







# IL-17A Levels and Progression of Kidney Disease Following Hospitalization with and without Acute Kidney Injury

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## Key Points

- IL-17A was higher in patients with AKI versus without AKI during hospitalization and up to 1-year postdischarge.
- IL-17A was higher in patients with progression of kidney disease but not independently associated with subsequent progression of kidney disease.

## Abstract

**Background** AKI is associated with increased mortality and new or progressive CKD. Inflammatory cells play an important role in acute organ injury. We previously demonstrated that serum IL-17A levels were significantly elevated in critically ill patients with AKI and independently associated with hospital mortality. We hypothesize that IL-17A levels are elevated in hospitalized patients with AKI at diagnosis, and sustained elevation after discharge is associated with subsequent CKD incidence or progression.

**Methods** This was an observational convenience sampling study of hospital survivors of stage 2 or 3 AKI and controls without AKI from the Assessment, Serial Evaluation, and Subsequent Sequelae of AKI study. Patients were classified as progression or nonprogression on the basis of a composite of CKD incidence, progression, or ESKD. IL-17A levels were evaluated with S-Plex assay (Meso Scale Discovery) at 0 (during hospitalization), 3, and 12 months postdischarge and analyzed along with clinical and biomarker data up to 84 months after discharge.

**Results** Among 171 AKI and 175 non-AKI participants, IL-17A levels were elevated in AKI versus non-AKI patients at 0-, 3-, and 12-month time points ( $P < 0.05$  for all comparisons). Furthermore, IL-17A levels were elevated in the progression versus nonprogression group at the 3- and 12-month time points for outcomes occurring at 3–6 and 12–84 months, respectively ( $P < 0.05$  for both). In adjusted multivariable models, IL-17A levels were not independently associated with progression of kidney disease. IL-17A levels were positively correlated with kidney disease and immune activation biomarkers at all time points ( $P < 0.001$ ).

**Conclusions** IL-17A was higher in patients with AKI versus without AKI during hospitalization and up to 1-year postdischarge. IL-17A was higher in patients with progression of kidney disease after hospitalization, but not independently associated with subsequent progression of kidney disease in fully adjusted models.

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## Introduction

AKI is associated with high morbidity, mortality, and long-term adverse outcomes. Strategies for diagnosing and treating AKI have remained largely unchanged for more than 70

years, with mortality rates up to approximately 60% in the critical care setting.<sup>1</sup> Identification of biomarkers of AKI may provide valuable information to facilitate more timely intervention, as well as provide valuable insight into the

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pathophysiology of a complex condition. Although many patients recover from AKI, it is estimated that the rate of CKD in AKI survivors is 25.8 per 100 person-years.<sup>2</sup> Immune cell activation is known to contribute to the pathogenesis of AKI-induced fibrosis in the AKI-to-CKD transition.<sup>3</sup>

IL-17 is a proinflammatory cytokine that plays an important role in host immune defense, autoimmunity, tissue repair, and inflammatory disease progression.<sup>4–6</sup> CD4<sup>+</sup> cells expressing the proinflammatory cytokine IL-17A (TH17 cells) are rapidly expanded after kidney injury. Interventions that mitigate AKI severity in rats manifest reduced kidney TH17 cell expression.<sup>3,7–9</sup> We recently demonstrated that serum IL-17A levels were significantly elevated in critically ill patients with AKI and independently associated with hospital mortality and major adverse kidney events.<sup>10</sup> Limited data exist regarding the utility of IL-17A to serve as a potential biomarker after an episode of AKI for risk of further progression of kidney disease in subsequent months or years. The relationship of IL-17A with other biomarkers of kidney function or injury is also underexplored.

The Assessment, Serial Evaluation, and Subsequent Sequelae of AKI (ASSESS-AKI) study sought to investigate differences in kidney and cardiovascular outcomes and death within a diverse, matched cohort of patients with and without AKI.<sup>11</sup> We used plasma samples from a subset of this study to evaluate the relationship between IL-17A plasma levels and progression of kidney disease after hospitalization in patients with and without AKI.<sup>11</sup> We hypothesize that plasma IL-17A levels are elevated in patients with AKI (versus without AKI) during hospitalization and associate with subsequent risk of kidney disease progression in patients with or without AKI after hospitalization.

## Methods

### Study Participants and Outcome Definitions

An observational convenience sampling study was conducted including ASSESS-AKI study participants. AKI and non-AKI matched controls were defined as per ASSESS-AKI study protocol.<sup>11</sup> We included adult patients age 18 years or older with stage 2 or 3 AKI, along with control participants without AKI. To be included, participants must have had plasma for IL-17A analysis available from visit 0 months (24–48 hours after AKI onset for AKI patients or date of hospital discharge for controls). Participants must have had complete data on baseline eGFR using serum creatinine measured between 7 and 365 days before admission, as well as at least one subsequent serum creatinine measurement after hospital discharge, spanning a timeframe of 3–84 months. Additional blood samples were collected at 3 and 12 months postdischarge according to the ASSESS-AKI study protocol.

