Supplemental Material for:

Critical illness and systemic inflammation are key risk factors of severe AKI in patients with COVID-19

Running title: Severe AKI in COVID-19

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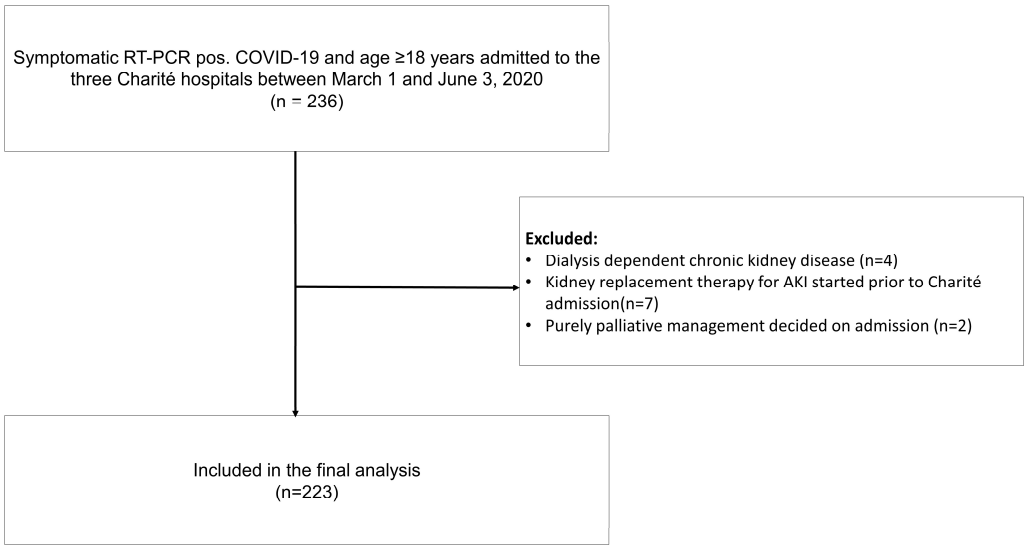
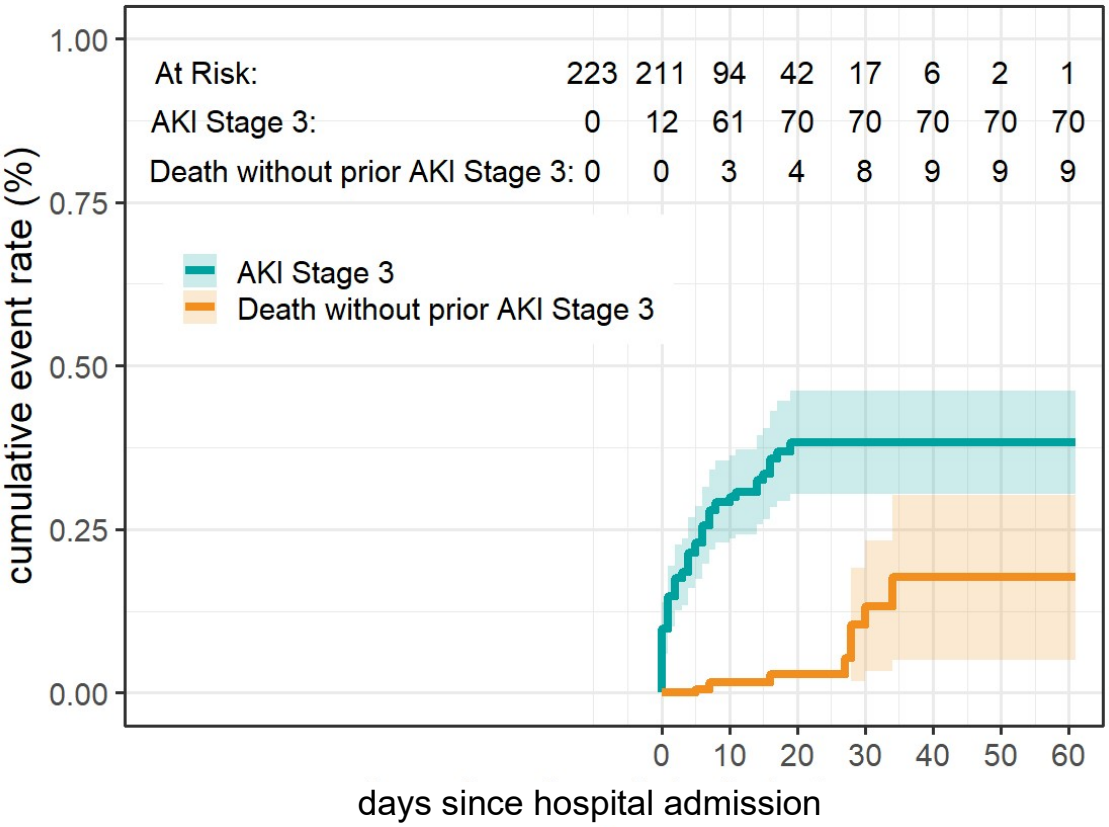
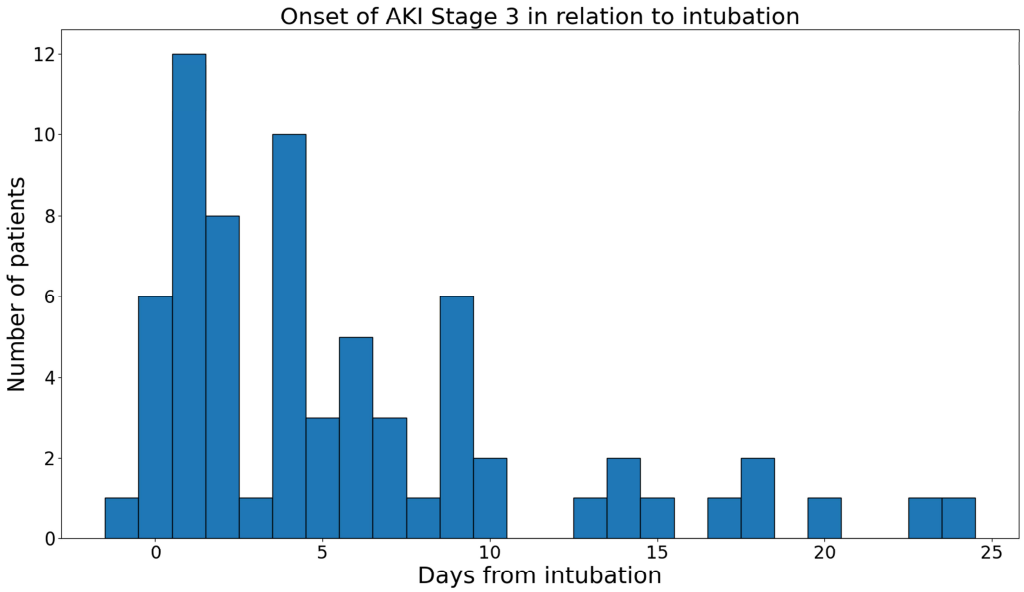


