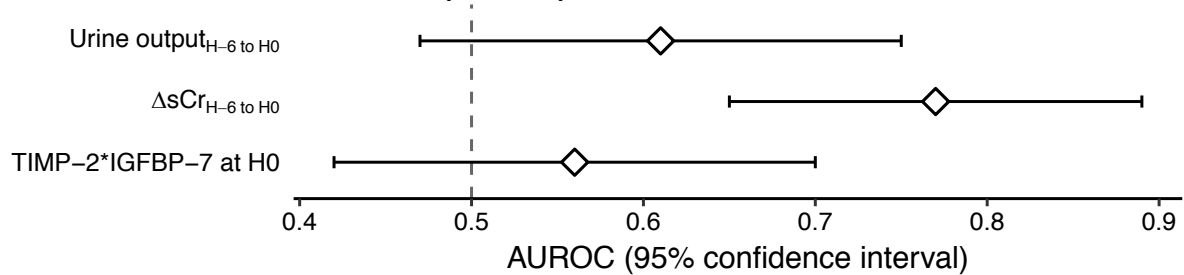


APPENDIX

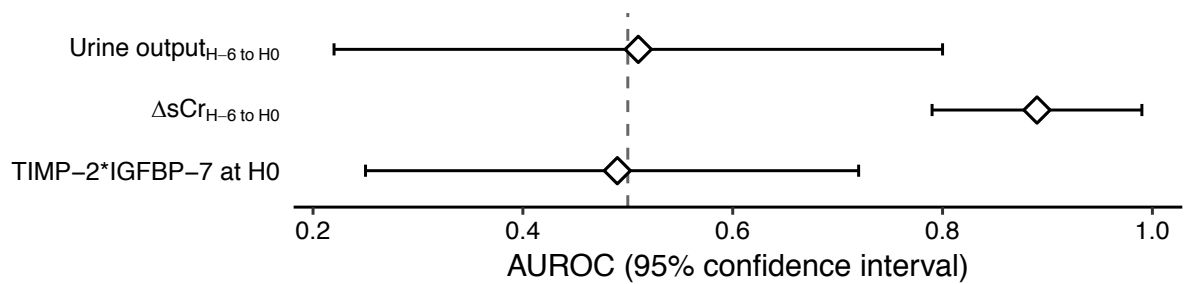
This appendix was part of the submitted manuscript and has been peer reviewed. It is posted as supplied by the authors.

Supplemental Figure 1

A. Severe AKI at 24h (N=19)



B. Renal replacement therapy (N=7)



Supplemental Figure 1. Predictive performance of studied biomarkers for secondary outcomes in all patients.

The figure shows the AUROC (with 95% confidence interval) of all studied biomarkers for the prediction of severe AKI at 24h of inclusion (panel A), and RRT during ICU admission (panel B). The dotted line represents the normal intercept, below which a biomarker performance is deemed non-valuable. H₀ represents time of inclusion, and corresponds to the time at which urine was sampled for TIMP-2 and IGFBP-7 measurements. H-6 corresponds to the 6 hours preceding inclusion.

AUROC: area under the receiver operator characteristics curve; $\Delta sCr_{H-6,H0}$: change in serum creatinine between H-6 and H₀; IGFBP-7: insulin-like growth factor binding protein type 7; RRT: renal replacement therapy; TIMP-2: tissue inhibitor of metalloproteinase type 2.

Supplemental Table 1. Characteristics of the primary and secondary outcomes				
	Whole cohort	AKI–	AKI+	
	N = 105	N = 73	N = 32	P
Main outcome description				
<i>AKI stages</i>				–
Stage 1	18 (17%)	18 (25%)	0 (0%)	
Stage 2	30 (29%)	0 (0%)	30 (94%)	
Stage 3	2 (2%)	0 (0%)	2 (6%)	
Severe AKI defining criteria (KDIGO)				
Serum creatinine	–	–	2 (6%)	
Oliguria	–	–	25 (78%)	
Both	–	–	5 (16%)	
AKI markers				
Urine output at 12h, ml/kg/h	0.7 [0.5; 1.1]	0.9 [0.7; 1.4]	0.4 [0.3; 0.5]	<0.01
Serum creatinine at 12h, µmol/L	87 [68; 118]	80 [64; 102]	109 [87; 188]	<0.01
Secondary outcomes				
Severe AKI at 24h	21 (20%)	4 (5%)	17 (53%)	–
RRT during ICU admission	7 (7%)	3 (4%)	4 (12%)	–
AKI: acute kidney injury; KDIGO: kidney disease: improving global outcome guidelines; RRT: renal replacement therapy				