The primary study outcome was the progression of kidney disease, as defined by the ASSESS-AKI study.<sup>11</sup> This outcome is the combination of CKD incidence, CKD progression, and the development of ESKD. For those without preexisting CKD (eGFR  $\geq 60$  ml/min per 1.73 m<sup>2</sup>) before hospitalization, CKD incidence consisted of a 25% or greater reduction in eGFR (compared with baseline) and achieving CKD stage 3 or worse (eGFR  $< 60$  ml/min per 1.73 m<sup>2</sup>). For

those with preexisting CKD (eGFR  $< 60$  ml/min per 1.73 m<sup>2</sup>) at the index hospitalization, CKD progression was defined as experiencing a 50% or greater reduction in eGFR (compared with baseline) or progression to stage 5 CKD (eGFR  $< 15$  ml/min per 1.73 m<sup>2</sup>). The development of ESKD during the follow-up period was defined as receiving outpatient dialysis after the 3-month visit, death while receiving inpatient dialysis lasting at least 28 days, or receiving a kidney transplant. Clinical data were obtained from the ASSESS-AKI database.

We established two distinct timeframes for defining the progression outcome of this study according to data collection time points prespecified in the ASSESS-AKI study. The first was 6-month progression, which was based on the latest eGFR within 3–6 months of discharge. The second was  $> 1$ -year progression, which was based on the latest eGFR within 12–84 months of discharge. Our calculations for the eGFR were derived using the 2021 race-free CKD Epidemiology Collaboration Creatinine Equation.<sup>12</sup>

### IL-17A and Biomarker Measurements

Available plasma samples collected at 0, 3, and 12 months posthospitalization were obtained from the ASSESS-AKI biorepository.<sup>11</sup> Plasma samples stored at  $-80^{\circ}\text{C}$  underwent a single controlled thaw, at which time plasma IL-17A measurements were obtained using a high sensitivity ELISA assay (S-Plex Human IL-17A Kit; Meso Scale Discovery). The expected lower and upper limits of detection in plasma are 13.36 and 235,000 fg/ml (coefficient of variation  $< 25\%$ ), respectively. All other experimental biomarkers of kidney disease and immune activation used in this study were retrieved from the ASSESS-AKI database.

### Statistical Analysis

Descriptive analyses are presented using medians and interquartile ranges for continuous variables and counts, and percentages for categorical variables. The progressor and nonprogressor groups were compared using the *t* test or Mann–Whitney *U* test as appropriate for continuous variables and the chi-squared test for categorical variables. Continuous variable distribution was assessed with the Shapiro–Wilk normality test, and the equality of variances was evaluated using *F*-test.

Trajectory plots of eGFR and IL-17A were developed using the mean values and SEM of each longitudinal time point. We compared the trajectories between progressor and nonprogressor groups at each time point using *t* tests or Mann–Whitney *U* tests as appropriate. A heat map was used to depict the relationship between IL-17A and other biomarkers of kidney disease and immune activation. Each heat map tile displays numerical values representing Pearson correlation coefficients between IL-17A and the reference biomarker, and *t* tests were used to evaluate the statistical significance of these correlations. Pairwise group difference was evaluated using the *t* test or Mann–Whitney *U* test as appropriate.

Multivariable logistic regression models were developed to analyze the kidney disease progression outcome, with IL-17A measurements as the primary independent variable. The IL-17A measurements were either included as

continuous values or divided into tertiles (low tertile as reference). The classifier of AKI versus no AKI was evaluated as an effect modifier with corresponding interaction term of IL-17A, AKI status, and kidney disease progression outcome. Demographic, comorbidity, kidney function characteristics (eGFR and albuminuria measurements), and the inflammatory marker C-reactive protein (CRP) were used in multivariable models. The models were constructed in a sequential additive manner, whereas model 1 included relevant demographics (race) and comorbidity (diabetes, cardiovascular disease) based on univariable analysis, and model 2 added kidney function characteristics and CRP. All covariates included in the model predated the kidney disease outcome. Specifically, for the 6-month kidney disease outcome, the fully adjusted model included race, prevalent diabetes, eGFR at 0 months (hospitalization) and 3 months, and urine albumin-to-creatinine ratio at 3 months. For the >1-year kidney disease outcome, the fully adjusted model included race; prevalent diabetes and cardiovascular disease; CRP at 3 months; eGFR at baseline and 0, 3, 6, and 12 months; and urine albumin-to-creatinine ratio at 3 and 12 months. The interaction term (IL-17A×AKI status×outcome) was not incorporated in the models because it was NS for both 6-month and >1-year outcomes (*P* > 0.05 for both). Statistical analyses were performed with R Programming (R Foundation for Statistical Computing, Vienna, Austria).

**Results**

**Clinical Characteristics**

The study evaluated 346 patients for the outcome of 6-month kidney disease progression and 303 patients for the outcome of >1-year kidney disease progression. In total, 35 of 346 (10.1%) experienced the 6-month progression outcome, and 46 of 303 (15.2%) experienced the >1-year progression outcome. Clinical characteristics, including kidney function parameters and albuminuria, are shown in **Table 1** according to kidney disease progression status. Overall, progressors were more likely to be Black, have diabetes and baseline CKD, and more frequently had AKI during the index hospitalization. The proportion of progressors versus nonprogressors according to AKI status is shown in **Supplemental Table 1**.

