CHEST Online Supplement

Corticosteroid Therapy Is Associated With Improved Outcome in Critically Ill Patients With COVID-19 With Hyperinflammatory Phenotype

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e-Appendix 1.

e-Method

Marginal structural Cox models (MSCM)

Marginal structural cox models (MSCM) are a new class of causal models for the estimation, from observational data, of the causal effect of a time-dependent exposure in the presence of time-dependent covariates that may be simultaneously confounders and intermediate variables. In our study, because corticosteroid therapy often was not started at the time of ICU admission, but rather during the course of the disease based on a change in the patient condition, so we used MSCM to provide the marginal causal relation between a time-varying exposure (Corticosteroid therapy) and a survival outcome (time to death), controlling for time-dependent variables (SPO_2/FiO_2 and model of ventilation) that are likely to influence the corticosteroid therapy initiation and at the same time are likely to be correlated with the risk of mortality. The parameters of MSCM could be estimated using inverse probability weighting (IPW) to correct both for confounding and for forms of selection bias such as informative censoring. By weighting each patient by IPW, two pseudo-populations are created, similar with regards to baseline and time-dependent confounding factors, and different in corticosteroid exposure. The MSCM allows a comparison of hazard functions for patients who had not received corticosteroids to those who had received corticosteroids. The partial likelihood function of Cox model was modified such that the contribution of patient i to the risk set was weighted by the stabilized inverse probability of treatment selection and censoring weights[1]. The analytical pipeline of MSCM had three steps.

First, the treatment selection weights are calculated according to a ratio of two weights, the numerator is the product of probabilities that a patient receives his observed treatment at time k, given the baseline confounders (age, sex, days from the onset of symptom to hospital admission, SOFA score on Day 1, respiratory rate, hypertension, coronary heart disease, diabetes, chronic obstructive pulmonary disease, and the mode of ventilation on Day 1), the denominator is calculated similarly by incorporating also the time-dependent variables (SPO_2/FiO_2 and model of ventilation) as

well. The weights are updated until the first day of corticosteroid therapy and kept constant afterwards. Second, the censoring weights are calculated in the same approach. We have censored patients at day 28. Third, the overall stabilized weight for each observation is obtained by multiplying the treatment selection weights and the censoring weights, and a MSCM was fit using robust variance estimator, and time-dependent variables were included in the model[2]. The ipw package (version 1.0–11) was employed for the MSCM analysis[3].

References:

1. Karim ME, Gustafson P, Petkau J, et al.: Marginal structural Cox models for estimating the association between β-interferon exposure and disease progression in a multiple sclerosis cohort. Am J Epidemiol. 2014; 180:160–171.

2.Hernán MA, Brumback B, Robins JM: Marginal structural models to estimate the causal effect of zidovudine on the survival of HIV-positive men. Epidemiology. 2000; 11:561–570

3.van der Wal WM, Geskus RB: ipw: An R Package for Inverse Probability Weighting. Journal of Statistical Software. 2011; 43:1–23

Statistical analyses

Subgroup analyses and sensitivity analyses

Several prespecified subgroup analyses were performed according to sex, age, comorbidity, SOFA score, SPO_2/FiO_2 and invasive mechanical ventilation use. Considering that the corticosteroid therapy effect may vary according to the dosage and the time of initiation, we conducted serial sensitivity analyses based on the following groups: patients who received a maximum daily doses of 40 mg (methylprednisolone-equivalent); patients who received a maximum daily doses of 80 mg (methylprednisolone-equivalent); patients who had corticosteroid therapy initiated within the first 4 days from hospital admission, each compared to patients who did not receive any corticosteroid therapy. We also compared corticosteroid therapy initiated after day 4 compared to patients who did not receive any corticosteroid therapy and were alive after day 4. To explore whether imputation of

missing data had an impact on the association observed in the MSCM, given that 91.4% of patients initialized corticosteroid therapy within 7 days when imputation was relatively limited, we compared patients who had corticosteroid therapy initiated within the first 7 days with patients in the no corticosteroid therapy group with imputation up to day 7 only.