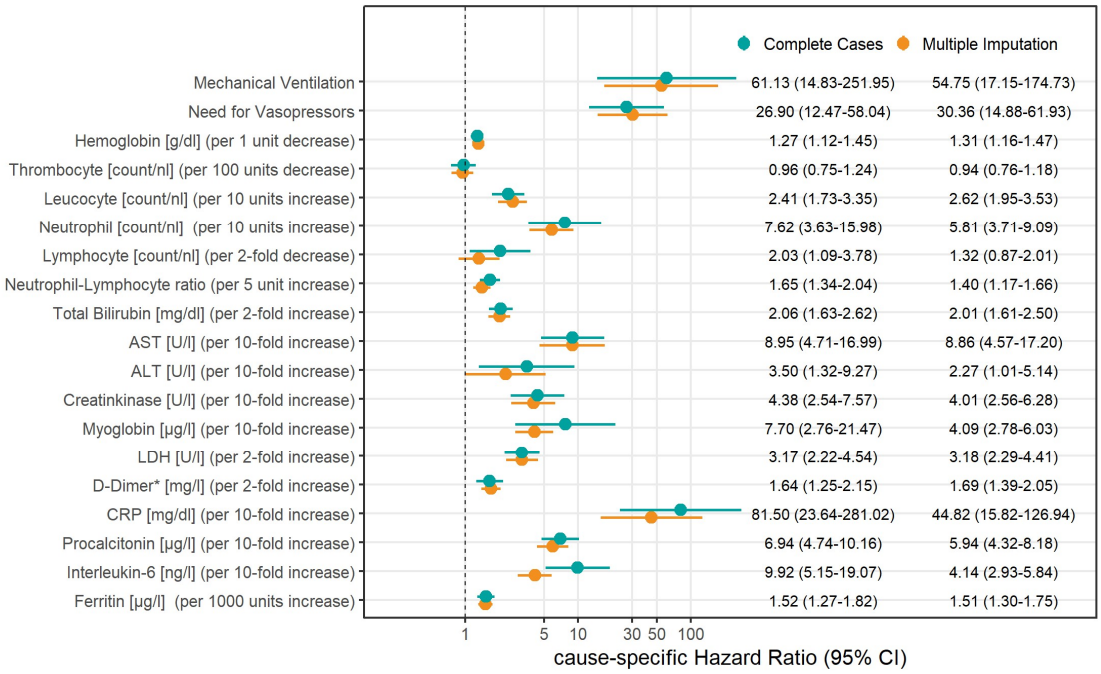
Supplemental Figure 1: Study flowchart
COVID-19, corona virus disease 2019. RT-PCR, reverse transcription polymerase chain reaction



Supplemental Figure 2: Cumulative incidence of AKI Stage 3 (severe AKI) and death without prior severe AKI in COVID-19 patients. Day 0 refers to the day of hospital admission.