**IL-17A Levels in Progressors versus Nonprogressors during Follow-Up**

When comparing progressors versus nonprogressors, IL-17A levels were not different when measured during the index hospitalization; however, IL-17A levels were significantly higher at 3 and 12 months in patients experiencing postdischarge progression of kidney disease versus those who did not (**Table 2**). Trajectory plots with data up to 12 months (**Figure 1**) and up to 84 months (**Supplemental Figure 1**) of follow-up were constructed to evaluate differences of IL-17A levels and eGFR during longitudinal time points.

**Table 1. Patient characteristics according to progression versus no progression of kidney disease**

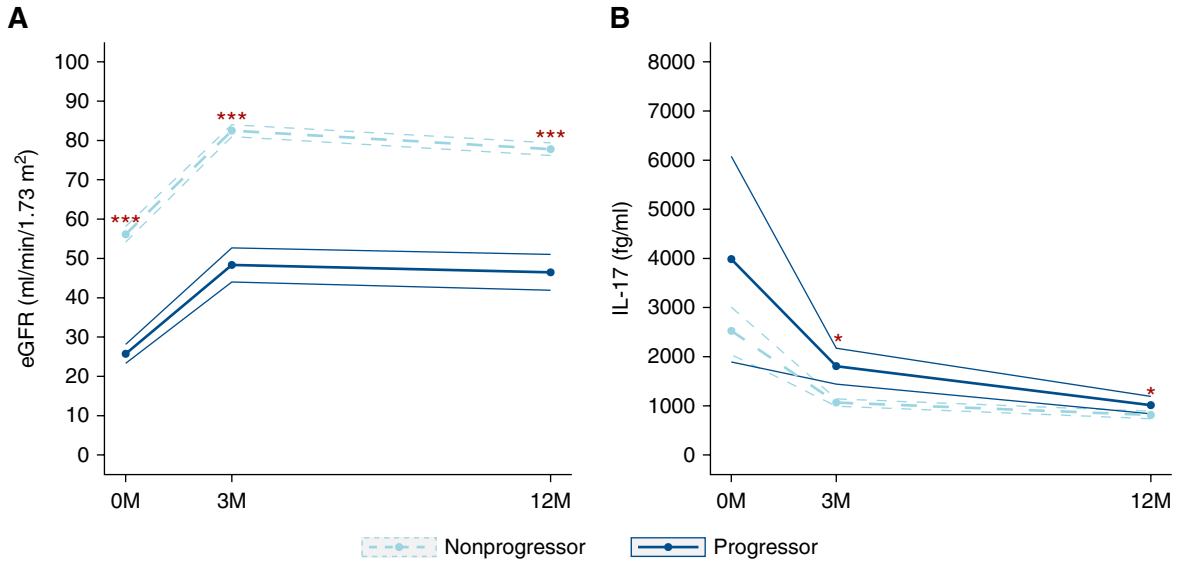
Characteristic	6-mo Kidney Disease Progression (n=346)		P Value	>1-yr Kidney Disease Progression (n=303)		P Value
	Progressor (n=35)	Nonprogressor (n=311)		Progressor (n=46)	Nonprogressor (n=257)	
Age	61.6 (53.0–65.8)	62.0 (52.8–70.4)	0.664	62.8 (57.3–67.2)	61.8 (51.0–70.7)	0.723
Male	17 (48.6)	196 (63.0)	0.096	24 (52.2)	166 (64.6)	0.109
Black	10 (28.6)	33 (10.6)	0.002 <sup>a</sup>	11 (23.9)	26 (10.1)	0.008 <sup>a</sup>
Hispanic/Latino	0 (0.0)	11 (3.5)	0.258	1 (2.2)	8 (3.1)	0.730
BMI (3 mo) kg/m <sup>2</sup>	35.0 (28.6–38.4)	30.2 (25.8–36.6)	0.072	33.8 (28.3–38.5)	30.1 (25.8–36.4)	0.041 <sup>a</sup>
Smoker (0 mo) (current or ex)	17 (48.6)	180 (57.9)	0.292	28 (60.9)	143 (55.6)	0.510
COPD (0 mo)	9 (25.7)	57 (18.3)	0.292	10 (21.7)	47 (18.3)	0.581
CVD (0 mo)	15 (42.9)	124 (39.9)	0.733	25 (54.4)	97 (37.7)	0.034 <sup>a</sup>
SEPSIS (0 mo)	6 (17.1)	45 (14.5)	0.672	7 (15.2)	39 (15.2)	0.994
DM (0 mo)	27 (77.1)	131 (42.1)	0.000 <sup>a</sup>	33 (71.7)	101 (39.3)	0.000 <sup>a</sup>
CKD (0 mo)	34 (97.1)	175 (56.3)	0.000 <sup>a</sup>	40 (87.0)	144 (56.0)	0.000 <sup>a</sup>
<b>AKI stage (0 mo)</b>			0.000 <sup>a</sup>			0.001 <sup>a</sup>
Non-AKI	5 (14.3)	170 (54.7)		12 (26.1)	143 (55.6)	
Stage 2	19 (54.3)	89 (28.6)		18 (39.1)	68 (26.5)	
Stage 3	11 (31.4)	52 (16.7)		16 (34.8)	46 (17.9)	
<b>AKI (0 mo)</b>			0.000 <sup>a</sup>			0.000 <sup>a</sup>
Non-AKI	5 (14.3)	170 (54.7)		12 (26.1)	143 (55.6)	
AKI	30 (85.7)	141 (45.3)		34 (73.9)	114 (44.4)	
Baseline eGFR, ml/min per 1.73 m <sup>2</sup>	67.2 (36.9–89.2)	81.1 (60.5–99.8)	0.008 <sup>a</sup>	65.2 (42.2–80.8)	83.7 (63.3–100.6)	0.000 <sup>a</sup>
eGFR (0 mo), ml/min per 1.73 m <sup>2</sup>	26.8 (13.7–33.7)	50.0 (25.2–88.7)	0.000 <sup>a</sup>	22.0 (11.1–36.6)	50.0 (26.7–88.2)	0.000 <sup>a</sup>
eGFR (3 mo), ml/min per 1.73 m <sup>2</sup>	45.9 (34.0–56.0)	87.7 (63.5–102.3)	0.000 <sup>a</sup>	51.6 (35.9–70.5)	88.1 (64.0–103.1)	0.000 <sup>a</sup>
eGFR (6 mo), ml/min per 1.73 m <sup>2</sup>	22.5 (16.1–34.7)	80.6 (64.5–101.4)	0.000 <sup>a</sup>	33.3 (22.7–68.2)	68.9 (54.5–97.5)	0.022 <sup>a</sup>
eGFR (12 mo), ml/min per 1.73 m <sup>2</sup>	50.8 (30.9–61.0)	81.6 (58.8–97.1)	0.000 <sup>a</sup>	51.0 (29.4–63.4)	82.1 (61.2–97.7)	0.000 <sup>a</sup>
UACR (3 mo), mg/g	55.7 (12.8–2067.5)	12.5 (6.7–40.7)	0.000 <sup>a</sup>	131.7 (8.6–1339.9)	11.3 (6.6–31.1)	0.000 <sup>a</sup>
UACR (12 mo)	28.3 (6.7–695.2)	12.2 (5.9–47.1)	0.052	85.8 (7.7–791.3)	11.7 (5.7–34.5)	0.000 <sup>a</sup>
CRP (0 mo), mg/L	85.5 (26.4–241.6)	85.1 (23.4–196.1)	0.574	59.8 (9.6–140.4)	100.2 (24.2–209.5)	0.078
CRP (3 mo), mg/L	5.5 (3.1–11.9)	3.7 (1.5–8.7)	0.079	6.9 (3.9–10.9)	3.5 (1.3–7.7)	0.001 <sup>a</sup>