Parsimonious model to predict phenotype

A biomarker-based parsimonious model was shown to have high accuracy in predicting phenotypes of ARDS. Based on the previous research, we attempt to construct a three variable model to predict phenotype of COVID-19, variables were chosen because they contributed most significantly to defining phenotypes using forward stepwise modeling. Receiver operator characteristics (ROC) and area under the curve (AUC) were calculated for the model. Subjects with missing data were excluded.

e-Table 1: Baseline and time-dependent variables in marginal structural Cox model

Baseline variables	Time-dependent variables
Age	SPO_2/FiO_2
Sex (Female)	Model of ventilation*
Days from onset of symptom to hospital admission	
SOFA score on Day 1	
Respiratory rate	
Hypertension	
Coronary heart disease	
Diabetes	
COPD	
Mode of ventilation on Day 1*	

*we coded the mode of ventilation as follows: 0=no ventilation, 1=non-invasive ventilation, 2=invasive ventilation, 3= advanced ventilation support (e.g. ECMO, oscillatory, prone)
SOFA=Sequential Organ Failure Assessment; COPD=chronic obstructive pulmonary disease; SPO_2/FiO_2 ratio= ratio of pulse oxygen saturation to the fractional concentration of oxygen in inspired air.

e-Table 2. Percentage of missing data in the variables of interest

	Missing, n (%)
Age, years	1 (0.2%)
Sex (Female)	0 (0)
Days from onset of symptom to Hospital admission, days	0 (0)
Fever (temperature>37.3°C)	0 (0)
Dyspnea	0 (0)
SOFA score	0 (0)
Hypertension	0 (0)
Coronary heart disease	0 (0)
Chronic heart failure	0 (0)
Diabetes	0 (0)
COPD	0 (0)
Chronic renal diseases	0 (0)
Liver disease	0 (0)
Malignancy	0 (0)
Immunodeficiency	0 (0)
Temperature, °C	3 (0.7%)
Heart rate, beats per min	1 (0.2%)
Respiratory rate, breaths per min	2 (0.46%)
MAP, mmHg	1 (2.3%)
SPO_2/FiO_2 ratio	25 (5.8%)
White blood cell count, $\times 10^9$ per L	10 (2.3%)
Lymphocyte count, $\times 10^9$ per L	12 (2.8%)
Platelet count, $\times 10^9$ per L	28 (6.5%)
Haemoglobin, g/L	14 (3.3%)
Erythrocyte sedimentation rate, mm/h	117 (27.3%)
High-sensitive C-reactive protein, mg/L	23 (5.4%)
Procalcitonin, ng/mL	103 (24%)
Interleukin-1β, pg/ml	86 (20.1%)
Interleukin-2R, U/ml	83 (19.4%)
Interleukin-6, pg/ml	80 (18.7%)
Interleukin-8, pg/ml	83 (19.4%)
Interleukin-10, pg/ml	83 (19.4%)
TNF-α, pg/ml	83 (19.4%)
Antiviral treatment	0 (0)
Antibiotics	0 (0)
Intravenous immunoglobin	0 (0)
Neuromuscular blockade	0 (0)
High-flow nasal cannula oxygen therapy	0 (0)
Non-invasive mechanical ventilation	0 (0)
Invasive mechanical ventilation	0 (0)

ECMO	0 (0)
Renal replacement therapy	0 (0)
Any vasopressor	0 (0)
ARDS	0 (0)
Septic shock	0 (0)
Coagulopathy	0 (0)
Acute kidney injury	0 (0)
Acute cardiac injury	0 (0)
28-day mortality	0 (0)
Hospital duration, days	0 (0)

SOFA=Sequential Organ Failure Assessment; COPD=chronic obstructive pulmonary disease; MAP= mean arterial pressure; SPO_2/FiO_2 ratio= ratio of pulse oxygen saturation to the fractional concentration of oxygen in inspired air; TNF-α= tumour necrosis factor-α; ECMO=extracorporeal membrane oxygenation; ARDS=acute respiratory distress syndrome.

e-Table 3. Additional baseline characteristics between corticosteroid group and no corticosteroid group

