Evaluation of urine biomarkers of kidney injury in Polycystic Kidney Disease

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Abstract

Progressive disruption of renal tubular integrity in the setting of increased cellular proliferation and apoptosis is a feature of ADPKD. Here we evaluated the effect of these processes on the expression of NGAL and IL-18, markers of tubular injury, in rodent models and in the cyst fluid and urine of patients with ADPKD. Two mouse models where Pkd2 was inactivated which resulted in early or adult onset cysts, were used to evaluate NGAL levels. Further, the Han:SPRD rat model of polycystic disease was used to study IL-18 levels. In four annual serial urine samples from 107 patients with ADPKD in the Consortium for Radiologic Imaging for the Study of Polycystic Kidney Disease (CRISP) study, NGAL and IL-18 excretion rates were determined in conjunction with measures of total kidney volume and estimated GFR (eGFR) by the MDRD equation. Kidneys from affected mice and rats showed prominent expression of NGAL and IL-18/IL-18R, respectively, in epithelial cells lining kidney cysts. In human ADPKD cyst fluid, both NGAL and IL-18 were elevated. In CRISP patients, the mean percentage increase in total kidney volume was 5.4/year and the mean decline in eGFR 2.4 mL/min/year. The trend of increased mean urine NGAL and IL-18 over three years was statistically significant; however, there was no association of tertiles of IL-18 or quartiles of NGAL and the change in total kidney volume or eGFR over this period. Thus, urinary NGAL and IL-18 excretion are mildly and stably elevated in ADPKD, but do not correlate with changes in total kidney volume or kidney function. This may
be due, in part, to the lack of communication between individual cysts and the urinary collecting system in this disorder.

**Keywords**
surrogate; NGAL; IL-18; biomarker

**Introduction**

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disease, affecting 1:500 to 1:1000 people and is the fourth leading cause of end-stage renal disease (ESRD) in the United States. ADPKD is characterized by massively enlarged kidneys caused by lifelong cyst expansion and is associated with a late decline in kidney function progressing to renal failure in the majority of patients. Cyst growth requires processes of cell proliferation, fluid secretion and remodeling of the surrounding milieu. A broad range of mechanisms have been proposed to underlie these changes and the apically located primary cilium, a single hair-like organelle protruding into the luminal space, has been implicated as being central to altered intracellular signaling processes responsible for increased cellular proliferation and apoptosis.

Specific therapies for ADPKD are lacking and one of the challenges in both clinical care and trial design is the absence of appropriate biomarkers to monitor disease progression early in the course of disease before significant impairment of renal function occurs. Currently, magnetic resonance (MR) imaging based total kidney volume (TKV) measurements detect early progression. However, MRI is expensive, TKV measures are not yet automated and structural progression as evidenced by MR imaging may lag behind important intrarenal functional changes. Importantly, clinical measures of kidney dysfunction, such as elevated serum creatinine concentration occur late in the disease and are inadequate with regard to identification of worsening cystic disease measured by TKV in the early stages. Therefore, non-invasive biomarkers developed for diagnostic purposes or that accurately identify increased renal cyst burden or increased TKV as well as progressive kidney damage are important. Identification of such markers could also assist in identification of patients at increased risk for progression to ESRD and could be utilized to determine the therapeutic benefit of an intervention.

Urine IL-18 and urine NGAL activity are established biomarkers of acute and chronic kidney injury. These are biomarkers of proximal and distal tubular injury and ADPKD is a chronic tubulointerstitial disorder. Given that tubular disruption and cyst formation are key features of ADPKD, we tested the hypothesis that levels of urinary NGAL and IL-18 are higher in PKD kidneys and correlate with increased TKV, decreased kidney function, faster rates of increase in TKV, and decline in kidney function. In order to test this hypothesis, we conducted series of preclinical experiments to establish the presence of IL-18 and NGAL in PKD models. In addition, urinary IL-18 and NGAL concentrations were measured in patients from the Consortium for Radiologic Imaging for the Study of Polycystic Kidney Disease (CRISP) study where a large cohort of ADPKD patients with relatively intact
kidney function were studied in prospective longitudinal fashion with regard to changes in TKV and kidney function or estimated GFR (eGFR) utilizing the MDRD equation $^{10,11}$.