BMI, body mass index; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; CVD, cardiovascular disease; DM, diabetes mellitus; UACR, urine albumin-to-creatinine ratio.  
<sup>a</sup>Statistically significant.

**Table 2. IL-17A levels according to progression versus no progression of kidney disease**

Biomarker	6-mo Kidney Disease Progression (n=346)		P Value	>1-yr Kidney Disease Progression (n=303)		P Value
	Progressor (n=35)	Nonprogressor (n=311)		Progressor (n=46)	Nonprogressor (n=257)	
IL-17A (0 M) fg/ml	860.5 (481.8–2247.8)	767.4 (387.3–1797.7)	0.374	910.5 (473.7–1945.2)	763.8 (391.0–1805.4)	0.457
IL-17A (3 mo) fg/ml	908.9 (532.7–2339.7)	677.0 (399.4–1221.1)	0.037 <sup>a</sup>	975.9 (539.2–1676.6)	675.8 (377.8–1205.5)	0.018 <sup>a</sup>
IL-17A (12 mo) fg/ml	732.7 (498.6–1097.2)	487.0 (299.1–865.9)	0.022 <sup>a</sup>	713.6 (387.1–1239.6)	464.2 (299.1–853.6)	0.015 <sup>a</sup>
Delta IL-17A (3–0 mo) fg/ml	–153.0 (–1677.6 to 236.1)	–128.4 (–826.9 to 189.1)	0.867	–82.7 (–1662.1 to 303.7)	–163.1 (–897.3 to 164.4)	0.539
Delta IL-17A (12–0 mo) fg/ml	–131.0 (–545.3 to 227.0)	–233.2 (–1000.1 to 73.0)	0.293	–154.3 (–720.9 to 168.4)	–238.1 (–1016.5 to 67.3)	0.314

<sup>a</sup>Statistically significant.



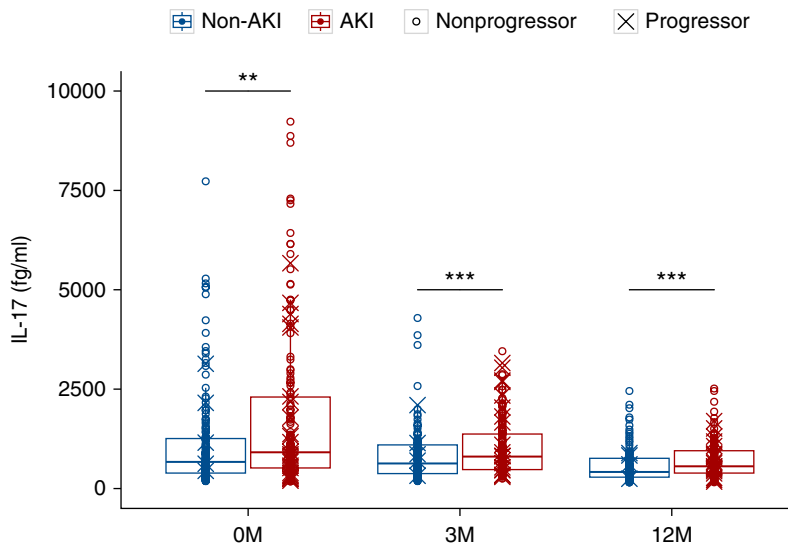
**Figure 1. Change in eGFR and IL-17 levels over time.** Trajectory plots of eGFR (A) and IL-17A (B) at 0, 3, and 12 months posthospital discharge in progressors and nonprogressors (3–6 months outcome, including non-AKI and AKI). Statistical comparisons between progressors and nonprogressors were made at each time point using *t* tests or Mann–Whitney *U* tests as appropriate. \**P* < 0.05, \*\*\**P* < 0.001.

