SUPPLEMENTARY MATERIAL

Title: A Prediction Model for Severe AKI in Critically Ill Adults That Incorporates Clinical and Biomarker Data

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Additional Methods

Methods for Biomarker Analysis

Blood for plasma biomarker measurements in both groups was collected during the first 24-48 hours of study enrollment. Biomarkers were measured using electrochemiluminescent immunoassays (Meso Scale Discovery, Rockville, MD) or an enzyme-linked immunosorbent assay. The blood was collected in EDTA-treated sterile tubes and centrifuged. Plasma was then aliquoted and frozen at -80°C. The samples were stored for different durations but they were thawed simultaneously and only once for running the biomarker measurements for this study. The biomarkers were measured for research purposes. Samples were diluted to fit within the dynamic range of each assay. Samples were measured in singlets in the discovery group and doublets in the validation group. Samples that fell below the lower limit of detection or above the upper limit of detection were assigned the value of the lowest standard or the highest standard multiplied by the dilution factor, respectively. As an additional quality control measure, we freeze/thawed a random subset of these samples and re-measured all analytes. The replication results were excellent with averaged Pearson Correlation for all assays at 0.95 with a standard deviation of 0.06 (data not shown). The intra-assay coefficients of variation ranged from 3.8 to 5.8 for the biomarker measurements in all cohorts. The inter-assay coefficients of variation ranged from 5.0 to 7.6.

SIRS Criteria

Adult patients admitted to an ICU who met two or more criteria (temperature >38 C or < 36 C, 2) heart rate > 90 beats per minute, 3) respiratory rate > 20 breaths per minute or arterial pCO_2 < 32 mmHg, 4) white blood cell count > 12,000 mm^3, <4,000 mm^3 or >10% immature (band) forms) for the systemic inflammatory response syndrome (SIRS) were prospectively enrolled.

Cohorts

In the derivation and internal validation cohorts the study was approved by the University of Washington Human Subjects Research Committee who granted a waiver of consent. In the external validation cohort the study was approved by the Massachusetts General Hospital Human Subjects Research Committee and signed consent was obtained from each patient or legal surrogate.

Supplemental Table 1. Number of subjects above or below the limit of detection for each biomarker

Biomarker	Below LLOD* (n)	Above ULOD** (n)
G-CSF	0	23
IL-6	0	33
IL-8	0	5
sFas	0	0
sTNFR-1	2	23
Ang-1	0	0
Ang-2	0	59

*LLOD – lower limit of detection

**ULOD – upper limit of detection

Supplemental Table 2. Risk of 28-day mortality with severe AKI within 72 hours after study enrollment in the Derivation cohort

				Odds Ratio (95% CI)	
		Total, n	**Deaths, n (%)**	**Unadjusted Model**	**Adjusted Model***
Derivation	No Severe AKI	687	70 (10)	Reference	Reference
	Severe AKI	62	17 (27)	3.3 (1.8 -6.1)	3.5 (1.8 – 6.7)

*Adjusted for basic demographics including age, gender, race/ethnicity, body mass index

Supplemental Table 3: Model performance of individual variables in Derivation, Internal Validation and External Validation cohorts for severe AKI within 72 hours after study enrollment

	Derivation	**Internal Validation**	**External Validation**
	C statistic (95% CI)	C statistic (95% CI)	C statistic (95% CI)
Age only	0.58 (0.51-0.65)	0.59 (0.48-0.69)	0.55 (0.41-0.68)
Cirrhosis only	0.52 (0.50-0.55)	0.50 (0.44-0.55)	0.49 (0.42-0.54)
sTNFR-1 only	0.93 (0.89-0.97)	0.88 (0.78-0.95)	0.92 (0.87-0.96)

Supplemental Table 4. Univariate performance of each biomarker in the Derivation Cohort to Predict Severe AKI within 72 hours after Study Enrollment.