**Results**

**Preclinical Studies**

Two orthologous gene mouse models based on Pkd2 inactivation were used to examine NGAL (Lcn2) expression in cystic and non-cystic tissues. Adult *Pkd2* $^{ws25/-}$ mice$^{12}$ showed strong expression of *Lcn2* transcripts detected by *in situ* hybridization in cyst lining cells (Fig. 1a-c). Weaker *Lcn2* expression was also present in some pericystic tubules that appeared otherwise normal (Fig. 1a,b). The second postnatal orthologous gene cystic model combined a novel transgenic mouse line expressing the human *PKD2* sequence from the *pCAGGS* vector$^{13}$ (*pCAGGS-PKD2*) with the previously described *Pkd2* $^{-/-}$ null mice that are embryonically lethal$^{14}$. Transgenic expression of *PKD2* in *Pkd2* $^{-/-}$; *pCAGGS-PKD2* mice partially rescued the embryonic lethality, cardiac defects and left-right axis formation defects observed in *Pkd2* $^{-/-}$ mice$^{14,15}$ (data not shown). Surviving mice develop markedly cystic kidneys during postnatal life (Fig. 1d) due to failure of the transgene to reconstitute expression of *PKD2* in kidney tissues. Immunocytochemical staining of cystic kidneys with antisera against NGAL$^{16}$ showed localisation of the *Lcn2* protein product in the epithelial cells lining the walls of the majority of cysts in *Pkd2* $^{-/-}$; *pCAGGS-PKD2* mice (Fig. 1e,f). A similar pattern of cyst-associated NGAL expression was found in kidneys from *Pkd2* $^{ws25/-}$ animals (data not shown), although there was significant variation in expression within both models (Fig. 1).

IL-18 and IL-18-receptor (IL-18R) expression was examined in the Han:SPRD (Cy+/+) cystic rats at one year of age. Cyst lining epithelial cells showed strong expression of IL-18 (Fig. 2a) and IL-18R was expressed in a subset of cells in regions surrounding the cysts (Fig. 2b).

**Human Cyst Fluid**

The levels of NGAL and IL-18 in the five pooled cystic fluid samples are provided in Table 1. Both NGAL and IL-18 levels are highly elevated compared to both serum and urine concentrations in normal subjects or in patients with acute kidney injury$^{17,18}$.

**Longitudinal Patient Studies**

Urine samples were available at baseline in 209 of 241 CRISP participants. The mean age of these CRISP participants was 32.1 years and 41.2 % were male. The baseline mean eGFR was 89.39 mls/min/1.73m$^2$ and the mean TKV was 1080 ml (308-3197). Urine samples were available for 156 participants at year 1, for 133 at year 2 and for 211 at year three. One hundred and seven patients had urine samples from baseline and all three follow-up visits. The urinary NGAL and IL-18 raw concentration levels at baseline and follow-up visits are given in Figure 3. The majority of patients demonstrated levels considered to be within the normal range or low at baseline with approximately 35% and 16% of patients with levels >20 units/ml for NGAL and IL-18 respectively. Only 7% and 2% of patients had levels of NGAL and IL-18 over 100 similar to levels seen in patients with acute kidney injury$^{17-20}$. 

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Table 2 demonstrates that among those with biomarker data at every visit, the corrected and uncorrected biomarker changes over 3 years. Tests for a linear and quadratic trend over 3 years in both biomarker levels (corrected and uncorrected) were statistically significant (p ≤ .05) implying an initial increase in the levels which then dropped in years 2 and 3.

**Biomarkers and Outcomes**—Baseline tertiles for biomarker concentrations and biomarkers corrected for creatinine concentrations of IL-18 and quartiles for NGAL were not associated with percent change in TKV or change in eGFR at 3 years of follow-up (Table 3a and 3b). No associations were found between baseline TKV and eGFR or change in TKV or eGFR over time when grouped by detectable versus not detectable limits (≥10 pg/ml for IL-18 and ≥1 ng/ml for NGAL; data not shown).