	No Corticosteroid	Corticosteroid	p value	SMD
PT, s	14.0 (13.3-15.0)	14.6 (13.7-16.0)	<0.0001	0.029
APTT, s	40.3 (36.1-44.0)	40.7 (35.7-45.7)	0.80	0.067
D-dimer, μg/ml	0.9 (0.4-2.6)	1.8 (0.8-12.3)	<0.0001	0.345
Fib, g/L	4.4 (3.6-5.7)	5.0 (4.0-6.0)	0.042	0.197
INR	1.1 (1.0-1.2)	1.1 (1.0-1.3)	<0.0001	0.128
Hypersensitive troponin I, pg/ml	3.3 (1.9-15.3)	13.6 (4.4-130.0)	<0.0001	0.249
Myohemoglobin, ng/ml	46.1 (22.9-111.6)	128.2 (58.7-201.7)	<0.0001	0.147
Creatine kinase–MB, U/L	1.0 (0.4-2.5)	2.2 (1.0-6.0)	0.00060	0.030
ALT, IU/L	21.0 (14.0-36.0)	29.0 (17.0-46.0)	0.00040	0.062
AST, IU/L	26.0 (20.0-39.0)	38.0 (24.0-57.0)	<0.0001	0.039
Albumin, g/L	35.6 (31.0-38.6)	32.5 (29.5-35.9)	<0.0001	0.251
Total bilirubin, μmol/L	8.6 (6.3-12.5)	11.0 (7.8-16.7)	0.00010	0.003
Creatinine, μmol/L	65.5 (53.0-89.0)	77.0 (61.5-96.5)	0.0025	0.067
Urea nitrogen, mmol/L	4.7 (3.4-7.1)	6.1 (4.3-9.5)	0.00020	0.011
Creatine kinase, U/L	76.0 (41.5-188.3)	129.0 (68.0-291.0)	0.027	0.008
Lactate dehydrogenase, U/L	244.5 (194.3-319.3)	427.5 (302.0-568.0)	<0.0001	0.794
eGFR, ml/min	93.6 (75.8-109.20)	82.1 (66.9-96.8)	<0.0001	0.373
Sodium, mmol/L	140.4 (137.8-142.8)	138.5 (135.8-142.0)	0.0017	0.203
Potassium, mmol/L	4.2 (3.9-4.5)	4.3 (3.8-4.7)	0.81	0.038
Chlorine, mmol/L	102.2 (100.2-104.5)	100.4 (97.2-103.4)	<0.0001	0.295
Glucose, mmol/L	6.0 (5.1-7.2)	7.7 (6.2-10.3)	<0.0001	0.432

PT=Prothrombin time; APTT=Activated partial thromboplastin time; Fib= Fibrinogen; INR= International Normalized Ratio; ALT=Alanine transaminase; AST= Aspartate transaminase; eGFR= glomerular filtration rate.

e-Table 4. Multivariate Cox proportional-hazards regression model assessing the impact of corticosteroid therapy on 28-day mortality in patients with COVID-19.

	HR	Lower.95	Upper.95	p value
Corticosteroid therapy	1.97	1.25	3.11	0.0034
Age	1.02	1.00	1.04	0.0018
Sex (Female)	0.66	0.48	0.91	0.011
Days from onset of symptom to hospital admission	1.01	0.99	1.03	0.25
SOFA score on Day 1	1.46	1.37	1.55	<0.0001
Respiratory rate	0.98	0.96	1.00	0.19
Hypertension	0.95	0.67	1.33	0.77
Coronary heart disease	1.02	0.64	1.63	0.93
Diabetes	1.37	0.93	2.02	0.11
COPD	2.18	1.21	3.91	0.0092
Mode of ventilation on Day 1 (0)*	Reference	—	—	Reference
Mode of ventilation on Day 1 (1)	1.42	0.98	2.04	0.062
Mode of ventilation on Day 1 (2)	1.93	1.03	3.62	0.041
Mode of ventilation on Day 1 (3)	0.61	0.14	2.67	0.51

*we coded the mode of ventilation as follows: 0=no ventilation, 1=non-invasive ventilation, 2=invasive ventilation, 3= advanced ventilation support (e.g. ECMO, oscillatory, prone)
SOFA=Sequential Organ Failure Assessment; COPD=chronic obstructive pulmonary disease.

e-Table 5. Univariate and multivariate models assessing impact of variables on classification of phenotypes.

Variables	Univariate models		Multivariate models	
	Odds Ratio (95% CI)	P value	Odds Ratio (95% CI)	P value
TNF-α	1.18 (1.12-1.24)	<0.001	1.16 (1.08-1.24)	<0.001
D-dimer	1.61 (1.40-1.86)	<0.001	1.32 (1.16-1.49)	<0.001
NLR	1.42 (1.32-1.53)	<0.001	1.30 (1.21-1.40)	<0.001

TNF-α= tumour necrosis factor-α; NLR= neutrophil to lymphocyte ratio.

e-Table 6. Association between corticosteroids therapy and 28-day mortality in patients with COVID-19 using various adjustment methodologies.

