Urine tumor necrosis factor-α and interleukin-9 for clinical diagnosis of acute interstitial nephritis

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SUPPLEMENTAL TABLES AND FIGURES

Supplementary Table 1. Participants selected for adjudication by three pathologists and adjudication results

Official biopsy report	Total	Selected for adjudication	Number of pathologists diagnosing AIN		
			All 3	2 out of 3	1 or 0
AIN	79	79 (100%)	32 (41%)	23 (29%)	24 (30%)
1st Diagnosis[1]	38	38	21 (66%)	10 (44%)	7 (29%)
2nd or 3rd Diagnosis	41	41	11 (34%)	13 (57%)	17 (71%)
Not AIN	186	28 (15%)	0 (0%)	0 (0%)	28 (100%)

[1]1st, 2nd, and 3rd diagnosis refer to the numerical order in which AIN was listed on the official biopsy report. Agreement on AIN diagnosis between pairs of pathologists ranged from 63-70% and overall Fleiss kappa was 0.35.

Supplementary Table 2. Comparison of urine and plasma biomarker levels between AIN and controls in the two sub-cohorts of the study

	Sub-cohort 1: Jan 2015-Jan 2017			Sub-cohort 2: Jan 2017-June 2018		
Characteristic	**AIN**	**NOT AIN**	**P**	**AIN**	**NOT AIN**	**P**
N	**22**	**105**		**10**	**81**	
Urine						
TNF-α	2.25 (0.70, 16.13)	0.33 (0.14, 1.23)	0.0001	3.76 (0.34, 15.03)	0.32 (0.10, 1.07)	0.01
IL9	1.60 (0.45, 3.60)	0.38 (0.17, 0.63)	0.0002	2.61 (0.86, 4.52)	0.41 (0.16, 1.16)	0.001
IL12p70	0.26 (0.17, 0.34)	0.12 (0.07, 0.21)	0.0004	0.10 (0.04, 0.22)	0.08 (0.04, 0.22)	0.90
IL2	0.68 (0.43, 2.72)	0.32 (0.20, 0.55)	0.0005	0.29 (0.14, 0.83)	0.27 (0.10, 0.52)	0.41
IL6	12.50 (4.89, 48.15)	2.64 (1.02, 9.99)	0.0006	9.05 (4.34, 15.44)	3.14 (1.27, 8.73)	0.04
IL4	0.07 (0.06, 0.13)	0.05 (0.02, 0.08)	0.01	0.03 (0.03, 0.08)	0.03 (0.01, 0.06)	0.44
IFN-γ	1.63 (0.36, 4.08)	0.51 (0.25, 0.85)	0.01	0.26 (0.17, 5.34)	0.59 (0.26, 1.76)	0.38
IL13	1.76 (1.24, 3.02)	1.00 (0.57, 1.72)	0.01	1.06 (0.23, 2.08)	0.33 (0.16, 1.33)	0.26
IL1β	2.97 (1.59, 10.85)	1.29 (0.44, 3.68)	0.02	1.70 (1.05, 7.99)	0.62 (0.36, 2.09)	0.07
IL8	93.39 (59.23, 177.22)	50.63 (16.09, 168.16)	0.03	94.18 (28.11, 306.20)	27.07 (7.46, 129.75)	0.07
IL5	0.12 (0.08, 0.81)	0.09 (0.04, 0.22)	0.05	0.19 (0.06, 0.54)	0.10 (0.06, 0.19)	0.31
IL10	0.15 (0.08, 0.24)	0.09 (0.04, 0.17)	0.10	0.23 (0.15, 0.37)	0.20 (0.14, 0.35)	0.56
Plasma						
IL13	0.48 (0.48, 0.48)	0.48 (0.48, 0.48)	0.15	0.19 (0.19, 0.48)	0.19 (0.19, 0.55)	0.81
IL8	9.06 (4.15, 14.23)	9.72 (5.41, 17.70)	0.26	10.40 (5.90, 14.28)	10.13 (5.53, 16.47)	0.84
IL4	0.01 (0.01, 0.04)	0.01 (0.01, 0.01)	0.33	0.03 (0.02, 0.04)	0.03 (0.02, 0.04)	0.63
IFN-γ	6.61 (3.33, 85.49)	7.28 (2.91, 17.37)	0.35	6.74 (2.40, 14.84)	3.18 (1.56, 6.51)	0.16
IL6	3.18 (2.04, 19.69)	3.52 (1.53, 7.78)	0.36	3.30 (1.85, 9.95)	3.47 (1.38, 8.33)	0.68
IL2	0.13 (0.06, 0.42)	0.06 (0.06, 0.30)	0.42	0.39 (0.16, 0.67)	0.22 (0.13, 0.34)	0.17
IL1β	0.20 (0.06, 0.33)	0.15 (0.06, 0.28)	0.45	0.30 (0.18, 0.71)	0.17 (0.02, 0.35)	0.11
IL10	0.47 (0.27, 0.90)	0.57 (0.32, 1.32)	0.50	0.93 (0.49, 1.42)	0.52 (0.32, 1.04)	0.06
IL12p70	0.08 (0.03, 0.13)	0.08 (0.03, 0.18)	0.55	0.10 (0.08, 0.19)	0.15 (0.09, 0.24)	0.29
TNF-α	6.61 (4.54, 10.07)	6.31 (4.04, 8.56)	0.56	9.52 (7.46, 13.53)	6.33 (4.48, 9.88)	0.01