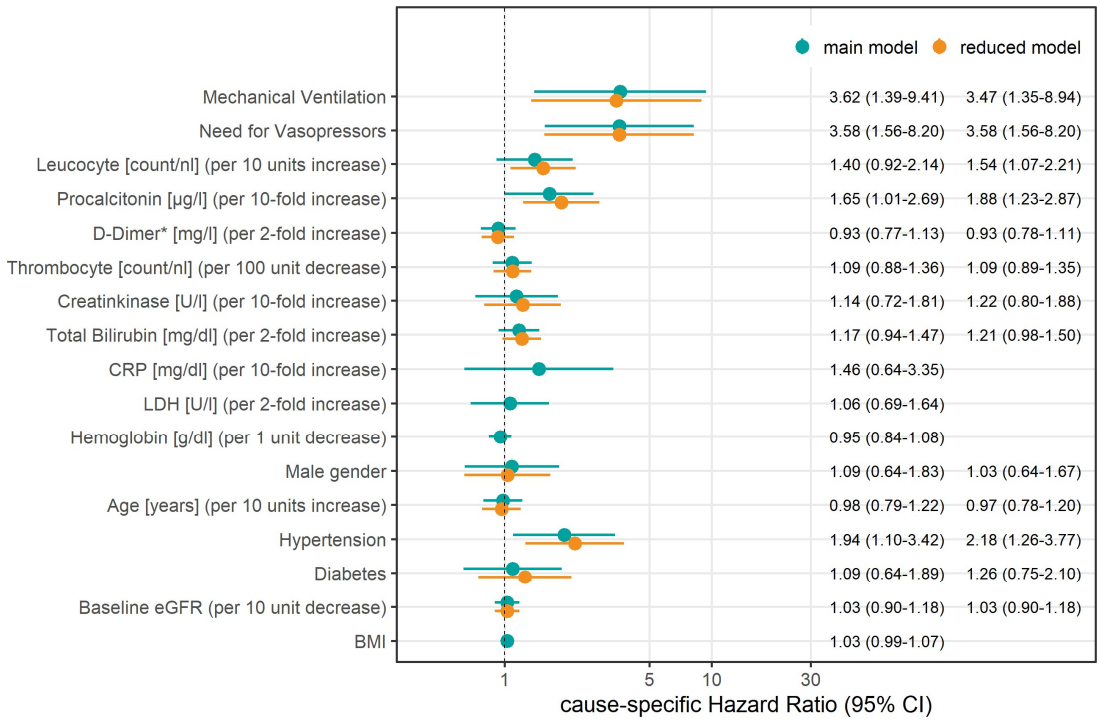


Supplemental Figure 3: Temporal relation of AKI Stage 3 onset and intubation
Day 0 marks the day of intubation.