Variables	Cox proportional hazard model			Marginal structural modeling		
	Number of patients	HR (95% CI)	p value	Number of patients	HR (95% CI)	p value
Patients treated with 40 mg (maximum daily dosage) vs not treated with corticosteroids (ref)	275	2.85 (1.68-4.85)	<0.001	275	1.00 (0.56-1.80)	0.98
Patients treated with 80 mg (maximum daily dosage) vs not treated with corticosteroids (ref)	262	1.33 (0.76-2.33)	0.31	262	0.87 (0.49-1.54)	0.62
Patients treated for <= 4 days vs not treated with corticosteroids (ref)	375	2.05 (1.30-3.22)	0.0018	375	0.78 (0.44-1.03)	0.071
Patients treated for >4 days vs not treated with corticosteroids and who survived >4 days (ref)	198	1.14 (0.50-2.60)	0.75	198	1.97 (0.92-4.22)	0.082

HR= hazard ratio

e-Table 7. Clinical characteristics and outcomes between hypoinflammatory and hyperinflammatory phenotype

	Hypoinflammatory phenotype	hyperinflammatory phenotype	p value	SMD
N	223	205		
Age, years	58.0 (48.0-68.0)	68.0 (59.0-75.0)	<0.0001	0.644
Sex (Female)	114 (51.4%)	87 (42.4%)	0.081	0.179
Days from onset of symptom to Hospital admission, days	11.0 (8.0-16.0)	12.0 (8.0-16.0)	0.41	0.043
Fever (temperature>37.3°C)	192 (86.1%)	184 (89.8%)	0.31	0.112
Dyspnea	129 (57.8%)	139 (67.8%)	0.043	0.207
SOFA score	2.0 (1.0-3.0)	7.0 (4.0-9.0)	<0.0001	1.599
Comorbidity				
Hypertension	78 (35.0%)	98 (48.0%)	0.0083	0.267
Coronary heart disease	18 (8.1%)	30 (14.7%)	0.046	0.209
Chronic heart failure	4 (1.8%)	16 (7.8%)	0.0064	0.285
Diabetes	33 (14.8%)	48 (23.6%)	0.028	0.226
COPD	8 (3.6%)	12 (5.9%)	0.37	0.108
Chronic renal diseases	1 (0.4%)	3 (1.5%)	0.35	0.105
Liver disease	2 (0.9%)	8 (3.9%)	0.053	0.198
Malignancy	8 (3.6%)	5 (2.5%)	0.69	0.066
Immunodeficiency	3 (1.3%)	3 (1.5%)	1.0	0.011
Temperature, °C	36.8 (36.5-37.5)	36.9 (36.5-38.0)	0.25	0.110
Heart rate, beats per min	89.0 (80.0-102.0)	93.0 (81.0-108.0)	0.016	0.304
Respiratory rate, breaths per min	20.0 (20.0-22.0)	23.0 (20.0-30.0)	<0.0001	0.515
MAP, mmHg	96.7 (90.7-105.0)	98.5 (87.9-105.7)	0.60	0.050
SPO_2/FiO_2 ratio	297.0 (154.0-274.5)	167 (122.0-230.0)	<0.0001	1.517
White blood cell count, $\times 10^9$ per L	5.1 (3.9-6.4)	9.8 (7.4-13.3)	<0.0001	1.391
Lymphocyte count, $\times 10^9$ per L	1.0 (0.7-1.5)	0.6 (0.4-0.8)	<0.0001	0.906
NLR	3.2 (1.9-5.1)	14.9 (8.6-25.5)	<0.0001	1.131
Platelet count, $\times 10^9$ per L	209.0 (154.0-274.5)	167.0 (122.0-230.0)	<0.0001	0.455
Haemoglobin, g/L	126.0 (114.5-137.0)	128.0 (113.5-143.0)	0.12	0.145
High-sensitive C-reactive protein, mg/L	23.6 (5.3-57.5)	92.1 (55.9-142.7)	<0.0001	0.193
Interleukin-1β, pg/ml	5.0 (5.0-5.0)	5.0 (5.0-5.0)	0.23	0.141
Interleukin-2R, U/ml	587.0 (404.5-829.5)	1128.0 (830.0-1530.0)	<0.0001	1.028
Interleukin-6, pg/ml	5.8 (1.7-19.4)	64.9 (30.8-138.7)	<0.0001	0.444
Interleukin-8, pg/ml	10.1 (5.9-17.7)	30.3 (18.1-68.3)	<0.0001	0.361
Interleukin-10, pg/ml	5.0 (5.0-6.6)	8.9 (5.2-15.0)	<0.0001	0.250
TNF-α, pg/ml	7.4 (5.5-9.6)	11.5 (8.4-17.5)	<0.0001	0.547
D-dimer, μg/ml	0.7 (0.4-21.3)	6.1 (1.8-21.0)	<0.0001	1.201
Corticosteroid therapy	111 (49.7%)	169 (82.4%)	<0.0001	1.292