Wilcoxon rank sum test. Sorted by P-value (low to high). Median and interquartile range are shown. TNF-α and IL-9 were selected for further analysis based on consistency and strength of association in both cohorts and biological plausibility of their association with AIN based on our hypothesis. TNF, tumor necrosis factor; IFN, interferon; IL, interleukin

Supplementary Table 3. Alternate splitting of cohort to determine validity of biomarkers

Biomarker	AIN	Not AIN	P-value	AIN	Not AIN	P-value
Year of enrollment	**Sub-cohort 1: Jan 2015-Jan 2017**			**Sub-cohort 2: Jan 2017-June 2018**		
	22	**105**		**10**	**81**	
TNF-α	2.25 (0.70, 16.13)	0.33 (0.14, 1.23)	<0.001	3.76 (0.34, 15.03)	0.32 (0.10, 1.07)	0.01
IL9	1.60 (0.45, 3.60)	0.38 (0.17, 0.63)	<0.001	2.61 (0.86, 4.52)	0.41 (0.16, 1.16)	0.001
Site of enrollment	**Site 1**			**Site 2**		
	24	**146**		**8**	**40**	
TNF-α	2.25 (0.58, 0.58)	0.30 (0.13, 0.83)	<0.001	3.97 (0.37, 11.15)	0.41 (0.17, 2.80)	0.04
IL9	1.62 (0.55, 0.55)	0.37 (0.16, 0.68)	<0.001	2.08 (0.55, 3.60)	0.46 (0.22, 1.15)	0.02

Median (IQR) shown. Wilcoxon Ranksum test. TNF, tumor necrosis factor; IL, interleukin.

Supplementary Table 4. Alternate approaches to acute interstitial nephritis diagnosis and biomarkers

Biomarker	AIN	Not AIN	P-value	AIN	Not AIN	P-value
	Phase 1: Jan 2015-Jan 2017			**Phase 2: Jan 2017-June 2018**		
A. Consensus diagnosis (Primary analysis)						
	22	105		10	81	
TNF-α	2.25 (0.70, 0.70)	0.33 (0.14, 1.23)	<0.001	3.76 (0.34, 15.03)	0.32 (0.10, 1.07)	0.01
IL9	1.60 (0.45, 0.45)	0.38 (0.17, 0.63)	<0.001	2.61 (0.86, 4.52)	0.41 (0.16, 1.16)	0.001
B. Majority diagnosis (Sensitivity Analysis 1)						
	35	120		20	90	
TNF-α	1.45 (0.41, 0.41)	0.34 (0.16, 1.35)	<0.001	2.37 (0.33, 11.37)	0.37 (0.10, 1.18)	0.01
IL9	0.66 (0.29, 0.29)	0.36 (0.17, 0.64)	0.001	1.47 (0.26, 2.85)	0.41 (0.16, 1.28)	0.04
C. Clinician's post-biopsy diagnosis (Sensitivity Analysis 2)						
	48	102		31	76	
TNF-α	1.13 (0.38, 0.38)	0.34 (0.15, 1.23)	<0.001	1.07 (0.14, 6.44)	0.34 (0.11, 1.14)	0.03
IL9	0.59 (0.27, 0.27)	0.34 (0.17, 0.60)	<0.001	1.00 (0.28, 2.96)	0.41 (0.16, 1.27)	0.03
D. AIN reported on official biopsy report (Sensitivity Analysis 3)						
	50	105		29	81	
TNF-α	1.16 (0.39, 0.39)	0.33 (0.14, 1.23)	<0.001	2.37 (0.32, 13.45)	0.32 (0.10, 1.07)	0.008
IL9	0.56 (0.22, 0.22)	0.38 (0.17, 0.63)	0.01	1.13 (0.26, 3.01)	0.41 (0.16, 1.16)	0.01