	Discovery
	C statistic (95% CI)
IL-6	0.63 (0.56-0.70)
IL-8	0.65 (0.58-0.71)
G-CSF	0.55 (0.48 – 0.63)
sTNFR-1	0.93 (0.90-0.97)
sFas	0.86 (0.80-0.91)
Ang-1	0.72 (0.65-0.78)
Ang-2	0.79 (0.73-0.85)

Supplemental Table 5: Model Performance in Derivation, Internal Validation and External Validation Cohorts for severe AKI within 7 days after study enrollment

	APACHE III C statistic (95% CI)	Baseline SCr C statistic (95% CI)	LASSO[a] C statistic (95% CI)	ACT[b] C statistic (95% CI)	ACT vs. APACHE III *P*-value	ACT vs. Baseline SCr *P*-value
Discovery	0.70 (0.62-0.77)	0.90 (0.85-0.95)	0.95 (0.92-0.97)	0.94 (0.91-0.97)	<0.001	0.08
Internal Validation	0.60 (0.49 - 0.71)	0.81 (0.68-0.92)	0.87 (0.78-0.95)	0.88 (0.79-0.95)	<0.001	0.10
External Validation	0.71 (0.58-0.82)	0.83 (0.69-0.94)	-	0.88 (0.78-0.94)	0.003	0.32

[a]LASSO model includes age, sex, medical source of ICU admission, smoking, diabetes mellitus, chronic kidney disease, cirrhosis, BMI, Ang-1, Ang-2/Ang-1, IL-8, IL-6, sFAS, and sTNFR-1
[b]ACT includes age, cirrhosis, and sTNFR-1.
APACHE III, acute physiology and chronic health evaluation. SCr, serum creatinine. Chronic kidney disease unavailable in the External Validation cohort to calculate LASSO C-statistic.

Supplemental Table 6: Model Performance in Derivation, Internal Validation and External Validation cohorts for severe AKI within 72 hours after study enrollment in patients with sepsis

	APACHE III C statistic (95% CI)	Reference SCr C statistic (95% CI)	LASSO C statistic (95% CI)	ACT C statistic (95% CI)	ACT vs. APACHE III *P*-value	ACT vs. Reference SCr *P*-value
Discovery	0.67 (0.57-0.76)	0.93 (0.88-0.97)	0.96 (0.93-0.98)	0.96 (0.93-0.98)	<0.001	0.11
Internal Validation	0.57 (0.40-0.73)	0.87 (0.74-0.98)	0.93 (0.85-0.98)	0.93 (0.87-0.98)	<0.001	0.21
External Validation	0.71 (0.57-0.83)	0.92 (0.81-0.99)	-	0.93 (0.89-0.96)	0.001	0.88

[a]LASSO model includes age, sex, white race, smoking, diabetes mellitus, chronic kidney disease, cirrhosis, Ang-2/Ang-1, IL-8, IL-6, sFAS, sTNFR-1, and sVCAM.
[b]ACT includes age, cirrhosis, and sTNFR-1
APACHE III, acute physiology and chronic health evaluation. SCr, serum creatinine. Chronic kidney disease unavailable in the External Validation cohort to calculate LASSO C-statistic.