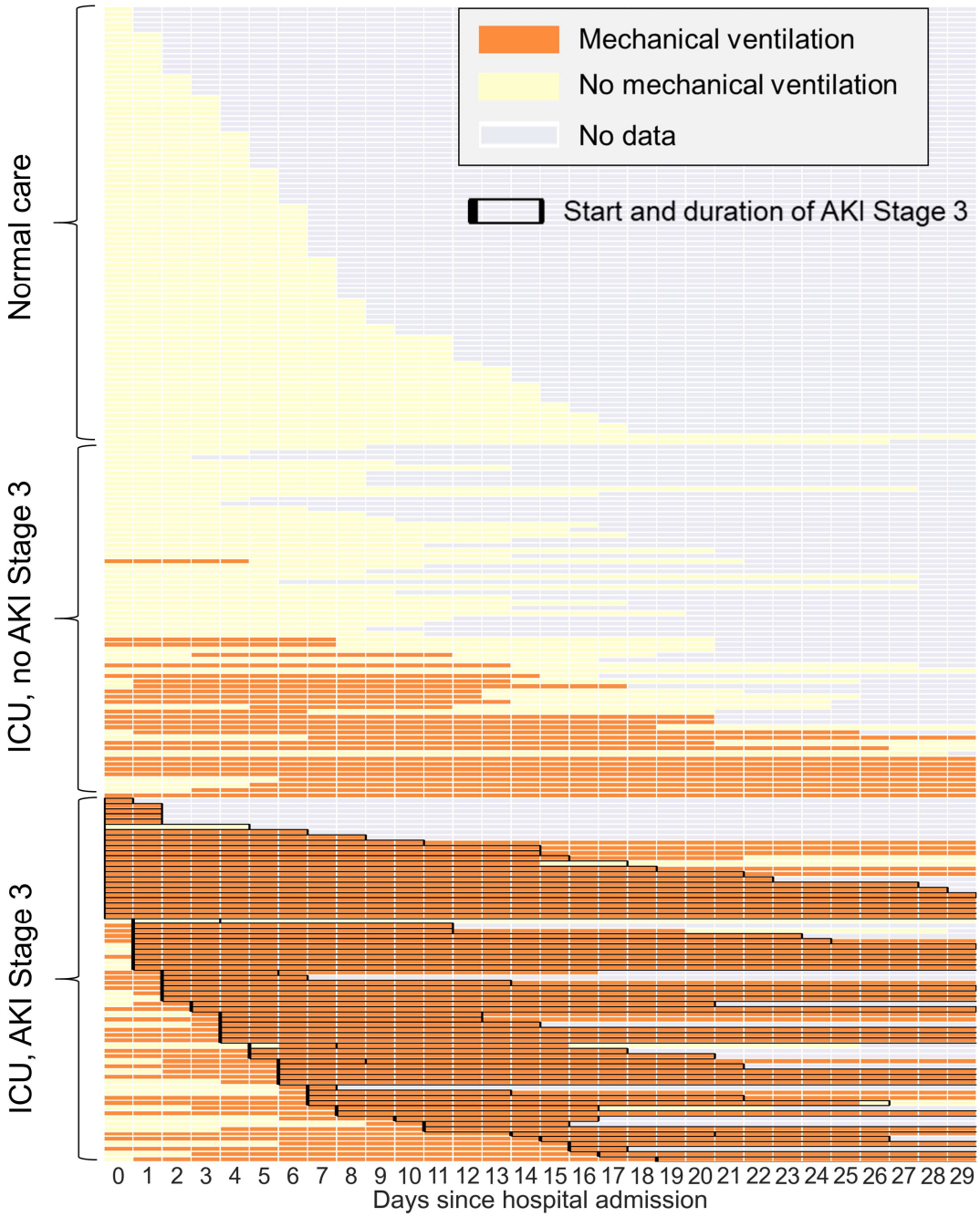
Supplemental Figure 4: Individual minimally adjusted risk model of AKI Stage 3 risk factors with time-varying covariates

Estimates were derived from individual competing risk models with time-varying covariates, adjusted only for baseline characteristics (gender, age, hypertension, diabetes, eGFR, and BMI). Models were either based on multiple imputation or complete cases. Dots indicate hazard ratio estimates, horizontal bars indicate 95% confidence intervals (CI).