AIN case definitions: A) AIN as consensus among the pathologists (primary analysis); B) AIN diagnosis based on diagnosis of majority of pathologists; C) Clinician's diagnosis after biopsy; D) AIN as reported on official biopsy report. Median (IQR) shown. Wilcoxon Ranksum test. TNF, tumor necrosis factor; IL, interleukin.

Supplementary Table 5. Drug-induced acute interstitial nephritis and all controls

Biomarker	AIN	Not AIN	P-value
N	**20**	**186**	
TNF-α	3.16 (0.34, 43.61)	0.32 (0.13, 1.18)	0.001
IL9	1.85 (0.66, 6.07)	0.39 (0.17, 0.78)	<0.001

Drug induced AIN was thought to be due to antibiotics (n=6), proton pump inhibitors (n=3), non-steroidal anti-inflammatory drugs (n=2), cancer immunotherapy (n=3), and others (n=6). Median (IQR) are shown. Wilcoxon Ranksum test

Supplementary Table 6. Comparison of biomarkers between drug-related and other causes of acute interstitial nephritis

Biomarker	Drug-related	Other AIN	P-value
N	**20**	**12**	
TNF-α	3.16 (0.34, 43.61)	2.05 (0.87, 6.79)	0.78
IL9	1.85 (0.66, 6.07)	1.78 (0.55, 2.85)	0.61

Median (IQR) are shown. Wilcoxon Ranksum test.

Supplementary Table 7. Post-test probabilities of acute interstitial nephritis at a range of pre-test probabilities

Pre-test Prob.	Post-test Probabilities							
	All controls				Acute tubular injury controls			
	0.41 ng/g		2.53 ng/g		0.41 ng/g		2.53 ng/g	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
0.90	0.95	0.68	0.97	0.85	0.95	0.65	0.99	0.84
0.75	0.85	0.41	0.92	0.66	0.87	0.39	0.98	0.64
0.50	0.66	0.19	0.80	0.39	0.69	0.17	0.94	0.37
0.25	0.40	0.07	0.58	0.18	0.43	0.07	0.84	0.17
0.10	0.18	0.03	0.31	0.07	0.20	0.02	0.65	0.06

Supplementary Table 8. Correlation coefficients between cells on biopsy and urine biomarkers

	TNF-α, cells	**FcεRI, cells**	**Urine TNF-α**	**Urine IL-9**
TNF-α, cells	1			
FcεRI, cells	0.77*	1		
Urine TNF-α	0.48*	0.41	1	
Urine IL-9	0.21	0.29	0.54*	1

*Spearman correlation coefficients (rho). * indicates P<0.05. TNF, tumor necrosis factor; IL, interleukin.*

Supplementary Table 9. Urine biomarker to albumin ratio and fractional excretion of biomarker

Biomarker	AIN	Not AIN	P-value
N	**31**	**176**	
Urine TNF-α to plasma TNF-α ratio	0.24 (0.05, 0.55)	0.04 (0.02, 0.10)	<0.001
Urine TNF-α to urine albumin ratio	18.21 (2.34, 123.18)	0.64 (0.18, 3.30)	<0.001
Urine IL-9 to urine albumin ratio	13.11 (1.27, 38.23)	0.47 (0.17, 2.71)	<0.001

Wilcoxon Ranksum test. Median (IQR) are shown.

