Appendix

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Title: Reduced urinary levels of angiotensin-converting enzyme 2 activity predict acute kidney injury in the critically ill

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Supplemental Methods

1. Modified STROBE Statement

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract Reported in the abstract (page 2).
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found Abstract provided on page 2
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Reported in paragraph #2 of the Introduction section (page 3)
Objectives	3	State specific objectives, including any prespecified hypotheses Reported in the last 2 sentences of the Introduction section (page 3)
Methods		
Study design	4	Present key elements of study design early in the paper Reported in the first sentence of the Methods section (page 4)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Reported in the "Study cohort" paragraph of Methods (page 4)
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Cohort study: Reported in the "Study cohort" paragraph of Methods (page 4)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Reported in the "Acute kidney injury" and the "Methodology of acute renal function assessment" sections of Methods (pages 6 and 7)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Reported in the "Methodology of acute renal function assessment" section of Methods (page 7)
Bias	9	Describe any efforts to address potential sources of bias

		Reported in the "Methodology of acute renal function assessment" section of Methods (page 7)
Study size	10	Explain how the study size was arrived at (if applicable) Convenient sample of 105 patients, reported in the first sentence of the "Study cohort" section of Methods, page 4, and justified in the Statistical Methods section on page 8.
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Reported in the third sentence of the Statistical Methods section (pages 7 and 8)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding A Statistical Methods paragraph is included (starts on page 7)
		(*b*) Describe any methods used to examine subgroups and interactions Subgroups are specified in the Statistical paragraph (page 8)
		(*c*) Explain how missing data were addressed Reported in the paragraphs describing AKI adjudication and uACE2 measurements
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed Not applicable, the primary outcome being assessed at 12 hours of inclusion.
		(*e*) Describe any sensitivity analyses Reported in the second sentence of the second paragraph of the statistical section (starts on page 7)
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed Reported in the Flow diagram (Figure 1), in the first sentence of the Results section, in all tables and in the "Acute kidney injury incidence and severity section" section of results (page 8)
		(c) **Use of a flow diagram** Reported in Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Reported in the first paragraph of the Results section (page 8), and in Table 1
		(b) Indicate number of participants with missing data for each variable of interest Missing data of the main measurement and the primary outcome are reported in the first sentence of the "Description of urinary ACE2" and "Acute kidney injury incidence and severity" sections of Results (page 8), respectively, and in the footnote of Table 3 (in relation with variables included in the multivariate models).
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) Reported in the footnote of Figure 4
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time Reported in the first sentence of the "Acute kidney injury incidence and severity" section (page 8), and in Table 2.

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Reported in the statistical section of Methods (page 7), the last paragraph of the Results section (page 10), and in the footnote of Table 3
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses Reported in Figure 4, and in Figure S2
Discussion		
Key results	18	Summarise key results with reference to study objectives Reported in the first paragraph of the Discussion (page 11)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Discussed on page 13 in the “Strengths and limitations” section
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Cautious interpretation and generalizability of results is discussed throughout the Discussion section
Generalisability	21	Discuss the generalisability (external validity) of the study results Discussed on page 13 in the “Strengths and limitations” section

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

2. STARD Checklist

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	Page 1
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	Page 2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	Page 3
	4	Study objectives and hypotheses	Page 3
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	Page 4
Participants	**6**	Eligibility criteria	Page 4
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	Page 4
	8	Where and when potentially eligible participants were identified (setting, location and dates)	Page 4
	9	Whether participants formed a consecutive, random or convenience series	Pages 4 and 8
Test methods	**10a**	Index test, in sufficient detail to allow replication	Page 5
	10b	Reference standard, in sufficient detail to allow replication	Page 6
	11	Rationale for choosing the reference standard (if alternatives exist)	Page 6
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	Page 8
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	Page 6
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	Page 6
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	Page 6
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	Page 8
	15	How indeterminate index test or reference standard results were handled	Page 6
	16	How missing data on the index test and reference standard were handled	Pages 6 and 8 – no imputation
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	Page 8
	18	Intended sample size and how it was determined	Page 8

RESULTS			
Participants	**19**	Flow of participants, using a diagram	Figure 1
	20	Baseline demographic and clinical characteristics of participants	Table 1
	21a	Distribution of severity of disease in those with the target condition	Page 9 and Table 2
	21b	Distribution of alternative diagnoses in those without the target condition	Not reported
	22	Time interval and any clinical interventions between index test and reference standard	Page 6 – predefined fixed interval
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Table S3
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	Page 9 & 10, Figure 3
	25	Any adverse events from performing the index test or the reference standard	Not reported
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	Page 13
	27	Implications for practice, including the intended use and clinical role of the index test	Page 12
OTHER INFORMATION			
	28	Registration number and name of registry	Not reported
	29	Where the full study protocol can be accessed	Page 16
	30	Sources of funding and other support; role of funders	Page 16