**Discussion**

In our series of preclinical and clinical studies we demonstrated that NGAL and IL-18 are present and highly expressed in the epithelial cells lining kidney cysts. We also found that urinary NGAL and IL-18 excretion were mildly and constantly elevated in ADPKD, change over time but did not correlate with worsening in kidney function or increase in TKV. The reason for the discrepant findings between cyst epithelial expression and fluid vs. urinary excretion of these biomarkers is possibly due to the lack of communication between the cystic space and the urinary collecting system. It is well known that renal cysts in ADPKD kidneys detach from the parent nephron soon after reaching two centimeters in diameter. Some patients had very high levels in the urine which may be related to acute fluctuations in these cytokines that occur during periods of rapid cyst expansion. As such annual measurements in TKV may not be sufficiently sensitive to detect changes associated with acute cyst expansion. Also, eGFR in the majority of these ADPKD patients remained stable, consistent with the natural history of this disorder. Typically cyst expansion is relatively slow with detectable changes in size limited to six months in most ADPKD patients. Capturing patients with acute rapid rates of cyst expansion (i.e., during acute cyst hemorrhage) may demonstrate usefulness for these markers.

NGAL expression increases in renal epithelial cells in response to injury. Increases in urinary NGAL levels have been found to be predictive of acute kidney injury in patients undergoing cardiac surgery and other hospitalized settings. NGAL gene expression is also increased in human ADPKD-derived cell lines and tissue, in the HAN:SPRD (Cy/+), rat model of ADPKD and cpk mouse model of recessive PKD. Thus NGAL emerged as a potentially interesting biomarker of progression in ADPKD. In the present study, NGAL (Lipocalin 2; Lcn2) was found to be highly expressed in two distinct orthologous mouse models of ADPKD. This increased expression is seen at both the mRNA and protein level and is associated with the cells lining cysts in these mouse models.

Urinary IL-18 is elevated during apoptosis and necrosis of renal tubular cells that is associated with acute kidney injury in rodent and human studies. Given that apoptosis contributes to the pathogenesis of cyst formation in ADPKD, we measured IL-18 and its receptor in the murine and human renal cysts. IL-18 expression was prominent in the cystic epithelial cells in kidneys of 1 year old Han:SPRD (Cy+) rats. As IL-18 signals via the
IL-18 receptor (IL-18R), the expression of IL-18R in the epithelial cells lining cysts, suggests that IL-18 may be having a biological effect during cyst formation or expansion. IL-18 and NGAL levels are also highly enriched in cyst fluid derived from ADPKD patients, demonstrating translation of the findings from murine and rat models to human disease. This prompted us to evaluate whether IL-18 and NGAL had clinical utility as biomarkers for progression of ADPKD using samples from the CRISP cohort.

The results of the current study are in contrast to results from Bolignano et al. in which 26 patients with ADPKD were evaluated by serum and urinary measurements of NGAL. Urinary and serum NGAL levels were higher in patients with ADPKD compared to controls, and proportionately higher in those patients with advanced disease. However, the patients with ADPKD already had a substantial reduction in renal function with a mean creatinine clearance of $59 \pm 38$ ml/min, far lower than the CRISP population studied here and lower compared to the control group with a creatinine clearance of $115 \pm 32$ ml/min. It is possible that elevations in serum and urinary NGAL concentrations found in this study were related more to the level of renal dysfunction than the presence of renal cystic disease. Thus, NGAL may have utility as a biomarker during later stages of ADPKD in the presence of a GFR decline associated with advanced ADPKD disease.

Although we conducted systematic evaluation of the kidney injury biomarkers in both preclinical and clinical settings of ADPKD, there are few limitations to consider. NGAL and IL-18 are relatively stable through multiple freeze-thaw cycles and the levels are known to be stable for at least 3 years at -80°C. In addition, we have found predictable results with these biomarkers in other studies where samples were collected and stored for several years so it is likely that these biomarkers were stable in PKD urine samples. Although the samples were handled uniformly across the study visits, the reasons for the fall in the biomarker levels during the follow-up period were unclear. Other potential biomarkers for progression in ADPKD include monocyte chemoattractant protein-1, B-N-acetylhexosaminidase, and KIM-1. Kistler et al. found that a unique urinary proteomic profile and pathway could correctly identify patients with ADPKD. It is possible that a combination of urinary biomarkers as opposed to a single biomarker could predict disease progression better in PKD.