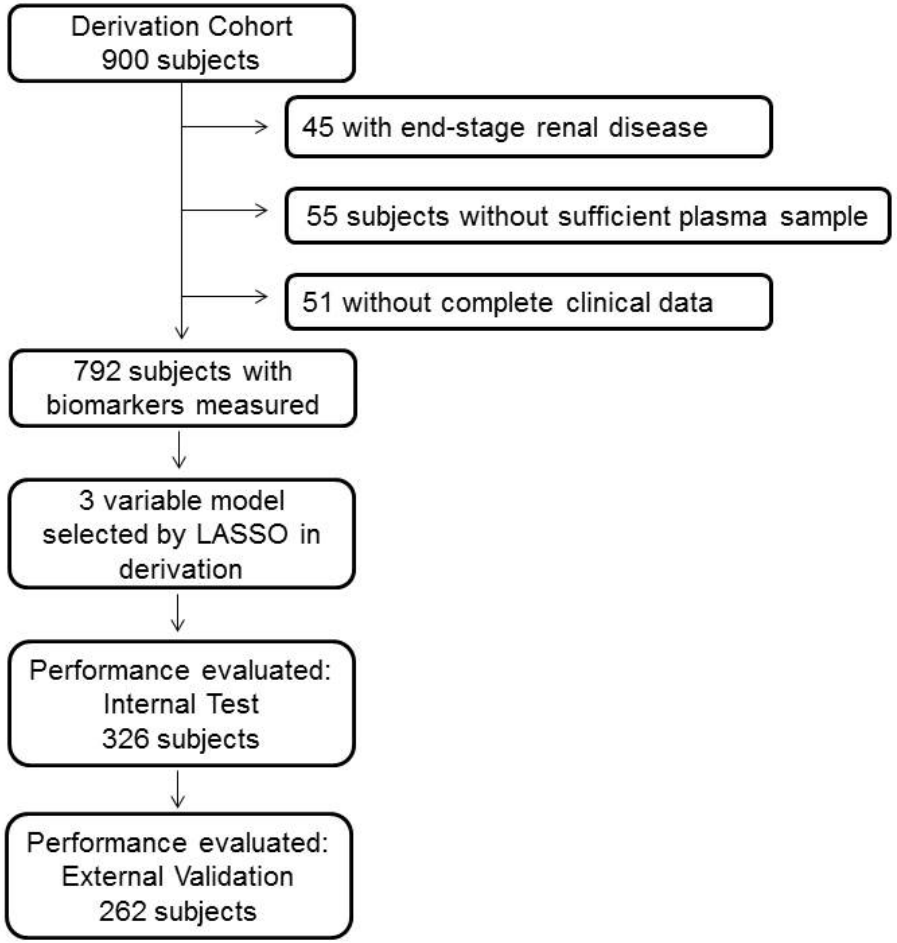
Supplemental Table 7: Negative and positive predictive values for the ACT model and severe AKI within 7 days after study enrollment

Cohort	Performance Goal	Patients Above/Below Threshold (N)	Proportion of Patients with Severe AKI, n (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Derivation	Maximizing NPV	37/712	67 (9)	0.46 (0.34-0.58)	0.99 (0.98-0.99)	0.84 (0.72-0.94)	0.95 (0.94-0.96)
	Maximizing PPV	17/732	67 (9)	0.24 (0.13-0.34)	0.99 (0.99-1.00)	0.94 (0.81-1.00)	0.93 (0.92-0.94)
Internal validation	Maximizing NPV	16/310	30 (9)	0.40 (0.23-0.57)	0.99 (0.97-0.99)	0.75 (0.54-0.94)	0.94 (0.93-0.96)
	Maximizing PPV	7/319	30 (9)	0.20 (0.07-0.33)	0.99 (0.99-1.00)	0.88 (0.50-1.00)	0.92 (0.91-0.94)
External validation	Maximizing NPV	11/245	21 (8)	0.33 (0.14-0.52)	0.98 (0.97-0.99)	0.64 (0.38-0.91)	0.94 (0.93-0.96)
	Maximizing PPV	2/254	21 (8)	0.05 (0.00-0.14)	0.99 (0.99-1.00)	0.50 (0.00-1.00)	0.92 (0.92-0.93)