Supplementary Table 10. Comparison of urine biomarker levels between those who did and did not receive corticosteroid therapy

Biomarkers	Steroids before urine	No steroids	P-val.
	35	**183**	
TNF-a	0.41 (0.16, 1.23)	0.38 (0.14, 1.72)	0.76
IL-9	0.36 (0.18, 0.78)	0.41 (0.17, 1.19)	0.42

Wilcoxon rank sum test. Includes participants who received steroids between 7 days and 6 hours before urine collection.

Supplementary Table 11. Association of urine biomarker levels with corticosteroid dose

Biomarker	**Comparison**	**Steroid Dose in mg (95% CI)**	**Steroid Dose in mg (95% CI)**
		Model 1	**Model 2**
TNF-a	Per log increase	-8 (-103, 87)	-19 (-120, 82)
	Below median	Ref.	Ref.
	Above median	-103 (-415, 208)	-131 (-453, 190)
IL-9	Per log increase	-105 (-237, 28)	**-180 (-333, -27)**
	Below median	Ref.	Ref.
	Above median	-276 (-576, 24)	**-330 (-640, -19)**

Linear regression analysis with biomarker quartile as outcome and steroid dose as predictor (Model 1). Model 2 controls for histological diagnosis (AIN yes/no).

Supplementary Table 12. Summary of the cytokines and chemokines measured in the study and their general function

Biomarker	Producer Cells	Action
T_H2 cell-related		
IL-4	T cells, mast cells, ILC2	B-cell activation, T_H2 differentiation
IL-5	T cells, mast cells, ILC2	Eosinophil growth and differentiation
IL-13	T cells, ILC2	B cell growth and differentiation, induces allergy/asthma, suppresses T_H1
T_H9 cell-related		
IL-9	T cells	Mast cell enhancing activating. Stimulates T_H2 and ILC2 cells
T_H1 cell-related		
IFN-γ	T cells, NK cells, neutrophils, ILC1	Macrophage activation, increase expression of MHC molecules
IL-12p70	Macrophages, dendritic cells	Activates NK cells, induces differentiation into T_H1 type
IL-2	T cells	T cell proliferation and differentiation
Other inflammatory		
TNF-α	Macrophages, T cells, NK cells	Promotes inflammation, endothelial activation
IL-1β	Macrophages, epithelial cells	Fever, T cell activation, macrophage activation
IL-6	T cells, B cells, macrophages, endothelial cells	T and B cell growth and differentiation
IL-8 (CXCL8)*	Neutrophil, basophil, CD8 cells	Chemotaxis of neutrophils, promotes angiogenesis
IL-10	macrophages, dendritic cells	Suppressor of macrophage function

**IL-8 is a chemokine whereas all of the others are cytokines. ILC, innate lymphoid cells; IL, interleukin; NK, natural killer. Adapted from (32)*

Supplementary Table 13. Ordinal scale used by pathologists to record interstitial histologic features of acute interstitial nephritis

Interstitial features	
1	Tubulointerstitial inflammation, preserved area*
2	Tubulitis^
3	Interstitial Eosinophils#
4	Tubulointerstitial inflammation, ***fibrosed*** area*
5	Acute tubular injury, overall*
6	Acute tubular injury, areas ***without*** inflammation*
7	Tubular atrophy / interstitial fibrosis*
*None (<10%), Mild (10-25%), Moderate (26-50%), Severe (>50%) ^0 (no cells), 1 (1-4 cells), 2 (5-10 cells), 3 (>10 cells) per tubule #0 (no cells), 1 (1-4 cells), 2 (5-10 cells), 3 (>10 cells) per high power field	