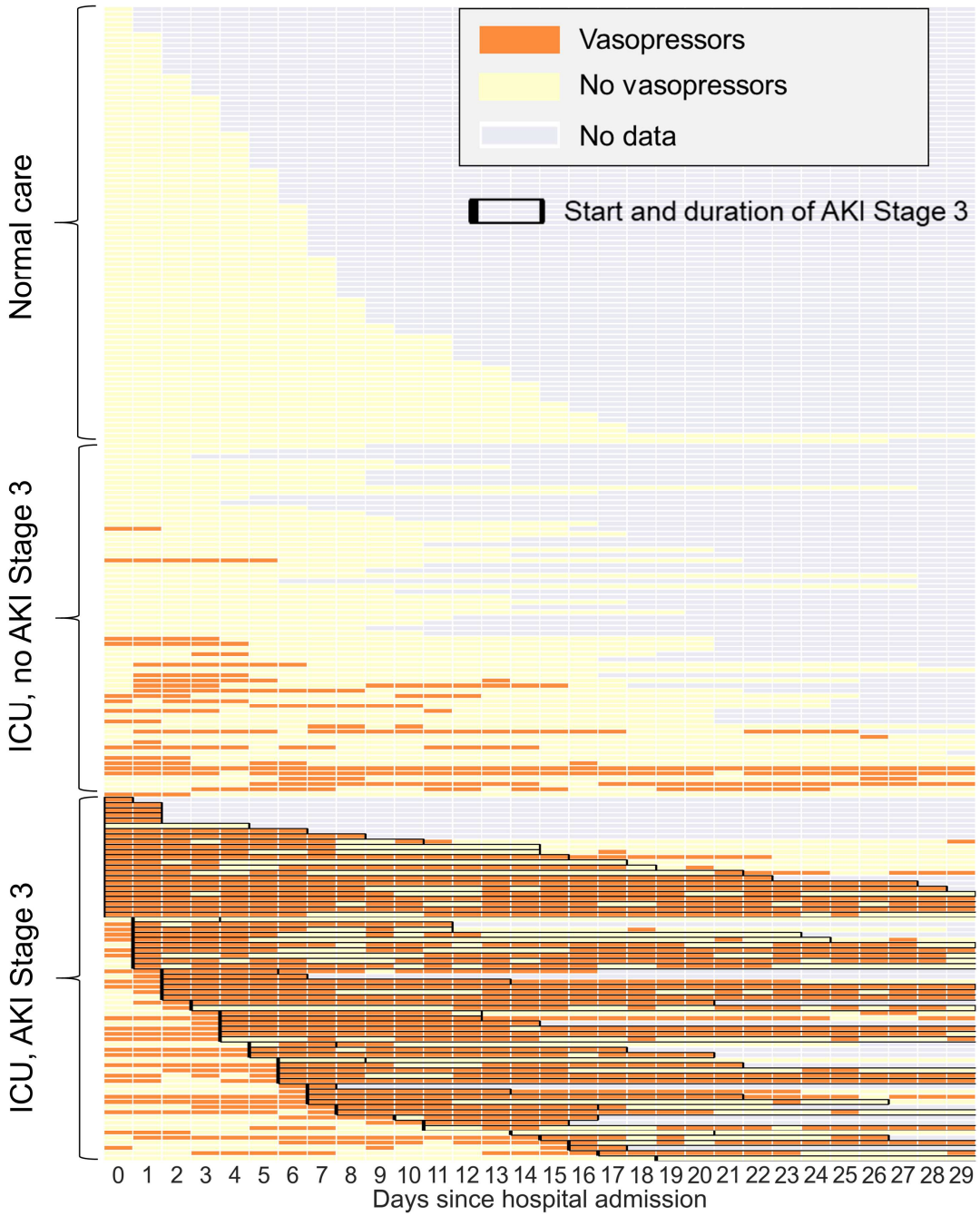


Supplemental Figure 5: Competing risk model with time-varying covariates of AKI Stage 2 or Stage 3 risk factors

The hazards of potential risk factors for time to AKI Stage 2 or Stage 3 were estimated, along with 95% confidence intervals (CI). Estimates were derived from two multivariable competing risk model with time-varying covariates, adjusted for all parameters displayed. Missing data were addressed by multiple imputation. A main multivariate model (including 11 time-varying risk factor covariates) and a reduced model (including 8 time-varying risk factor covariates) were fitted to account for partially overlapping clinical information content of the time-dependent risk factors of AKI Stage 3. Dots indicate hazard ratio estimates, horizontal bars indicate 95% confidence intervals (CI).