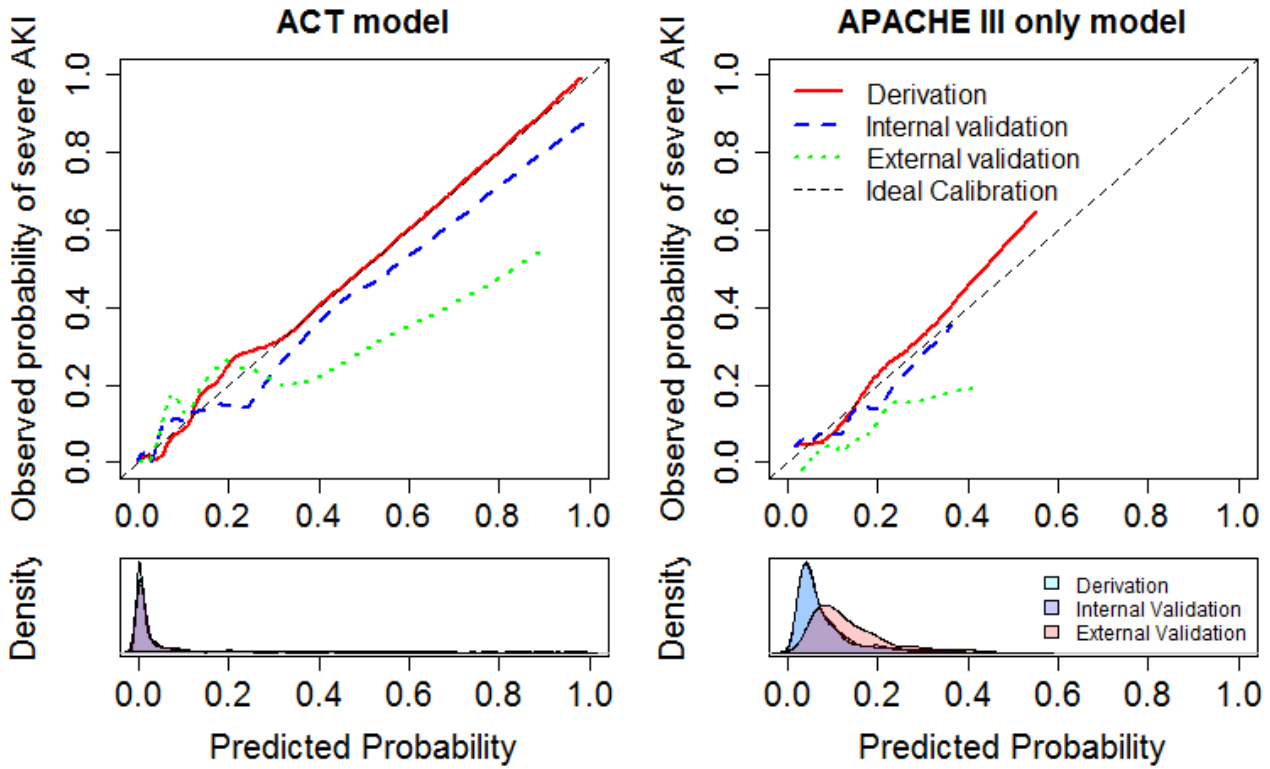
Supplemental Table 8. Risk of severe AKI within 72 hours after study enrollment. (ACT Model, Derivation Cohort)

		Predicted Probability Thresholds					
	All Patients	**5%**	**10%**	**20%**	**40%**	**60%**	**80%**
Proportion of patients predicted to have severe AKI (%)	100	23	17	12	8	5	3
Sensitivity, % (95% CI)	NA	94 (87-98)	92 (85-98)	84 (74-92)	65 (52-76)	47 (34-60)	32 (21-44)
Specificity, % (95% CI)	NA	84 (81-86)	90 (87-92)	94 (93-96)	98 (96-99)	99 (98-100)	99 (99-100)
Positive Predictive Value, % (95% CI)	NA	34 (30-39)	45 (39-50)	58 (51-66)	70 (60-81)	83 (71-94)	95 (85-100)
Negative Predictive Value, % (95% CI)	NA	99 (99-100)	99 (99-100)	98 (98-99)	97 (96-98)	95 (94-96)	94 (93-95)

Supplemental Figure 1. Patient flow diagram for severe AKI prediction model. Subject number for the derivation, internal test and validation cohorts.