Methods

Preclinical Studies

Animals and tissue samples—Three mouse strains based on Pkd2 inactivation and one rat model of autosomal dominant proximal cystic disease were used. NGAL experiments utilized the mouse strains Pkd2<sub>WS25</sub> and Pkd2<sub>WS183</sub> (null allele, a.k.a., Pkd2<sup>-/-</sup>) and a novel transgenic mouse line expressing the human PKD2 sequence from the pCAGGS vector (pCAGGS-PKD2). Cystic kidneys were obtained from eight weeks old Pkd2<sub>WS25/-</sub> mice and 10 day old Pkd2<sup>-/-;pCAGGS-PKD2</sup> mice. One year old polycystic Han:SPRD (Cy/+) rats were used for IL-18 studies.

RNA in situ hybridization—for in situ hybridization, serial cryostat sections (4-5 μm) of snap-frozen kidneys were mounted on aminopropylsilan-coated slides and fixed with 4%
paraformaldehyde. After acetylation, the slides were pre-hybridized at 42°C in 45% formamide, 0.6M NaCl, 2.5× Denhardt’s solution, 10 mM Tris-HCl, 1 mM EDTA, 0.1% SDS and 0.15 mg/ml tRNA. Digoxigenin-labeled Lcn2-specific anti-sense (and sense control) riboprobes were generated by in vitro transcription and hybridized to the sections dissolved in pre-hybridization solution containing an additional 10% dextran sulfate at 42°C for 16h. The slides were then washed in 50% formamide/1x SSC at 55°C, treated with RNAse, and washed twice in 0.1x SCC at room temperature. Signal was detected by an antibody reaction to digoxigenin according to the manufacturer’s protocol (Roche). Sections were lightly counterstained with hematoxylin and photographed using a Nikon TE2000U microscope.

**Immunocytochemistry**—Kidneys were obtained from anesthetized mice by perfusion fixation with 0.4% lidocaine and 0.01% heparin followed by 4% paraformaldehyde in PBS. Sections 4-5 μm thick were blocked with 0.1% BSA/10% goat serum in PBS for 1 hour at room temperature and incubated with primary antibodies overnight at 4°C followed by the secondary antibodies for 1 hour at room temperature. Images were obtained with a Nikon TE2000U inverted microscope. Polyclonal antibody to lipocalin-2 was a kind gift from Jonathan Barasch (Columbia University, New York) and was applied at a dilution of 1:500 [16]. Anti-α-tubulin antibody was obtained from Sigma. Secondary antibodies were conjugated with Cy5 or Cy3.

For immunofluorescence of rat samples, kidney tissues were embedded in OCT, snap-frozen in liquid nitrogen, and stored at -80°C. Cryostat sections (5 μm) were fixed in 70% acetone/30% methanol and prepared for immunofluorescence studies as previously described[31]. A rabbit polyclonal IL-18 antibody (Santa Cruz Biotechnology, Catalogue number sc-7954) antibody was used.

**Clinical Studies**

**Human Kidney Cyst Samples**—Samples of cyst fluid were collected from several cysts and pooled in each of five ADPKD nephrectomy specimens. All participants had established renal insufficiency or were receiving renal replacement therapy. The indications for nephrectomy in these patients were related to symptoms including pain, shortness of breath, early satiety, increases in abdominal girth or as a requirement in preparation for renal transplantation. Cyst fluid was obtained at the time of surgery and aliquoted and stored at -20°C immediately.

**CRISP Cohort: Longitudinal Studies of Human ADPKD**—These protocols were reviewed by the CRISP Ancillary Studies committee and were approved by the CRISP Steering committee and the Yale Institutional Review Board. The CRISP Consortium is an NIH funded U01 cooperative agreement that began in 2000. Two hundred and forty one ADPKD participants with relatively intact kidney function (creatinine clearance > 70 mls/min) with 2/3 at increased risk for progression to renal failure were enrolled. Participants were studied on an annual basis in standardized fashion as reported elsewhere[11]. Urine samples were obtained from CRISP participants in the fasting state as a first void, at baseline and at each annual visit for 3 consecutive years. The samples were handled
uniformly across enrolling sites as per the study protocol. The urine samples were centrifuged immediately after collection; the supernatant was separated, aliquoted and frozen at −80°C until assays were performed. No protease inhibitors or other additives were added to the urine samples after collection. Samples underwent one free-thaw cycle before biomarker measurement.