Treatments				
Antiviral treatment	196 (87.9%)	154 (75.1%)	0.0011	0.333
Antibiotics	158 (70.9%)	191 (93.2%)	<0.0001	0.607
Intravenous immunoglobin	79 (35.4%)	141 (68.8%)	<0.0001	0.708
Neuromuscular blockade	12 (5.4%)	36 (17.6%)	0.00012	0.389
High-flow nasal cannula oxygen therapy	16 (7.2%)	47 (22.9%)	<0.0001	0.452
Non-invasive mechanical ventilation	24 (10.8%)	91 (44.4%)	<0.0001	0.812
Invasive mechanical ventilation	30 (13.5%)	130 (63.4%)	<0.0001	1.197
ECMO	4 (1.8%)	18 (8.8%)	0.0023	0.316
Renal replacement therapy	9 (4.0%)	35 (17.1%)	<0.0001	0.434
Any vasopressor	24 (10.8%)	131 (63.9%)	<0.0001	1.315
Outcomes				
ARDS	46 (20.6%)	168 (82.0%)	<0.0001	1.554
Septic shock	29 (10.8%)	131 (63.9%)	<0.0001	1.315
Coagulopathy	12 (5.4%)	84 (41.0%)	<0.0001	0.930
Acute kidney injury	23 (10.3%)	106 (51.7%)	<0.0001	1.001
Acute cardiac injury	32 (14.3%)	133 (64.9%)	<0.0001	1.207
28-day mortality	34 (15.2%)	146 (71.2%)	<0.0001	1.369

SOFA=Sequential Organ Failure Assessment; COPD=chronic obstructive pulmonary disease; MAP= mean arterial pressure; SPO_2/FiO_2 ratio= ratio of pulse oxygen saturation to the fractional concentration of oxygen in inspired air; NLR= neutrophil to lymphocyte ratio; TNF-α= tumour necrosis factor-α; PT=Prothrombin time; APTT=Activated partial thromboplastin time; Fib= Fibrinogen; INR= International Normalized Ratio; ALT=Alanine transaminase; AST= Aspartate transaminase; eGFR= glomerular filtration rate; ECMO=extracorporeal membrane oxygenation; ARDS=acute respiratory distress syndrome.

e-Table 8. Results of marginal structural Cox model in overall population and phenotypes

	Number of patients	HR	Lower.95	Upper.95	p value
Overall population	428	0.80	0.54	1.18	0.26
Hypoinflammatory phenotype	223	1.15	0.45	2.94	0.76
hyperinflammatory phenotype	205	0.51	0.34	0.78	0.0018

HR= hazard ratio

e-Table 9. Subgroup analyses of the association between corticosteroid therapy and 28-day mortality in hyperinflammatory phenotype using MSCM

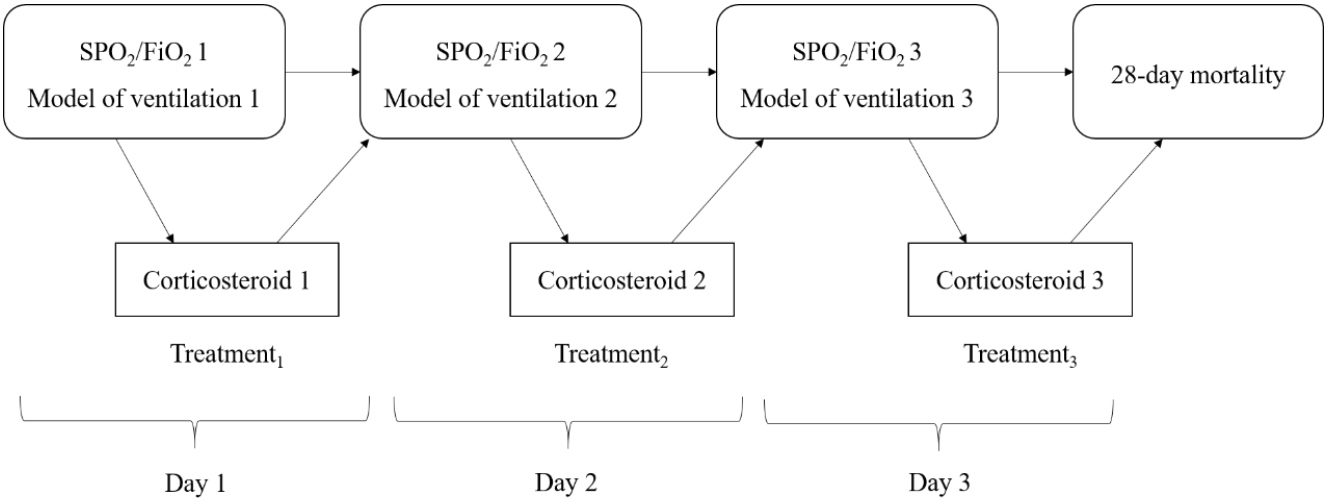
	Number of patients	HR	Lower.95	Upper.95	p value
Invasive Mechanical ventilation					
Yes	130	0.51	0.30	0.84	0.006
No	75	0.45	0.21	0.97	0.042
SPO_2/FiO_2 ratio					
≤150	114	0.30	0.13	0.68	0.004
>150	91	0.55	0.32	0.94	0.028

HR= hazard ratio; SOFA=Sequential Organ Failure Assessment; SPO_2/FiO_2 ratio= ratio of pulse oxygen saturation to the fractional concentration of oxygen in inspired air.