In fully adjusted models, IL-17A levels—both continuously and when the highest tertile (T3) was compared to the lowest tertile (T1) (tertiles shown in Supplemental Table 2)—were not independently associated with kidney disease progression, both at 6-month or >1-year follow-up (Supplemental Tables 3–6).

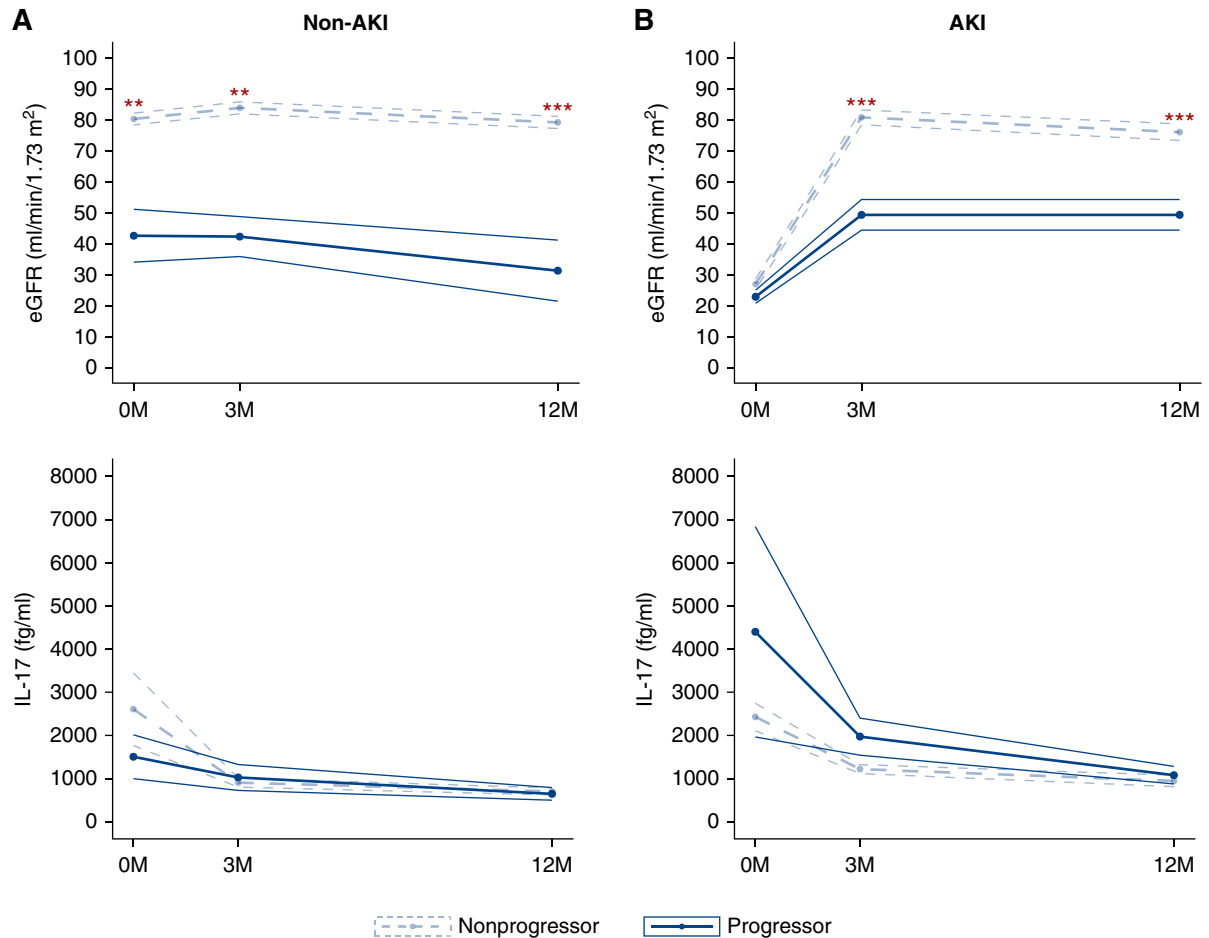
**IL-17A Levels According to AKI Status during Hospitalization**

Consistent with our previous findings, IL-17A levels were higher in patients with AKI versus those without AKI (Figure 2).<sup>10</sup> This was true not only for the hospitalization

measurement but also at 3 and 12 months of follow-up, suggesting persistent elevation over time of IL-17A levels among AKI versus non-AKI patients. Despite similar eGFR after AKI diagnosis (0 months), plasma IL-17A levels in post-AKI progressors were numerically higher, albeit not statistically different, at 0 and 3 months when compared with nonprogressors (Figure 3B). Trajectory plots with data up to 12 months (Figure 3) and up to 84 months (Supplemental Figure 2) of follow-up stratified by AKI status were developed to evaluate differences of IL-17A levels and eGFR during longitudinal time points.