**Biomarker Assays**—Specimens were randomly ordered for biochemical analysis, and the personnel performing the biochemical assays were blinded to clinical information and subject identification. The urine NGAL ELISA was performed as described previously for urine and cyst fluid\textsuperscript{18}. Briefly, microtiter plates pre-coated with a mouse monoclonal antibody raised against human NGAL (HYB211–05, AntibodyShop, Gentofte, Denmark) were blocked with buffer containing 1% BSA, coated with 100 μL of patient samples and incubated with a biotinylated monoclonal antibody against human NGAL (HYB211–01B, AntibodyShop) followed by addition of avidin-conjugated HRP (Dako, Carpinteria, CA, USA). TMB substrate (BD Biosciences, San Jose, CA, USA) was added for color development, which was read after 30 min at 450 nm with a microplate reader (Benchmark Plus, BioRad, Hercules, CA, USA). The inter- and intra-assay coefficient variations were 5% and 10% respectively for samples analyzed on the same day. Urine creatinine was measured using a quantitative colorimetric assay kit (Sigma, St. Louis, MO, USA). IL-18 was measured in human urine and cyst fluid using a human IL-18 ELISA kit (Medical and Biologic Laboratories, Nagoya, Japan) that specifically detects the mature form of IL-18 as described previously\textsuperscript{17}. The coefficient of variation of inter- and intra-assay reproducibility for IL-18 concentration were 7% and 10% respectively. Measurements of IL-18 and NGAL concentrations were repeated on 10% of randomly chosen samples to confirm the reliability of the measurement.

**Statistical Analysis**—The data were examined using SAS system software (SAS Institute, Inc., Cary, NC, USA 2002). Although 241 ADPKD patients were enrolled in CRISP, not every participant completed all three years of study and in select individuals, urine samples were either not available or the participant was pregnant and the urine sample was not appropriate for use. When comparing levels over time within individuals, we restricted the sample to the 107 patients who had results available at every visit.

Urine NGAL and IL-18 biomarker levels were measured at each study visit. TKV was measured using magnetic resonance imaging details of which are provided in the CRISP protocol and the MDRD formula was used to estimate eGFR To assess change in TKV and eGFR we calculated the percent change at each year from baseline. To test for a linear or quadratic trend, a general linear model with repeated measures was used and the results were supplemented with Friedman’s test due to the skewed distribution of the data. Baseline urine biomarkers were grouped by quartile for NGAL and tertile for IL-18. ANOVA was used to test for differences in total kidney volume and MDRD GFR by IL-18 tertiles and NGAL quartiles. As seen below, IL-18 concentrations were undetectable in over 50% of participants. To address data inclusion for all subjects, all raw IL-18 values were increased by 1 to avoid zero values.
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Fig. 1. Expression of LCN2 (NGAL) in orthologous gene mouse models of PKD
(a-c) RNA in situ hybridization for Lcn2 in a Pkd2^{WS25}−/− cystic kidney showing expression (dark reactivity) after hybridization with the Lcn2-specific antisense RNA probe (a, b) in epithelial cyst lining cells and in some pericycstic tubules; the sense probe lacks reactivity (c). The boxed area in panel a is shown in higher magnification in panel b (inset is digitally enlarged). (d) The appearance of kidneys from Pkd2^{−/−};pCAGGS-PKD2 mice at postnatal day 10. (e, f) Immunocytochemistry showing expression of NGAL in cyst lining epithelia of Pkd2^{−/−};pCAGGS-PKD2 mice (NGAL, green; α-tubulin, red; DAPI, blue. Original magnifications: a, c, 10×; b, 40×; e, f, 20×.
Figure 2. IL-18 Expression in Han:SPRD (Cy/+) Rats
(a) Immunocytochemistry showing strong expression of IL-18 (green) in cells lining the cysts in one year old Han:SPRD (Cy+) rats. (b) IL-18-receptor (IL-18R) expression (red) is also observed in cells adjacent to some cysts. Staining is also seen in the nucleus and interstitium. DAPI, blue. Original magnification, 400×.
Figure 3. Biomarker Distribution in the CRISP Cohort at each visit
Raw Values of NGAL values are in ng/ml and IL-18 values are in pg/ml.
Table 1
NGAL and IL-18 concentrations in the cyst fluid obtained during nephrectomy in PKD patients.