Supplemental Table 2. Optimal cutoffs of studied biomarkers for the risk prediction of the main outcome							
Variables	Optimal cutoff	Sensitivity	Specificity	PPV	NPV	Positive likelihood ratio	Negative likelihood ratio
Urine output _{H-6,H0}	≤ 0.38 ml/kg/h	0.41 [0.24; 0.59]	0.85 [0.75; 0.92]	0.54 [0.38; 0.72]	0.77 [0.60; 0.87]	2.70 [1.36; 5.36]	0.70 [0.52; 0.95]
ΔsCr _{H-6,H0}	≥ +15 μmol/L	0.34 [0.18; 0.54]	0.85 [0.74; 0.93]	0.53 [0.35; 0.72]	0.74 [0.54; 0.87]	2.38 [1.08; 5.21]	0.77 [0.58; 1.02]
TIMP-2•IGFBP-7	≥ 1.5 (ng/ml) ² /1000	0.41 [0.24; 0.59]	0.85 [0.75; 0.92]	0.54 [0.38; 0.72]	0.77 [0.60; 0.87]	2.70 [1.36; 5.36]	0.70 [0.52; 0.95]
<p>Optimal cutpoints was computed by selecting the value associated with a specificity of at least 0.85, using the ROC plot. 95% confidence intervals are presented between brackets.</p> <p>ΔsCr_{H-6,H0}: change in serum creatinine over 6 hours; IGFBP-7: insulin-like growth factor binding protein type 7; PPV: positive predictive value; NPV: negative predictive value; ROC: receiver operating characteristics; TIMP-2: tissue inhibitor of metalloproteinase type 2.</p>							

Supplemental Table 3. Association of novel and conventional biomarkers measured at inclusion with secondary outcomes				
Variables	Severe AKI at 24h N = 19		RRT during ICU admission N = 7	
	Unadjusted OR [95% CI]	P	Unadjusted OR [95% CI]	P
Urine output _{H-6,H0} , per 1 ml/kg/h increase	0.66 [0.22; 1.32]	0.36	1.17 [0.39; 2.19]	0.68
Δ sCR _{H-6,H0} , per 1 μ mol/L increase	1.09 [1.04; 1.15]	<0.01	1.11 [1.04; 1.19]	<0.01
TIMP-2•IGFBP-7, per 1 (ng/ml) ² /1000 increase	1.22 [0.92; 1.61]	0.15	1.26 [0.84; 1.77]	0.19

AKI: acute kidney injury; CI: confidence interval; Δ sCr_{H-6,H0}: change in serum creatinine over 6 hours; IGFBP-7: insulin-like growth factor binding protein type 7; OR: odds ratio; TIMP-2: tissue inhibitor of metalloproteinase type 2.