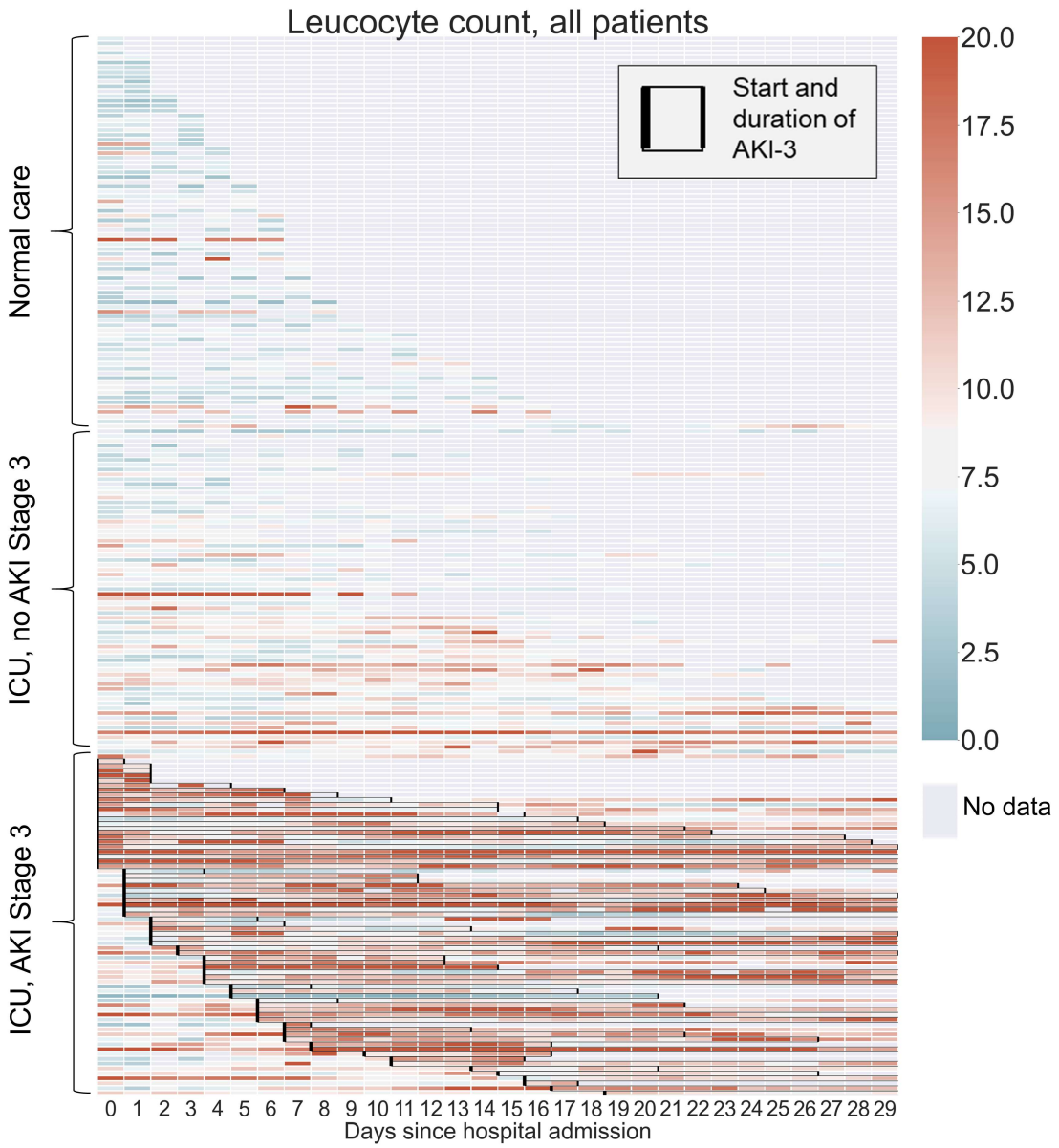


Supplemental Figure 6: Visualization of mechanical ventilation over time and its relationship to AKI Stage 3 in all 223 patients included into the study
Days with mechanical ventilation are indicated in red, days without mechanical ventilation in yellow (no data, grey). Days with AKI Stage 3 are labeled by black boxes. Each row represents one patient, each column one day of hospitalization. Day 0 refers to the day of admission to Charité university hospitals. Patients are sorted on the basis of whether they were ever admitted to an intensive care unit. Patients ever admitted to the ICU were then sorted by AKI Stage 3 status and by the start and duration of AKI Stage 3.



Supplemental Figure 7: Visualization of vasopressor use over time and its relationship to AKI Stage 3 in all 223 patients included into the study

Days on which vasopressors (norepinephrine and vasopressin) were administered are indicated in red, days without vasopressors in yellow (no data, grey). Days with AKI Stage 3 are labeled by black boxes. Each row represents one patient, each column one day of hospitalization. Day 0 refers to the day of admission to Charité university hospitals. Patients are sorted on the basis of whether they were ever admitted to an intensive care unit. Patients ever admitted to the ICU were then sorted by AKI Stage 3 status and by the start and duration of AKI Stage 3.



Supplemental Figure 8: Heat map visualization white blood cell levels over time and their relationship to AKI Stage 3 in all 223 patients included into the study
Displayed are blood leukocyte counts per nl over time (grey cells indicating missing data). Days with AKI Stage 3 are labeled by black boxes. Every row represents one patient, each column one day of hospitalization. Day 0 refers to the day of admission to Charité university hospitals. Patients are sorted on the basis of whether they were ever admitted to an intensive care unit. Patients ever admitted to the ICU were then sorted by AKI Stage 3 status and by the start and duration of AKI Stage 3.

Supplemental Table 1: Parameters screened and included in analysis

Putative mechanism of kidney injury	Time-varying parameter screened; one value per day, if available	No patients with available measurements	Parameter analyzed in the simplified models	Parameter analyzed in the main model	Mean number of measured values per patient (±SD)	Comment
Inflammation	CRP	223	yes	yes	15.8 (±16.2)	These parameters were selected in the main model as surrogates of inflammation and because they show a high degree of completeness and also reflect distinct aspects of inflammation.
	Procalcitonin	218	yes	yes	14.5 (±16.0)	
	Leukocyte count	222	yes	yes	17.3 (±17.0)	
	Interleukin 6	198	yes	no	7.9 (±9.4)	Interleukin-6 was not included into the main model because of a rather high degree of missingness.
	Ferritin	208	yes	no	9.2 (±10.8)	Ferritin was not included into the main model because of a rather high degree of missingness.
	Neutrophil/lymphocyte ratio	216	yes	no	10.8 (±11.8)	Neutrophil/lymphocyte ratio was not included in the main model because of a rather high degree of missingness.
	Neutrophil count	216	yes	no	10.8 (±11.8)	Neutrophil count was not included in the main model because of a rather high degree of missingness.
	Lymphocyte count	216	yes	no	10.8 (±11.8)	Lymphocyte ratio was not included in the main model because of a rather high degree of missingness.
Hypercoagulability	D-Dimer	137	yes	yes	7.6 (±12.0)	D-Dimer level was included in the main model despite a rather high degree of missingness because this is a suitable marker reflecting thrombosis/hyper-coagulability.
	Fibrinogen	126	no	no	6.9 (±12.4)	Fibrinogen was not included in the main model because it had a higher degree of missingness compared to D-Dimers.
Rhabdomyolysis	Creatinkinase	213	yes	yes	12.7 (±14.0)	Creatinkinase was included in the main model because it is a marker of rhabdomyolysis and showed the highest degree of completeness
	Myoglobin	84	yes	no	3.0 (±8.0)	Myoglobin was not included in the main model because of the rather high degree of missingness.
Liver failure	Total bilirubin	219	yes	yes	15.8 (±16.5)	Bilirubin was selected for the main model as a surrogate for liver failure because it had a high degree of data completeness. Furthermore, it is a component of critical illness score, thereby reflecting critical illness.
	ALT	197	yes	no	12.9 (±15.7)	ALT was not included in the main model because of a comparatively higher degree of missingness.
	AST	195	yes	no	12.3 (±15.3)	AST was not included in the main model because of a comparatively higher degree of missingness.
Hypotension/organ hypoperfusion	Need for vasopressors	223	yes	yes	18.1 (±17.4)	The need for vasopressors was determined on every patient-day and was available for the entire cohort without