Supplemental Figures

Figure S1

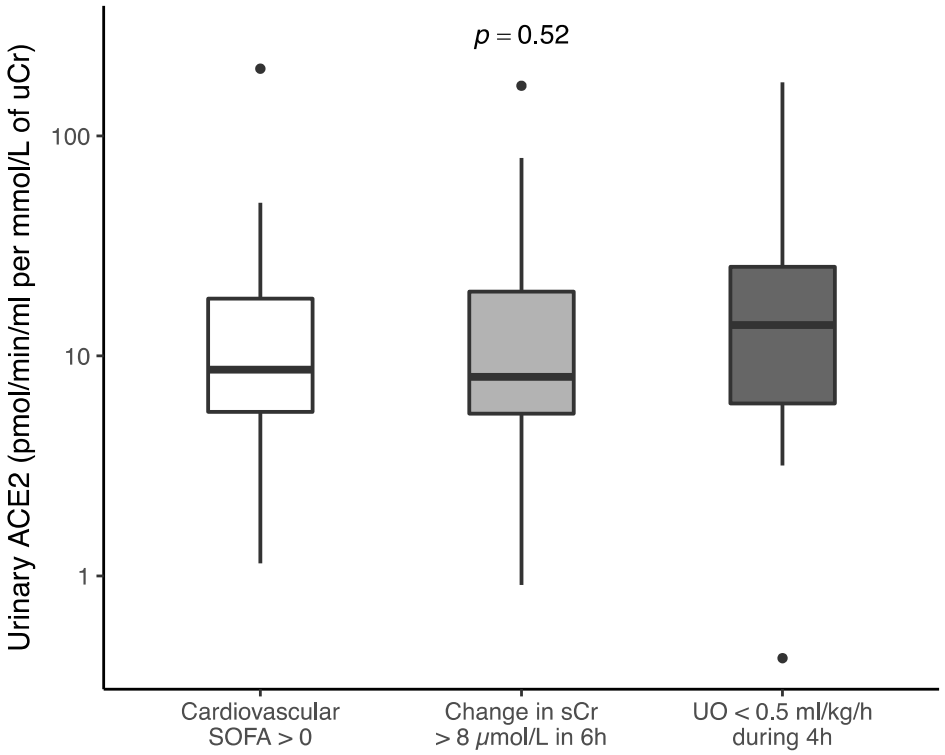

Figure S1. Urinary ACE2 levels in the critically ill cohort, based on inclusion criteria. The *p* value examines the overall difference across the 3 inclusion criteria. The only patient enrolled using the respiratory SOFA criterion was excluded from this analysis. The *y* axis was log-transformed to improve figure readability. ACE2: angiotensin-converting enzyme type 2; ICU: intensive care unit; SOFA: sepsis-related organ failure score; sCr: serum creatinine; uCr: urinary creatinine; UO: urine output.