Supplemental Table 4. Characteristics of cohorts A and B			
	Cohort A	Cohort B	
	N = 56	N = 49	P
Baseline characteristics			
<i>Demographics</i>			
Gender, male	31 (55%)	25 (51%)	0.7
Age, year	65 [55; 74]	64 [50; 73]	0.74
Weight, kg	79 [69; 90]	80 [74; 90]	0.10
<i>Admission category</i>			
Elective surgery	19 (34%)	8 (16%)	
Emergent surgery	18 (32%)	13 (27%)	
Medical	19 (34%)	28 (57%)	
<i>Comorbidities</i>			
Hypertension	29 (52%)	24 (49%)	0.85
Diabetes	15 (27%)	7 (14%)	0.15
Ischemic heart disease	20 (36%)	5 (10%)	<0.01
Congestive heart failure	6 (11%)	4 (8%)	0.75
COPD	9 (16%)	7 (14%)	>0.99
<i>Baseline renal function</i>			
Serum creatinine, $\mu\text{mol/L}$	74 [56; 93]	78 [59; 88]	0.97
eGFR, $\text{ml/min}/1.73\text{m}^2$	90 [59; 90]	90 [67; 90]	0.87
Serum creatinine at inclusion, $\mu\text{mol/L}$	88 [72; 127]	87 [71; 112]	0.72
<i>Severity of disease</i>			
APACHE III score	49 [41; 63]	46 [38; 65]	0.92
SOFA score	7 [6; 9]	5 [3; 6]	<0.01
Sepsis	16 (29%)	16 (33%)	0.68
Vasopressor support	53 (95%)	16 (33%)	<0.01
Lactate, mmol/L	2.9 [2; 5.5]	2.4 [1.5; 3]	0.01
Mechanical ventilation	42 (75%)	31 (63%)	0.21
Main outcome description			
<i>AKI stages</i>			
Stage 1	9 (16%)	9 (18%)	
Stage 2	13 (23%)	17 (35%)	
Stage 3	1 (2%)	1 (2%)	
<i>Severe AKI defining criteria (KDIGO)</i>			
Serum creatinine	1 (7%)	1 (6%)	
Oliguria	9 (64%)	16 (89%)	
Both	4 (29%)	1 (6%)	
<i>AKI markers</i>			
Urine output at 12h, ml/kg/h	0.7 [0.5; 1.3]	0.6 [0.5; 1]	0.15
Serum creatinine at 12h, $\mu\text{mol/L}$	88 [72; 135]	87 [67.2; 103.8]	0.53
Increase in serum creatinine at 12h, %	19 [6; 47]	20 [6; 40]	0.56
Secondary outcomes			
Severe AKI at 24h	14 (25%)	7 (14%)	0.22
RRT during ICU admission	5 (9%)	2 (4%)	0.44
AKI risk biomarkers			
Urine output _{H-6,H0} , ml/kg/h	0.8 [0.5; 1]	0.6 [0.4; 1]	0.15
$\Delta\text{sCr}_{\text{H-6,H0}}$, $\mu\text{mol/L}$	2 [-3; 13.5]	5.5 [2.5; 11.2]	0.12
TIMP-2•IGFBP-7, $(\text{ng/ml})^2/1000$	0.5 [0.2; 1.4]	0.4 [0.1; 1.2]	0.35
AKI: acute kidney injury; APACHE: acute physiology, age, chronic health evaluation; COPD: chronic obstructive pulmonary disease; $\Delta\text{sCr}_{\text{H-6,H0}}$: change in serum creatinine over 6 hours; eGFR: estimated glomerular filtration rate; IGFBP-7: insulin-like growth factor binding protein type 7; KDIGO: kidney disease: improving global outcome guidelines; RRT: renal replacement therapy; SOFA: sepsis-related organ failure assessment; TIMP-2: tissue inhibitor of metalloproteinase type 2.			

Supplemental Table 5. Association of studied biomarkers with severe AKI at 12 hours, in cohorts A and B						
	Cohort A N = 56			Cohort B N = 49		
Variables	Unadjusted OR [95% CI]	P	AUROC [95% CI]	Unadjusted OR [95% CI]	P	AUROC [95% CI]
Urine output _{t_{H-6,H0}} , per 1 ml/kg/h increase	0.02 [0.00; 0.24]	0.01	0.79 [0.67; 0.91]	0.32 [0.06; 0.95]	0.10	0.69 [0.53; 0.84]
ΔsCr _{H-6,H0} , per 1 μmol/L increase	1.07 [1.02; 1.14]	0.01	0.69 [0.47; 0.91]	1.04 [0.98; 1.12]	0.18	0.62 [0.44; 0.80]
TIMP-2•IGFBP-7, per 1 (ng/ml) ² /1000 increase	1.34 [0.97; 2.00]	0.10	0.65 [0.48; 0.82]	1.88 [1.15; 3.66]	0.03	0.73 [0.57; 0.88]
<p>The combination of biomarkers was not evaluated because of the low prevalence of secondary outcomes, restricting the acceptable number of variables included in the model. We observed no significant difference in AUROCs for the same biomarker between the 2 cohorts.</p> <p>AKI: acute kidney injury; AUROC: area under the receiver operator characteristics curve; CI: confidence interval; ΔsCr_{H-6,H0}: change in serum creatinine over 6 hours; IGFBP-7: insulin-like growth factor binding protein type 7; OR: odds ratio; TIMP-2: tissue inhibitor of metalloproteinase type 2.</p>						