						missing data. Therefore, this parameter was used as surrogate marker for hypotension and organ hypoperfusion in the main model.
	Mean arterial pressure (MAP) invasively measured; lowest moving average of 3 measurements per day	135	no	no	15.1 (±18.9)	These parameters illustrate the depth of our data collection and represent a component of the need for vasopressors. However, they were only available in ICU patients and could therefore could not be assessed in the entire cohort.
	Maximum dose of Norepinephrin	95	no	no	6.9 (±11.6)	
	Maximum dose of Enoximon,	17	no	no	0.5 (±2.0)	
	Maximum dose of Argipressin	41	no	no	1.0 (±3.5)	
	Maximum dose of Epinephrin	11	no	no	0.07 (±0.35)	
Hypoxemia/ respiratory failure/mechanical ventilation	Mechanical ventilation per day	223	yes	yes	18.1 (±17.4)	The need for mechanical ventilation was determined on every patient-day and was available for the entire cohort without missing data. Therefore, this parameter was used as surrogate marker for respiratory failure and impaired oxygenation.
	SpO2; lowest value per day	134	no	no	15.0 (±18.8)	These parameters illustrate the depth of our data collection. However, they were largely only available in ICU patients and could therefore could not be assessed in the entire cohort.
	Horowitz index; lowest value per day	96	no	no	12.1 (±18.0)	
	arterial SaO2; lowest value per day	137	no	no	14.3 (±17.9)	
	arterial PaO2; lowest value per day	130	no	no	14.8 (±18.8)	
	arterial pH; lowest value per day	136	no	no	14.3 (±17.9)	
	arterial pCO2; highest value per day	138	no	no	14.3 (±17.8)	
	Peak inspiratory Pressure (Ppeak); mean value per day	96	no	no	11.9 (±17.7)	
	PEEP; mean value per day	96	no	no	12.1 (±18.0)	
Other	Hemoglobin	223	yes	yes	17.3 (±17.0)	Hemoglobin is critical for oxygen delivery to the kidney and was therefore assessed as a potential risk factor of AKI in the main model.
	Thrombocyte count	223	yes	yes	17.3 (±17.0)	Low thrombocyte counts have been associated with severe COVID-19 and the thrombocyte count is a component of critical illness scores and was therefore assessed in the main model.
	LDH	220	yes	yes	13.3 (± 14.2)	Elevation in LDH have been associated with severe COVID-19 and were therefore assessed in the main model.

Rational for parameter selection in minimal and main models. Parameter selection was based on pathophysiological categories as indicated in the table.

ALT, Alanine transaminase; AST, Aspartate transaminase; CRP, C-reactive protein; GCS, Glasgow coma scale; LDH, Lactate dehydrogenase; PEEP, positive end-expiratory pressure; PaO2, partial pressure of oxygen in arterial blood; PaCO2, partial pressure of carbon dioxide in arterial blood; SaO2, arterial oxygen saturation; SOFA, sequential organ failure assessment;

Supplemental Table 2: Longitudinal parameters overview of missing values

Parameter	Total (n = 2010)	No severe AKI (n=1676)	Severe AKI (n = 334)
Hemoglobin			
Missing n (%)	118 (5.9%)	114 (6.8%)	4 (1.2%)
Thrombocyte count			
Missing n (%)	121 (6.0%)	116 (6.9%)	5 (1.5%)
Leucocyte count			
Missing n (%)	120 (6.0%)	115 (6.9%)	5 (1.5%)
Neutrophil count			
Missing n (%)	733 (36.5%)	581 (34.7%)	152 (45.5%)
Lymphocyte count			
Missing n (%)	733 (36.5%)	581 (34.7%)	152 (45.5%)
Neutrophil Lymphocyte ratio			
Missing n (%)	733 (36.5%)	581 (34.7%)	152 (45.5%)
Total Bilirubin			
Missing n (%)	333 (16.6%)	311 (18.6%)	22 (6.6%)
AST			
Missing n (%)	845 (42.0%)	755 (45.0%)	90 (26.9%)
ALT			
Missing n (%)	772 (38.4%)	683 (40.8%)	89 (26.6%)
Creatinkinase			
Missing n (%)	724 (36.0%)	639 (38.1%)	85 (25.4%)
Myoglobin			
Missing n (%)	1,770 (88.1%)	1,511 (90.2%)	259 (77.5%)
LDH			
Missing n (%)	551 (27.4%)	489 (29.2%)	62 (18.6%)
D-Dimer			
Missing n (%)	1,439 (71.6%)	1,271 (75.8%)	168 (50.3%)