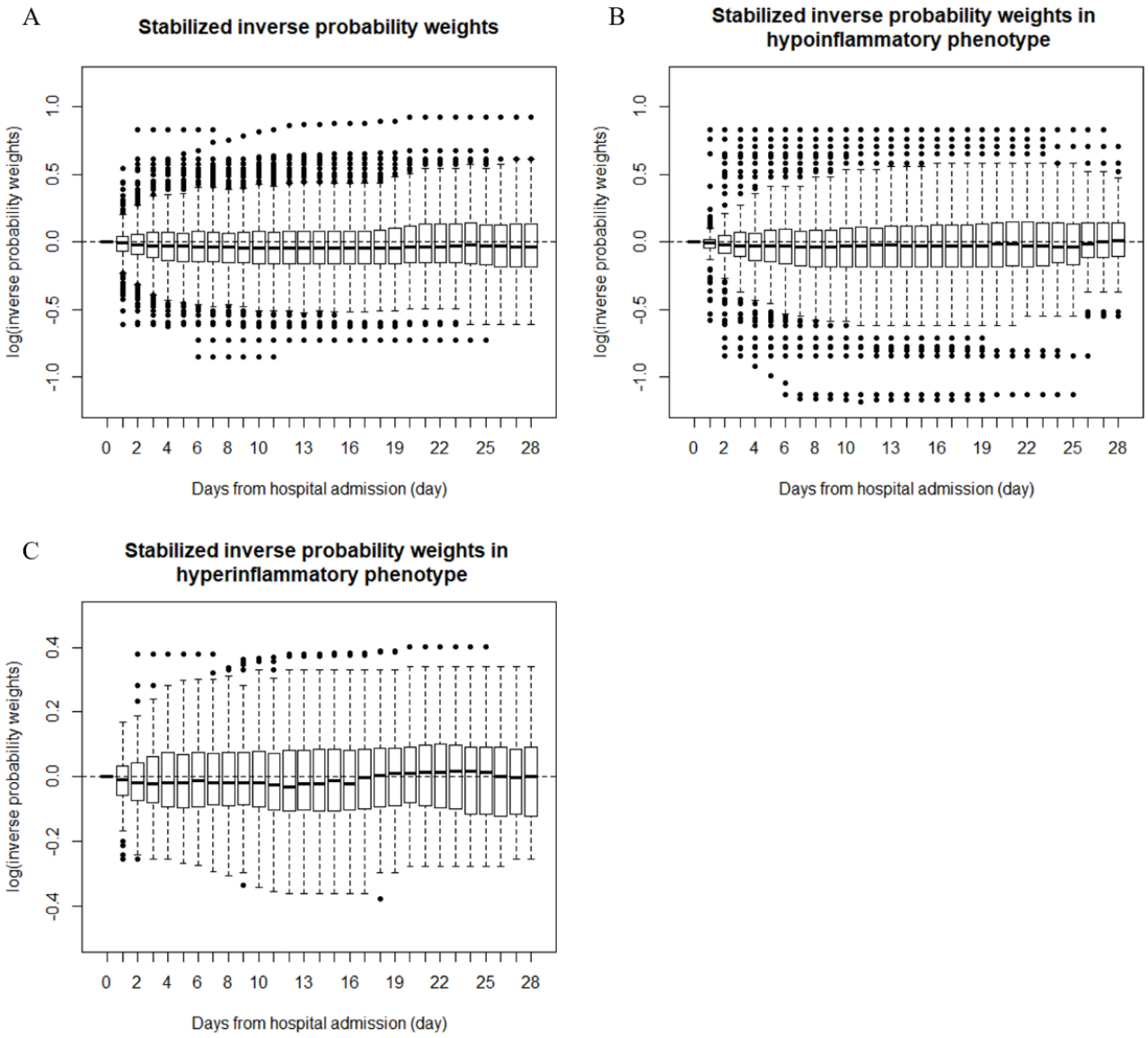
e-Table 10: The association between corticosteroid therapy and 28-day mortality in groups based on the cutoff value of TNF-α, D-dimer and NLR