**Figure 2. Comparison of plasma IL-17A levels at 0, 3, and 12 months posthospital discharge for patients with and without AKI.** Statistical comparisons between patients with versus without AKI were made using the Mann–Whitney *U* test. \*\**P* < 0.01, \*\*\**P* < 0.001.



**Figure 3. Change in eGFR and IL-17 levels over time.** Trajectory plots of eGFR and IL-17A at 0, 3, and 12 months posthospital discharge in progressors and nonprogressors (3–6 months outcome), stratified according to non-AKI (A) versus AKI (B) status. Statistical comparisons between progressors and nonprogressors were made at each time point using *t* tests or Mann–Whitney *U* tests as appropriate. \*\**P* < 0.01, \*\*\**P* < 0.001.

### IL-17A Levels and Other Biomarkers of Kidney Disease and Immune Activation

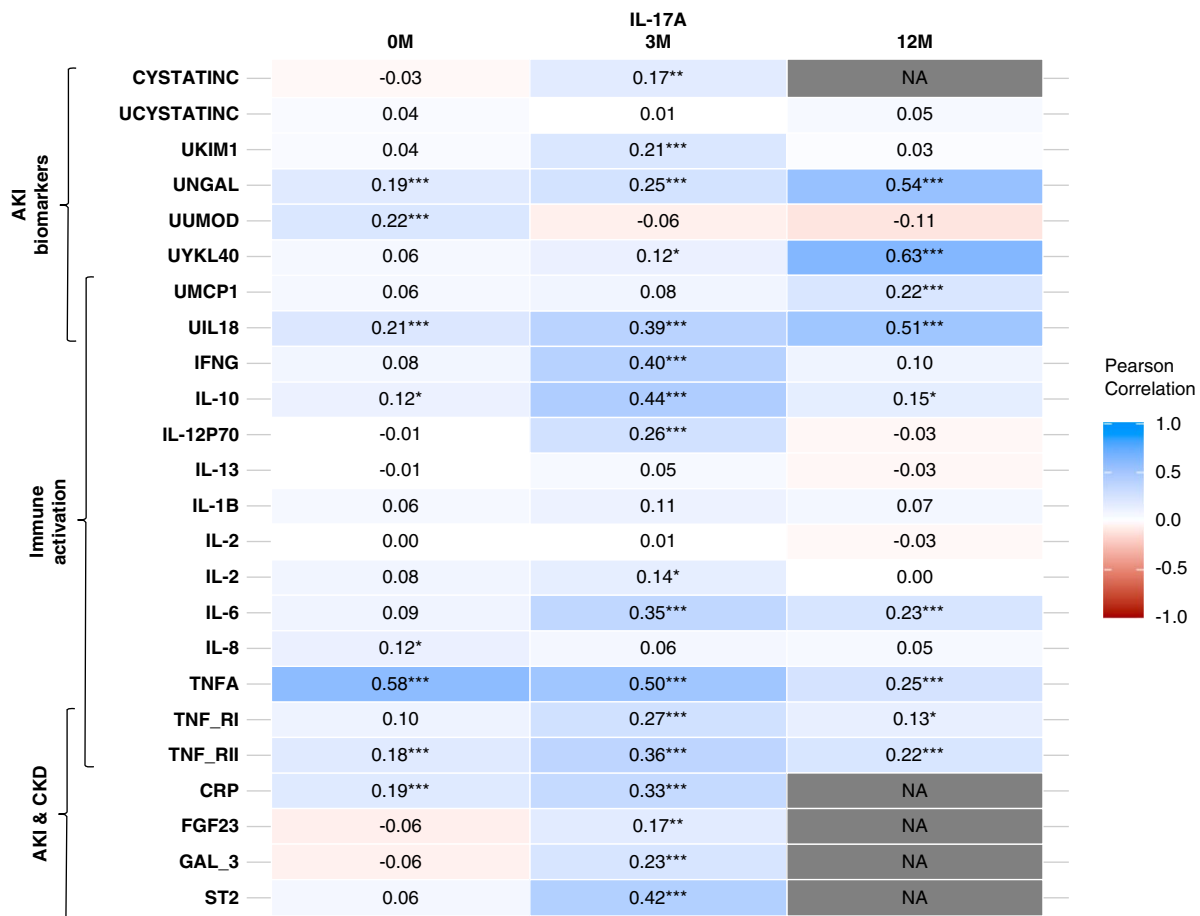
A heat map depicting the relationship of IL-17A levels with other biomarkers of kidney disease and immune activation is shown in [Figure 4](#). IL-17A was strongly correlated with TNF- $\alpha$  at initial measurement during hospitalization and at 3 months after discharge. During the later time period of 12 months posthospitalization, IL-17A levels were more highly correlated with urinary chitinase-3-like protein 1, urinary neutrophil gelatinase-associated lipocalin, and urinary IL-18.