| Kidney Status at Nephrectomy | Age | Gender | NGAL* ng/ml | IL-18* pg/ml |
|------------------------------|-----|--------|-------------|--------------|
| SCR: 1.7; Prior transplant; no dialysis | 60  | M      | 140         | 100          |
| SCR: 8.5; Dialysis          | 54  | F      | 4939        | 380          |
| SCR: 4.4; Dialysis          | 46  | F      | 979         | 152          |
| SCR: 5.1; Prior transplant; no dialysis | 54  | M      | 449         | 309          |
| SCR: NA; No dialysis; ESRD  | 49  | F      | 3625        | 588          |

* Urinary levels of IL-18 and NGAL are usually <25 pg/ml and <20 ng/ml respectively.
Table 2

Biomarker values over time for 107 participants who followed up for all visits

| Variable                      | Baseline visit |          |          |          |          |          |          |          |          |
|-------------------------------|---------------|----------|----------|----------|----------|----------|----------|----------|----------|
|                               | Mean | SD   | Median | Mean | SD   | Median | Mean | SD   | Median |
| Urine IL-18 (ng/ml) *         | 13.8 | 26.7 | 1.0    | 23.5 | 58.2 | 1.0    | 9.3 | 24.0 | 1.0    |
| Urine NGAL (pg/ml) *          | 32.3 | 52.7 | 14.3   | 43.2 | 86.7 | 11.3   | 20.2 | 42.9 | 6.2    |
| Urine Creatinine (mg/ml)      | 0.9  | 0.7  | 0.8    | 1.0  | 0.6  | 1.0    | 0.8  | 0.6  | 0.7    |
| NGAL/Creatinine (ng/mg) *     | 37.6 | 51.2 | 16.1   | 40.0 | 64.9 | 11.4   | 27.4 | 37.4 | 13.4   |
| IL-18/Creatinine (pg/mg) *    | 13.0 | 18.8 | 5.3    | 19.7 | 42.4 | 3.2    | 8.4  | 12.5 | 3.4    |

*p ≤ .05 for linear and quadratic trend and Friedman’s chi-squared test.
### Table 3

a. Mean annual % change in total kidney volume and eGFR at 1 year and 3 years from baseline by IL-18 Tertile

| Baseline IL-18 Tertile | N   | Mean  | SD  |
|------------------------|-----|-------|-----|
| ≤ 1                    | 127 | 4.12  | 6.61|
| >1 and < 19            | 37  | 4.95  | 7.83|
| ≥19 and ≤ 434          | 39  | 5.66  | 5.66|

| Baseline IL-18 Tertile | N   | Mean  | SD  |
|------------------------|-----|-------|-----|
| ≤ 1                    | 126 | 16.40 | 14.17|
| >1 and < 19            | 37  | 19.93 | 16.54|
| ≥19 and ≤ 434          | 38  | 17.11 | 12.65|

b. Mean annual % change in total kidney volume and eGFR at 1 year and 3 years from baseline by NGAL quartile

| Baseline NGAL quartile | N   | Mean  | SD  |
|------------------------|-----|-------|-----|
| < 3.65                 | 51  | 5.24  | 7.69|
| ≥3.65 and < 11.5       | 48  | 4.43  | 6.42|
| ≥11.5 and < 31.6       | 53  | 3.38  | 6.73|
| ≥31.6 and ≤ 315.62     | 51  | 5.26  | 5.73|

| Baseline NGAL quartile | N   | Mean  | SD  |
|------------------------|-----|-------|-----|
| < 3.65                 | 50  | 18.98 | 14.80|
| ≥3.65 and < 11.5       | 50  | 17.20 | 13.70|
| ≥11.5 and < 31.6       | 51  | 15.42 | 15.10|
| ≥31.6 and ≤ 315.62     | 50  | 17.15 | 13.99|

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| Baseline IL18 Tertile | N  | Mean | SD  |
|----------------------|----|------|-----|
| ≥11.5 and < 31.6     | 53 | -7.23| 14.81|
| ≥31.6 and ≤315.62    | 51 | -0.94| 13.27|

| Baseline NGAL quartile | N  | Mean | SD  |
|------------------------|----|------|-----|
| < 3.65                 | 51 | -5.83| 17.30|
| ≥3.65 and < 11.5       | 50 | -9.10| 17.00|
| ≥11.5 and < 31.6       | 51 | -12.59| 17.79|
| ≥31.6 and ≤315.62      | 50 | -6.42| 17.25|

* No significant differences by baseline IL18 tertile for variables assessed

* No significant differences by baseline NGAL quartile for variables assessed