	Number of patients	HR	Lower.95	Upper.95	p value
TNF-α					
>10.1	170	0.64	0.35	1.21	0.17
≤10.1	258	1.00	0.58	1.72	0.99
D-dimer					
>2.0	180	0.58	0.34	0.98	0.042
≤2.0	248	1.07	0.55	2.06	0.84
NLR					
>6.9	212	0.55	0.34	0.89	0.015
≤6.9	216	1.22	0.59	2.52	0.59

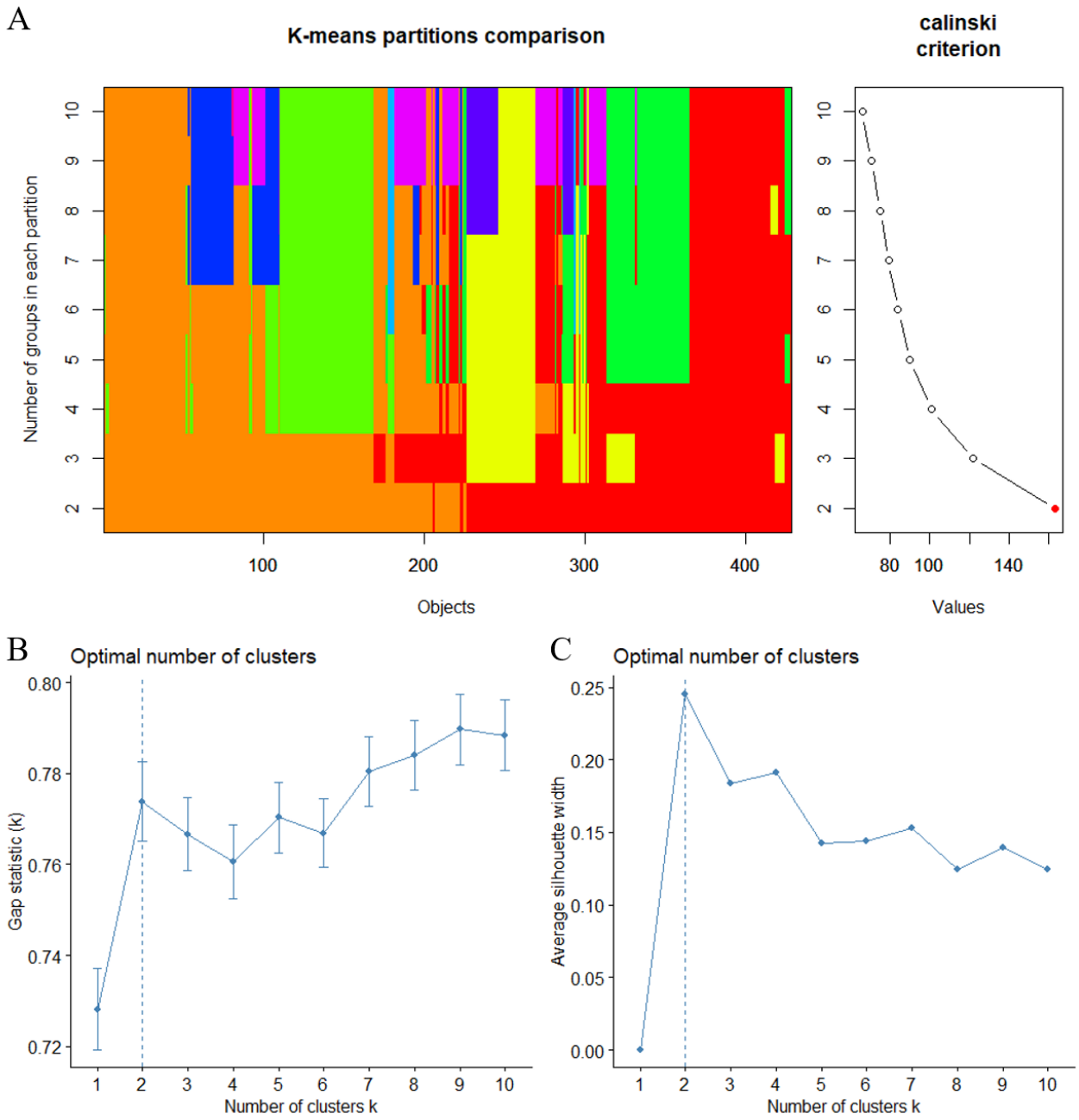
HR= hazard ratio; NLR= neutrophil to lymphocyte ratio; TNF-α= tumour necrosis factor-α;