Supplementary Table 14. Detection range and precision of biomarkers

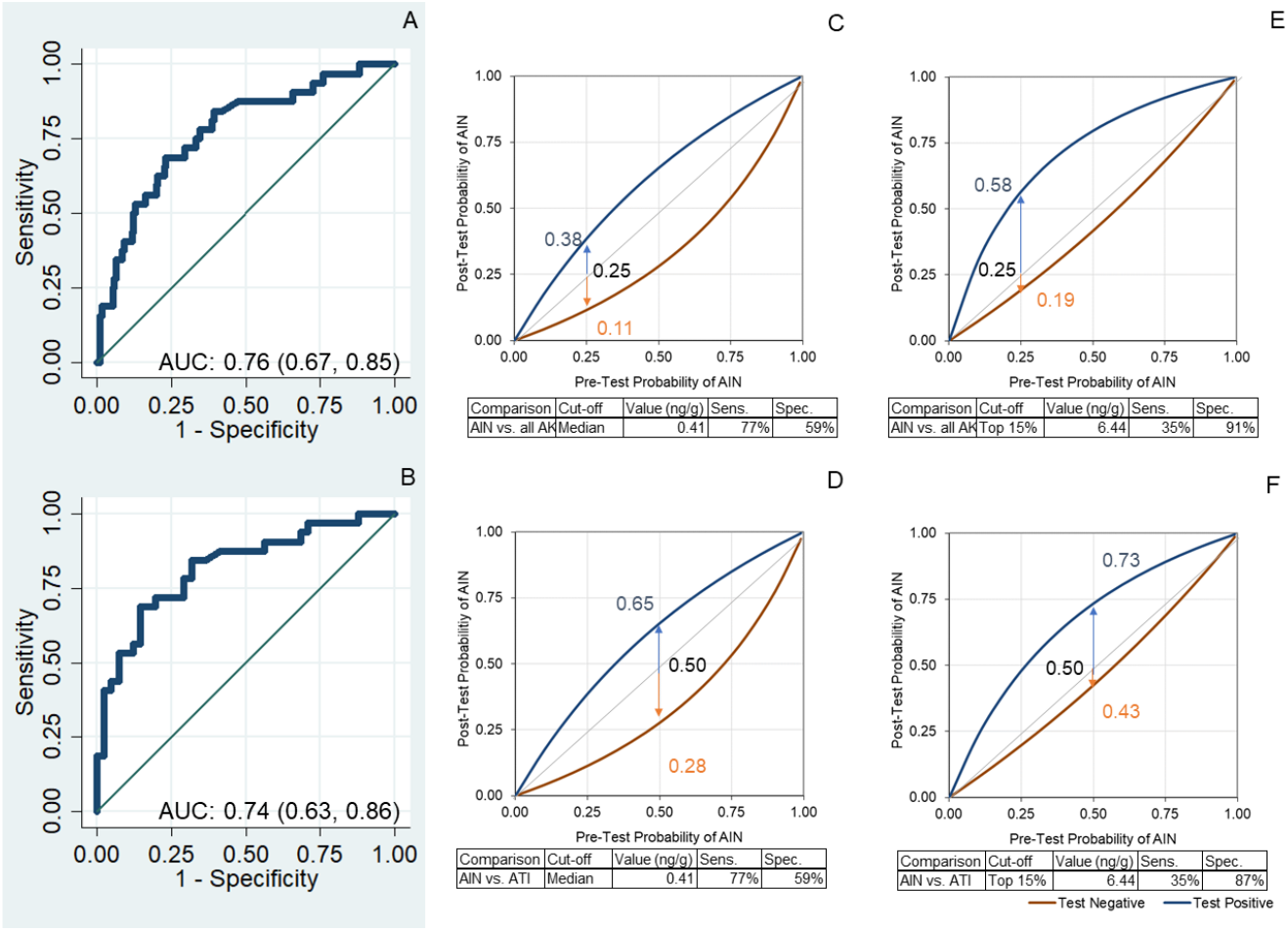
Biomarker	Detection range (pg/ml)		CV (%)	
	Urine	**Plasma**	**Urine**	**Plasma**
TNF-α	0.10-368	0.26-736	8.5	3.5
IFN-γ	0.27-1400	0.49-2800	10.9	3.6
IL-1β	0.05-575	0.11-1150	6.2	2.3
IL-2	0.04-1530	0.13-3060	7.5	10.4
IL-4	0.019-283	0.025-566	12.9	8.2
IL-5	0.10-2080	N/A	8.9	N/A
IL-6	0.048-765	0.096-1530	3.7	3.7
IL-8	0.055-599	0.175-1198	2.4	2.8
IL-9	0.03-580	N/A	7.1	N/A
IL-10	0.04-374	0.106-748	22.6	5.4
IL-12p70	0.06-501	0.058-1002	12.7	7.6
IL-13	0.446-496	0.96-992	8.5	5.8

Plasma IL-5 and IL-9 were not measured due to lack of availability of appropriate assay.