### Discussion

This study used data from the ASSESS-AKI consortium and found that plasma IL-17A levels were higher in hospitalized patients that had progression of kidney disease both at 6 months and >1 year postdischarge. Specifically, plasma IL-17A levels were higher during hospitalization in patients with AKI versus those without AKI and were persistently elevated both at 3 and 12 months after discharge. However, IL-17A levels were not independently associated with subsequent increased risk of kidney

disease progression in multivariable models that adjusted for demographics, comorbidity, inflammation, and kidney disease parameters, such as eGFR and albuminuria. IL-17A levels were also positively correlated with specific biomarkers of kidney disease and immune activation measured at similar time points, which provides additional validity to our observations. For example, TNF- $\alpha$  has been shown to promote Th17 differentiation through IL-6 and IL-1 $\beta$  in a TNFR1 and TNFR2 dependent manner, all of which were significantly correlated with IL-17A in this study.<sup>13,14</sup> Although this study does not highlight a relevant role of IL-17A as a biomarker of kidney disease progression, it does underpin the translation potential of targeting Th17 cell activation in AKI-to-CKD therapeutics.

AKI is associated with increased levels of inflammatory cytokines, such as IL-6, IL-18, and TNF- $\alpha$ .<sup>15–18</sup> Th17 cells and the cytokine IL-17A appear to play a relevant role in acute kidney disease.<sup>8,14,19–22</sup> It is thought that cytokines released from leukocytes and renal tubular cells are involved in both the initiation and extension phase of AKI.<sup>18</sup> IL-17 cytokines are produced by a variety of cell types, such as CD4<sup>+</sup>, CD8<sup>+</sup>, and  $\gamma\delta$  T cells, as well as several innate cell populations like neutrophils and macrophages.<sup>23,24</sup>



**Figure 4.** Heat map depicting the relationship of IL-17A with other kidney disease and immune activation markers evaluated in the ASSESS-AKI study. The biomarkers are organized based upon their putative role/function or experimental association with AKI, CKD, and/or immune activation. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . ASSESS-AKI, Assessment, Serial Evaluation, and Subsequent Sequelae of AKI; CRP, C-reactive protein; CYSTATINC, cystatin C; FGF23, fibroblast growth factor 23; GAL\_3, galectin 3; IFNG, IFN gamma; IL1B, IL-1 $\beta$ ; ST2, IL receptor-like 1; TNFA, TNF- $\alpha$ , TNF\_RI, TNF receptor 1; TNF\_RII, TNF receptor 2; UCYSTATINC, urinary cystatin C; UIL18, urinary IL-18; UKIM1, urinary kidney injury molecule 1; UMCP1, urinary monocyte chemoattractant protein-1; UNGAL, urinary neutrophil gelatinase-associated lipocalin; UUMOD, urinary uromodulin; UYKL40, urinary YKL-40.

However, others and we have demonstrated that Th17 cells are a major infiltrating lymphocyte in kidneys post-AKI in rodent models.<sup>25,26</sup> This observation has been also demonstrated in various models of AKI in humans. We recently reported elevated serum IL-17A levels in patients with AKI relative to acutely ill patients without AKI in the intensive care unit, and these levels were independently associated with hospital mortality and major adverse kidney events.<sup>10</sup> These results are consistent with our previous report demonstrating a four-fold increase in circulating TH17 cells and a ten-fold increase in Orai1+ cells in critically ill patients with AKI compared with those without AKI.<sup>8</sup> Maravitsa *et al.* identified elevated circulating IL-17A in nonsurvivors versus survivors of AKI after septic shock.<sup>27</sup> Furthermore, elevated IL-17A has been associated with chronic allograft nephropathy.<sup>28</sup> Our results are consistent with these previous reports of IL-17A as an important cytokine in the initiation and propagation of AKI.

AKI is a significant risk factor of new or progressive CKD.<sup>29–32</sup> The extent to which IL-17A is involved with

maladaptive repair and progression to CKD remains unknown, as others and we have shown associations with CKD, whereas others have shown a protective effect.<sup>33</sup> Our laboratory demonstrated sustained increases in renal T cells after recovery of AKI<sup>34</sup> and blockade of IL-17 activity using IL-17Rc decoy receptor significantly decreased fibrosis and neutrophil recruitment after ischemic AKI.<sup>35</sup> Furthermore, we demonstrated that Th17 cell activation in response to elevated dietary salt intake after AKI was associated with renal fibrosis and CKD progression, which was attenuated by IL17 blockade.<sup>26,35</sup> Consistent with this work, we also demonstrated increases in peripheral blood and bronchiolar lavage fluid Th17 cells after AKI in a CKD rodent model.<sup>36</sup> Recent work by Liang *et al.* demonstrated that group 2 innate lymphoid cells (ILC3s) increase expression of programmed cell death-1 and IL-17A production associated with renal fibrosis.<sup>37</sup> Biopsies of patients with renal fibrosis showed elevated renal IL-17 expression, and gene polymorphisms of IL-17 and IL-17 receptor are associated with ESKD.<sup>38</sup> This supports previous observations that patients

with ESKD exhibit alternate T cell-associated immunologic profiles.<sup>39</sup> The results of this study highlight that plasma IL-17A is not only elevated in patients with AKI, but also elevated in patients with kidney disease progression, even in the absence of *de novo* acute injury. Owing to its pleiotropic effects on various target cell types, IL-17A may be a valuable therapeutic target for both AKI and AKI-to-CKD.

