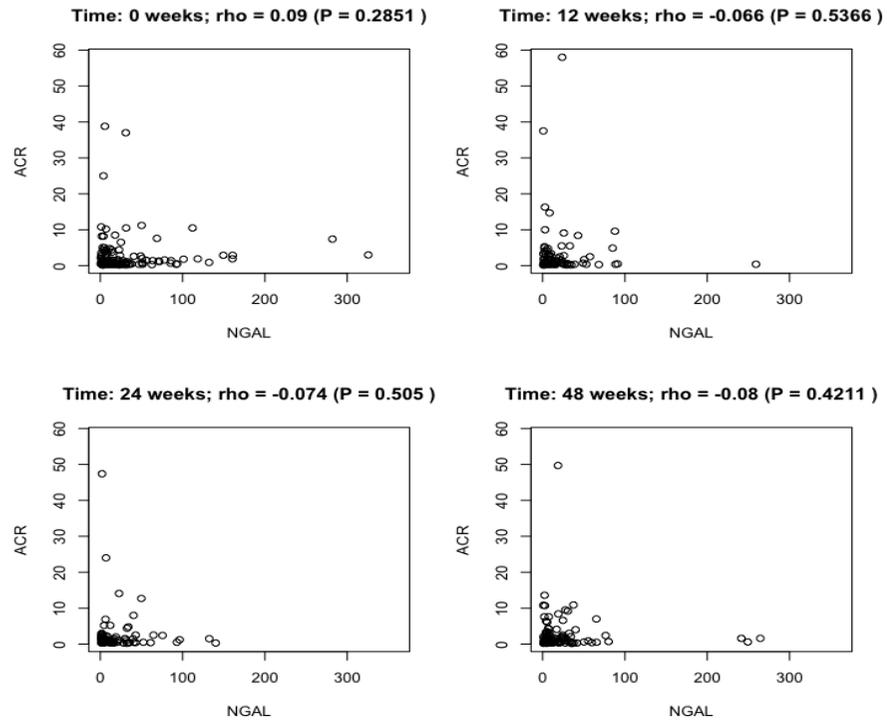


Figure 2 Longitudinal ACR mean profiles for Arm A (control) and Arm D (80mg) over threshold subgroups. Note the profile shown in ACR > 30 includes a single patient

Table 54 Estimates from linear mixed effect model for each subgroup for longitudinal ACR

	Parameter	SE	95% CI lower limit	95% CI Upper limit	p-value	Parameter	SE	95% CI lower limit	95% CI Upper limit	p-value
Marker subgroup	ACR below 3					ACR 3 or above				
Intercept	0.336	0.668	-0.999	1.672	0.6166	-1.779	0.908	-3.638	0.081	0.0601
TIME	0.002	0.004	-0.005	0.009	0.5727	0.007	0.007	-0.006	0.021	0.2882
Baseline ACR	0.564	0.131	0.301	0.826	0.0001	0.877	0.221	0.406	1.347	0.0012
Age	-0.007	0.013	-0.032	0.018	0.5804	0.014	0.015	-0.018	0.046	0.3696
Ethnicity	0.182	0.245	-0.308	0.672	0.4616	1.159	0.403	0.300	2.019	0.0116
Treatment B vs A	-0.320	0.223	-0.766	0.126	0.1567	-0.903	0.382	-1.718	-0.088	0.0321
Treatment C vs A	-0.111	0.258	-0.626	0.404	0.6676	0.643	0.480	-0.381	1.667	0.2008
Treatment D vs A	-0.074	0.253	-0.578	0.431	0.7715	-0.665	0.303	-1.310	-0.019	0.0443

(a)



(b)

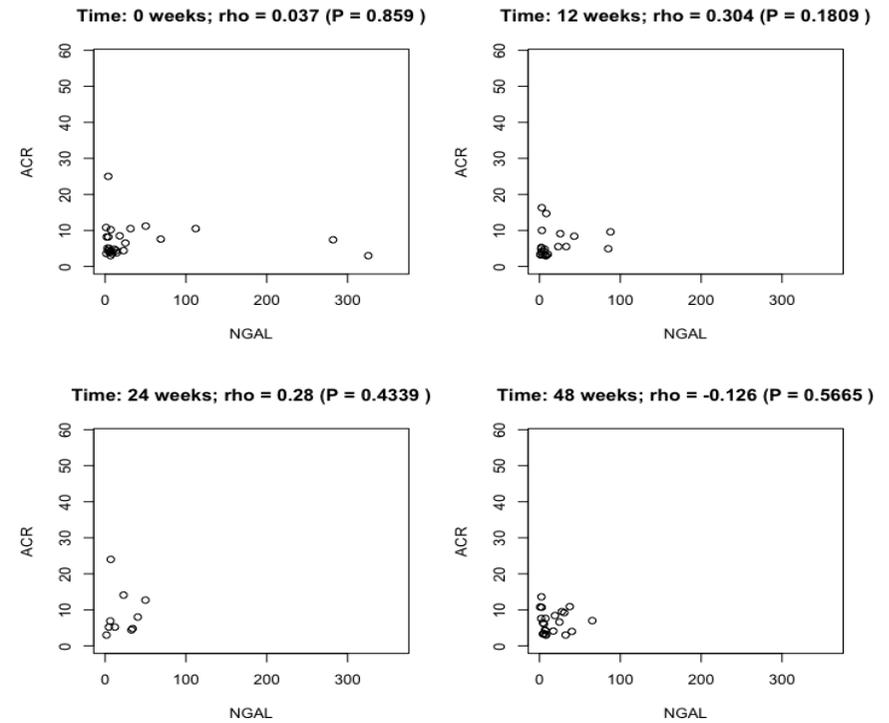


Figure 3 Scatter plots showing the correlation between NGAL and ACR at baseline and follow-up time points. (a) all pairs and (b) ACR 3 – 30. rho = correlation coefficient, P = p value