Figure S2

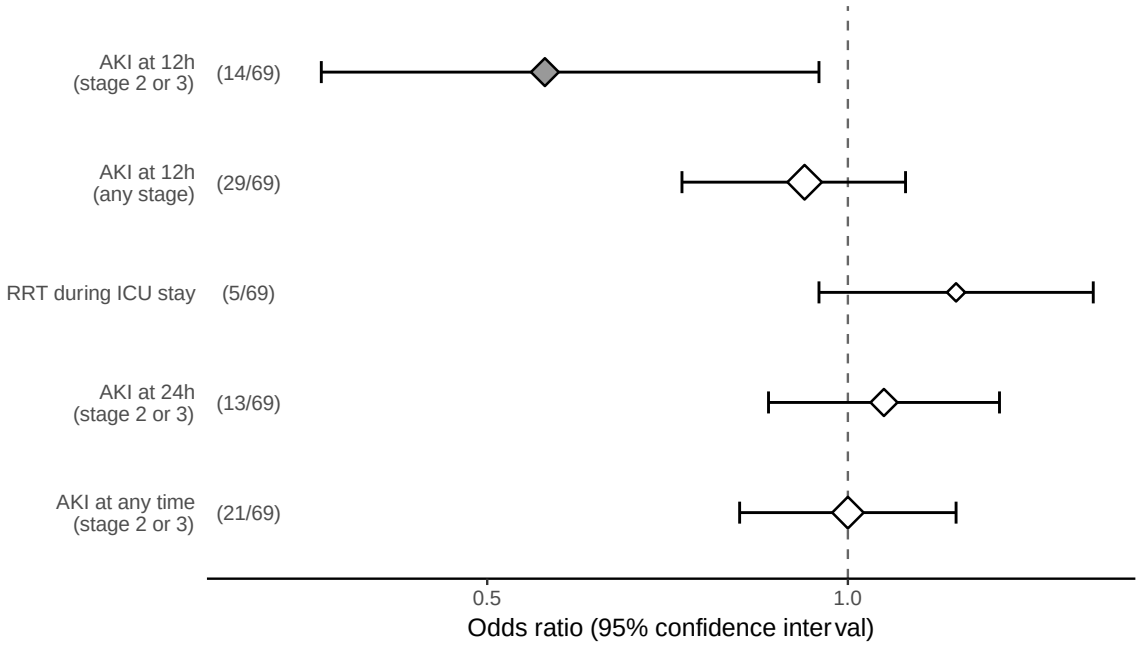

Figure S2. Association of urinary ACE2 levels with study outcomes, after exclusion of patients with oliguria at time of urine collection.
Oliguria was defined as a urine output <0.5 ml/kg/h over the 6h preceding urine collection. The figure shows the unadjusted odds ratio (with its 95% confidence interval) for the primary outcome (AKI stage 2 or 3 at 12h) and the secondary outcomes, for an increase in uACE2 levels of 10 pmol/min/ml per mmol/L of uCr. The size of the diamond is proportional to the number of events (also indicated in parenthesis). AKI: acute kidney injury; ICU: intensive care unit; RRT: renal replacement therapy; uACE2: angiotensin-converting enzyme type 2; uCr: urinary creatinine.

Supplemental Tables

Table S1. Distribution and frequency of inclusion criteria based on primary outcome

	AKI– N = 73	AKI+ N = 32	p value
Number of co-existing inclusion criteria			**0.03**
1	29 (40%)	8 (25%)	
2	29 (40%)	10 (31%)	
3	13 (18%)	11 (34%)	
4	2 (3%)	3 (9%)	

AKI– corresponds to AKI stage 0 or 1 at 12h, and AKI+ to AKI stage 2 or 3 at 12 hours of urine collection. AKI: acute kidney injury; sCr: serum creatinine; SOFA: sepsis-related organ failure score; UO: urine output

Table S2. Corrected urinary ACE2 levels in the critically-ill cohort and the healthy volunteer population.

	ICU study cohort N=105	Healthy volunteers N=10	p value
uACE2 (pmol/min/ml per mmol/L of uCr)	8.7 [5.4; 19.1]	7.5 [3.2; 10.2]	0.13

Data is shown as median with interquartile range in brackets.
ICU: intensive care unit; uCr: urinary creatinine; uACE2: urinary angiotensin-converting enzyme type 2

Supplemental Table S3. Comparison of urine ACE2 values between AKI and non-AKI patients, based on inclusion criteria.

Inclusion criteria	Severe AKI	uACE2 (pmol/min/ml per mmol/L of uCR)	P value
Cardiovascular SOFA ≥ 1 (N=55)	41	18.6	0.08
	14	8.2	
Respiratory SOFA ≥ 2 (N=1)	1	4.3	Not
	0	NA	applicable
Increase in sCr > 8 µmol/L over 6h (N=25)	16	28.9	0.03
	9	11.6	
Urine output < 0.4 ml/kg over 4h (N=24)	15	29.0	0.32
	9	14.2	

The P value (Wilcoxon Mann Whitney test) compares the uACE2 value between non-AKI and AKI patients within each inclusion category.
sCr: serum creatinine; SOFA: sepsis-related organ failure score; uACE2: urinary levels of angiotensin-converting enzyme type 2; uCr: urine creatinine

Table S4. Cross tabulation of the optimal corrected uACE2 cutoff with the primary outcome

	AKI –	AKI +	Total
uACE2 < 12.8 pmol/min/ml per mmol/L of uCr	36	26	61
uACE2 ≥ 12.8 pmol/min/ml per mmol/L of uCr	37	6	44
Total	73	32	105

Sensitivity and specificity are reported in the main body of the manuscript. Other

parameters were (reported with 95% confidence interval):

- Positive predictive value: 0.42 (0.30 to 0.55)
- Negative predictive value: 0.86 (0.72 to 0.95)
- Positive likelihood ratio: 1.65 (1.24 to 2.19)
- Negative likelihood ratio: 0.37 (0.17 to 0.79)

AKI: acute kidney injury; uCr: urinary creatinine; uACE2: urinary angiotensin-converting enzyme type 2