Numerous serum and urine biomarkers of early AKI identification have been described, such as urine IL-18, urine kidney injury molecule-1, and urine neutrophil gelatinase-associated lipocalin.<sup>40</sup> On the contrary, there is limited availability of prognostic biomarkers of kidney disease progression after AKI and certainly overall lack of effective implementation science and bedside applications of biomarkers of kidney health.<sup>41</sup> In our study, plasma IL-17A was positively correlated with several kidney disease and immune activation biomarkers, not only at hospitalization, but also after discharge, suggesting that the Th17 cell activation pathway may play a pivotal role in the AKI-to-CKD transition.<sup>42</sup> Specifically, IL-17A was strongly correlated to both TNFRI and TNFRII, both of which have been associated with CKD risk.<sup>43</sup>

Our study has several strengths including the use of systematically collected samples from ASSESS-AKI, with detailed longitudinal follow-up and prespecified time points of kidney function evaluation over several months after a hospitalization with or without AKI. This allowed for a temporal evaluation of repeated measures of IL-17A levels and eGFR over time. Furthermore, the availability of previously measured biomarkers of kidney disease and immune activation allows for additional examination of the relevance of IL-17A levels on kidney disease progression. Finally, we used advanced methods of IL-17A measurement, which enhances reproducibility of our results.

This study also has notable limitations. First, the study design was based on convenience sampling and is therefore susceptible to selection bias. Specifically, we only included patients with severe AKI (Kidney Disease Improving Global Outcomes stages 2 or 3) to avoid concerns of prerenal azotemia in some cases of stage 1 AKI. This exclusion reduced our sample size significantly because most patients in ASSESS-AKI had stage 1 AKI. Second, owing to the design of ASSESS-AKI, the timing of measurements at 0 months was different between patients with AKI (24–48 hours after AKI onset) and without AKI (hospital discharge), which may in part affect the interpretation of IL-17A comparisons at 0 months. Third, this study was not designed to identify the cellular sources of IL-17A. While reduced kidney filtration could potentially affect IL-17A levels, our previous data showing elevated Th17 cells in the early post-AKI period suggest that reduced filtration is not the primary mechanism for the observed elevations in IL-17A.<sup>8</sup>

One should acknowledge that plasma IL-17A was not independently associated with subsequent progression of kidney disease in fully adjusted models. The reasons for this observation are likely multifactorial: (1) IL-17A biology may differ depending on the etiology of AKI or other circumstances associated with AKI-to-CKD progression; (2) unmeasured clinical factors, including coregulated inflammatory factors, could mitigate the prognostic capacity of a single cytokine; and (3) the lack of power given the limited number of progression events in this study. Therefore, these findings

are hypothesis generating regarding the pathway of Th17 cell activation and its role in human AKI-to-CKD transition.

In conclusion, this study found that plasma IL-17A levels were higher in patients who had progression of kidney disease after hospitalization with or without AKI, both at 6 months and >1 year postdischarge. IL-17A levels were not independently associated with subsequent increased risk of kidney disease progression in multivariable models that adjusted for demographics, comorbidity, inflammation, and kidney disease parameters, such as eGFR and albuminuria. When compared according to AKI status during hospitalization, plasma IL-17 levels were higher in patients with AKI versus those without AKI, and persistently elevated both at 3 and 12 months after discharge. Furthermore, IL-17A levels were also positively correlated with other kidney disease and immune activation biomarkers measured at similar time points. The translation potential of targeting Th17 cell activation in AKI-to-CKD therapeutics warrants further investigation.

#### Disclosures

Disclosure forms, as provided by each author, are available with the online version of the article at <http://links.lww.com/KN9/A651>.

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#### Data Sharing Statement

A complete deidentified patient dataset can be made available through the National Institute of Diabetes and Digestive and Kidney Diseases data repository at <https://repository.niddk.nih.gov/studies/assess-aki/>.

#### Supplemental Material

This article contains the following supplemental material online at <http://links.lww.com/KN9/A650>.

**Supplemental Table 1.** Proportion of progressors and non-progressors according to AKI status.

**Supplemental Table 2.** Distribution of IL-17A and delta IL-17A tertiles in the study sample.

**Supplemental Table 3.** Multivariable logistic regression analysis for tertiles of IL-17A (0 or 3 months) as the independent variable and 6-month kidney disease outcome as the dependent variable.

**Supplemental Table 4.** Multivariable logistic regression analysis for tertiles of IL-17A (0, 3 or 12 months) as the independent variable and >1-year kidney disease outcome as the dependent variable.

**Supplemental Table 5.** Multivariable logistic regression analysis for continuous IL-17A (0 or 3 months) as the independent variable and 6-month kidney disease outcome as the dependent variable.

**Supplemental Table 6.** Multivariable logistic regression analysis for continuous IL17A (0, 3 or 12 months) as the independent variable and >1-year kidney disease outcome as the dependent variable.

**Supplemental Figure 1.** Trajectory plots of eGFR (A) and IL-17A (B) up to 84 months posthospital discharge in progressors and nonprogressors (>1-year outcome), regardless of AKI diagnosis.

**Supplemental Figure 2.** Trajectory plots of eGFR and IL-17A up to 84 months posthospital discharge in progressors and non-progressors (>1-year outcome), stratified according to non-AKI (A) versus AKI (B) status.

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