CRP			
Missing n (%)	293 (14.6%)	266 (15.9%)	27 (8.1%)
Procalcitonin			
Missing n (%)	519 (25.8%)	478 (28.5%)	41 (12.3%)
Interleukin-6			
Missing n (%)	1,160 (57.7%)	986 (58.8%)	174 (52.1%)
Ferritin			
Missing n (%)	1,078 (53.6%)	908 (54.2%)	170 (50.9%)

The n in the column headers represents the total number of patient-days per group. Missingness refers to all patient-days without an available measurement. For parameters in the AKI Stage 3 group only observations preceding AKI Stage 3 were considered. ALT; Alanine Aminotransferase. AST; Aspartate aminotransferase. CRP; C-reactive Protein. LDH; Lactate dehydrogenase.

Supplemental Table 3: Route of admission. Shown are N (% per column category)

	Total (n=223)	No AKI Stage 3 (n=153)	AKI Stage 3 (n=70)
Initial presentation to Charité emergency department	120 (53.8%)	98 (64.1%)	22 (31.4%)
Direct admission to normal ward	17 (7.6%)	17 (11.1%)	0
Direct admission to ICU	86 (38.6%)	38 (24.8%)	48 (68.6%)
Transfer from external ICU	57 (25.6%)	22 (14.4%)	35 (50%)
Intubation prior to admission at Charité*	58 (26%)	17 (11.1%)	41 (58.6%)

*includes intubation as part of initial remote emergency care, intubation in an external emergency department (outside Charité), and intubation in an external ICU (outside Charité).

Supplemental Table 4: AKI frequency

	Total (n=223)	No ICU care (n=85)	ICU care (n=138)
Any AKI (n, %)	117 (52.4%)	14 (16.5%)	103 (74.6%)
AKI Stage 1 (n, %)	24 (10.8%)	12 (14.1%)	12 (8.7%)
AKI Stage 2 (n, %)	23 (10.3%)	2 (2.4%)	21 (15.2%)
AKI Stage 3 (n, %)	70 (31.3%)	0 (0%)	70 (50.7%)
KRT (n, %)	67 (30%)	0 (0%)	67 (48.6%)

AKI, acute kidney injury; ICU, intensive care unit; KRT, kidney replacement therapy

Supplemental Table 5: Overview of the individual components of the KDIGO diagnosis criteria for Stage 3 AKI on a per patient level at the day of diagnosis.

Patients with KDIGO Stage 3 AKI	KDIGO Stage 3 creatinine criterion fulfilled	KDIGO Stage 3 urine output criterion fulfilled	Kidney replacement therapy criterion fulfilled
1	yes	yes	yes
2	yes	yes	yes
3	yes	yes	yes
4	no	yes	yes
5	no	yes	yes
6	yes	yes	yes
7	no	yes	no
8	no	no	yes
9	no	no	yes
10	no	no	yes
11	yes	yes	no
12	no	no	yes
13	yes	yes	yes
14	yes	yes	no
15	no	no	yes
16	yes	yes	no
17	yes	no	no
18	no	no	yes
19	no	yes	no
20	yes	yes	yes
21	yes	yes	yes
22	no	yes	no
23	no	yes	yes
24	no	no	yes
25	no	yes	yes
26	yes	yes	yes
27	no	yes	yes
28	yes	yes	yes
29	BL creatinine missing	yes	yes
30	BL creatinine missing	no	yes
31	no	yes	no
32	no	no	yes
33	yes	yes	yes
34	no	yes	no
35	no	yes	no
36	no	yes	no
37	no	yes	yes
38	no	no	yes
39	yes	yes	no
40	no	yes	yes
41	no	no	yes

42	no	yes	yes
43	no	yes	yes
44	no	yes	yes
45	yes	no	yes
46	no	no	yes
47	no	no	yes
48	no	yes	no
49	no	no	yes
50	no	yes	yes
51	BL creatinine missing	no	yes
52	yes	yes	no
53	no	yes	yes
54	no	no	yes
55	no	yes	yes
56	no	no	yes
57	no	no	yes
58	BL creatinine missing	yes	yes
59	no	yes	yes
60	no	yes	yes
61	no	yes	yes
62	no	yes	yes
63	yes	yes	no
64	no	yes	no
65	no	yes	no
66	no	yes	no
67	BL creatinine missing	yes	yes
68	no	no	yes
69	no	no	yes
70	BL creatinine missing	yes	yes

BL; Baseline.