Supplementary Figure 1. Comparison of urine biomarkers between AIN cases and controls

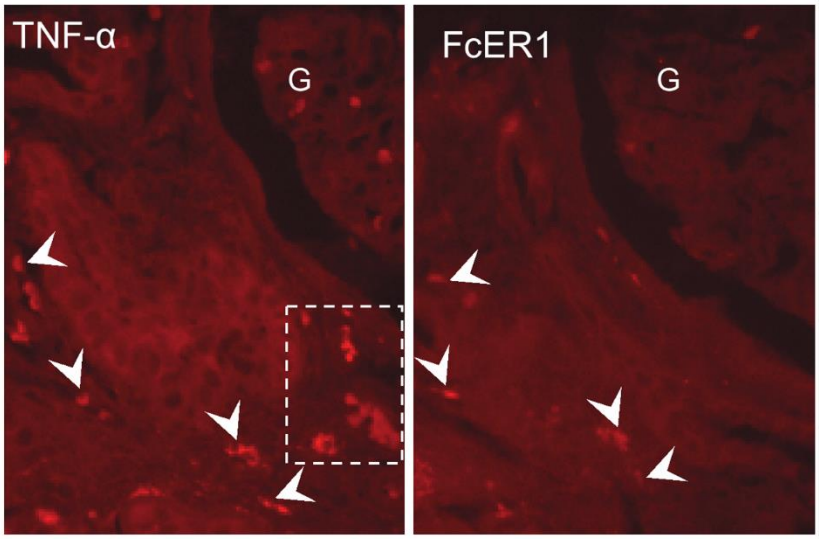
Dot plot of biomarkers (on log scale) are shown. Wilcoxon Ranksum test. Cohort 1 includes 22 AIN participants and 105 without AIN; cohort 2 includes 10 AIN participants, and 81 without AIN. Maroon line represents median value. AIN, acute interstitial nephritis; TNF, tumor necrosis factor; IL, interleukin, Cr, creatinine.

Supplementary Figure 2. Post-test probabilities of acute interstitial nephritis at a range of pre-test probabilities at two cut-offs of tumor necrosis factor-α



Area under receiver operating characteristics curve (AUC) for outcome of acute interstitial nephritis (AIN) vs. all causes of acute kidney disease (panel A) and AIN vs. acute tubular injury (panel B). Post-test probability of AIN at a range of pre-test probabilities at TNF-α cut-off equal to median (panels C and E) and top 15% values (panels D and F). Top 15% cut-off was chosen based on 15% prevalence of AIN in cohort.