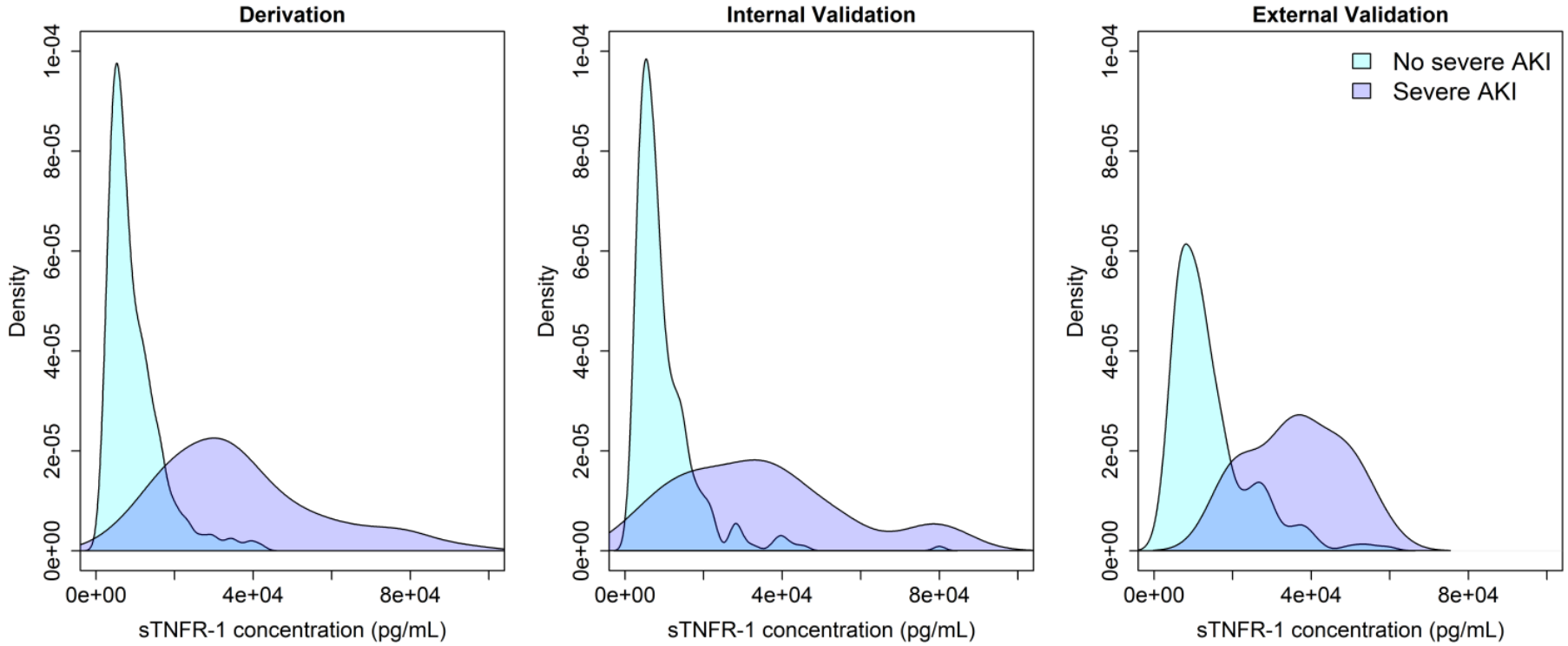
Supplemental Figure 2. Calibration plots for the ACT model and APACHE III scores.



	Derivation cohort (p-value)[a]	Internal validation cohort (p-values)	External validation cohort (p-values
Prediction Models			
APACHE III	0.14	0.31	0.57
ACT	0.43	0.46	0.29

[a]p-values from the Cessie-van Houwelingen goodness of fit test are testing the null hypothesis that models are appropriately calibrated.

Supplemental Figure 3. Distribution of sTNFR-1 plasma concentrations by severe AKI status.



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