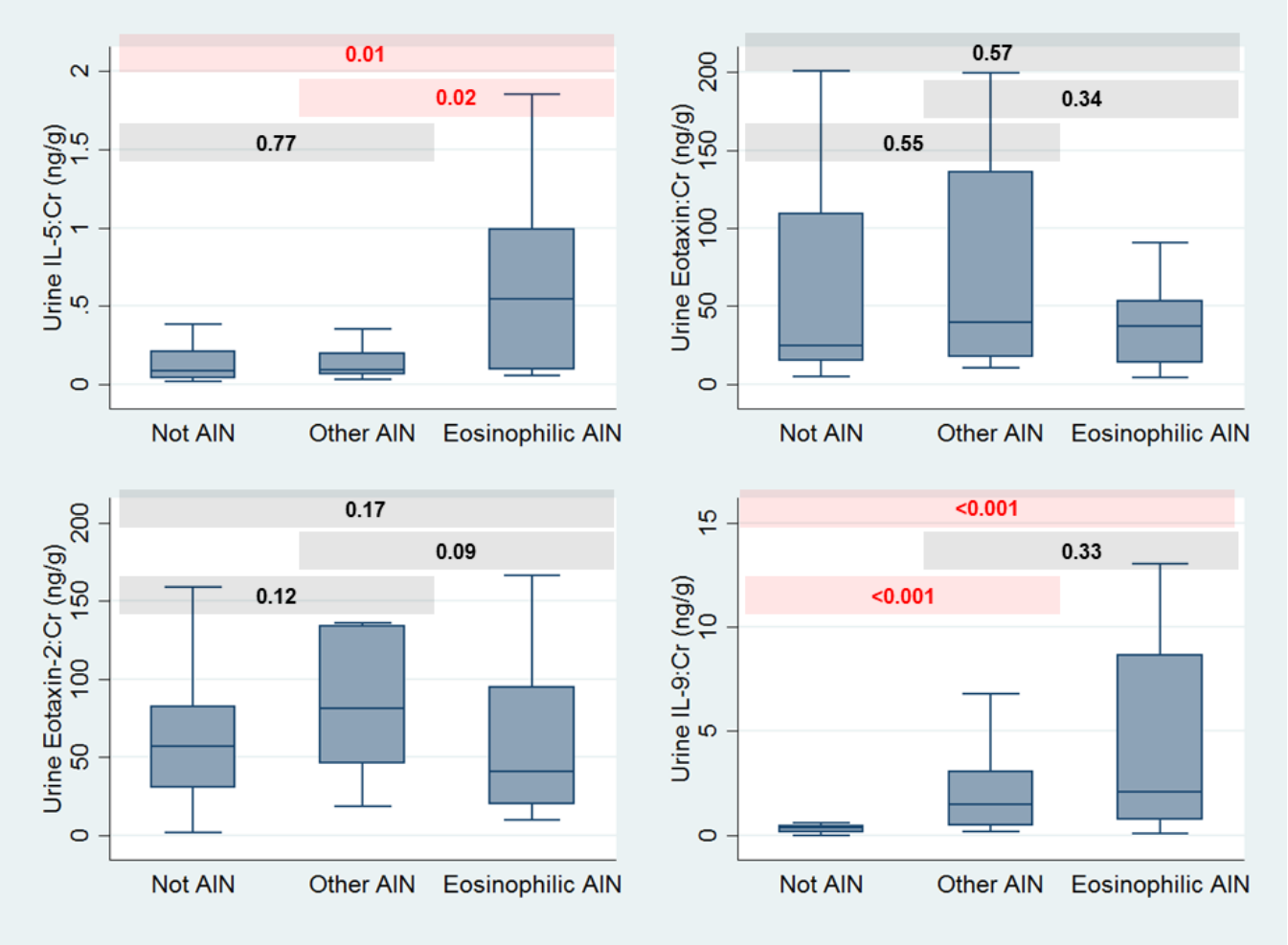
Supplementary Figure 3. Colocalization of TNF-α and FCER1 staining cells in patients with acute interstitial nephritis



TNF-α	FcER1	N
Pos.	Pos.	24
Pos.	Neg.	61
Neg.	Pos.	16
Total	N/A	101

Shown are high magnification (40x) representative images of 5µm adjacent sections stained as follows: Right panel: FCERI staining and left Panel: TNFa staining. Arrow heads denote cells which stain positive for both markers. Boxed inset shows a group of TNFa-positive, FCERI-negative cells. G: glomerulus. Table denotes results of 101 cells counted from AIN participants in whom sections were aligned (n=4)

Supplementary Figure 4. Association of eosinophil-related cytokine and chemokines in AIN



Median (horizontal line), 25th and 75th percentile (box), and 5th and 95th percentile (whiskers) of biomarkers are shown. Both urine biomarkers are normalized to urine creatinine and shown in pg/mg. Wilcoxon rank sum test comparing Eosinophilic AIN (n=16) to Non-eosinophilic AIN (n=16), non-eosinophilic AIN to non-AIN (n=24), and Kruskal Wallis test comparing biomarker levels between the three groups.