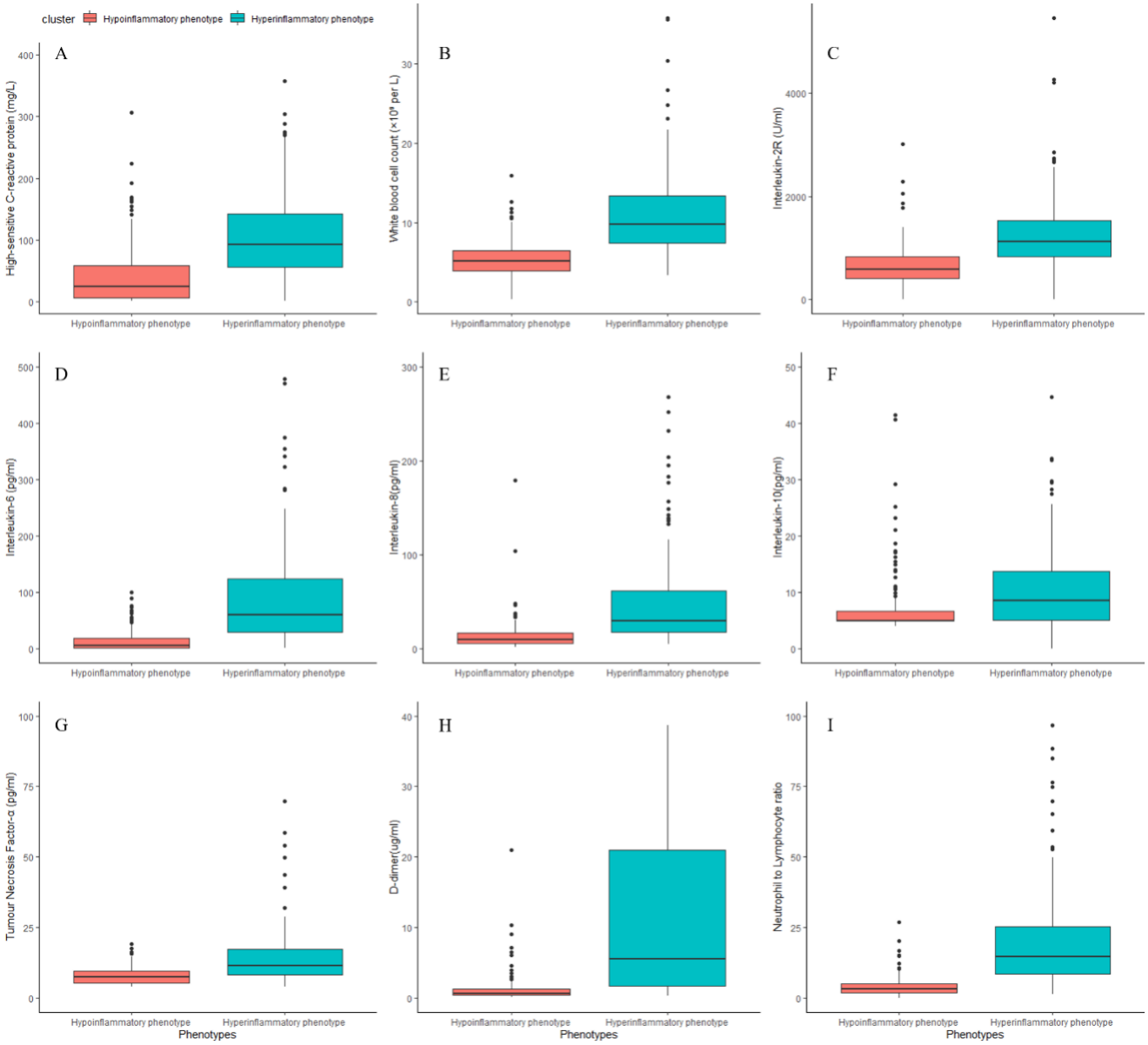
e- Figure 1: Causal diagram of corticosteroids therapy with 28-day mortality in marginal structural Cox model. Hypothetical relationship in the treatment of corticosteroids with 3 time points (days): j=1, 2, 3. Here $Treatment_j$ means binary corticosteroids exposure variable that is measured immediately after the time-dependent confounders SPO_2/FiO_2 j and model of ventilation j. The time-dependent confounder SPO_2/FiO_2 j and model of ventilation j at time j are affected by prior treatment ($Treatment_{j-1}$). According to the causal diagram, SPO_2/FiO_2 1 and model of ventilation 1 imposes confounding on the $Treatment_1$- 28day-mortality relationship, and SPO_2/FiO_2 2 and model of ventilation 2 is an intermediate variable for the same relationship, since the prior corticosteroids therapy may improve SPO_2/FiO_2 or change the model of ventilation, which may improve the final mortality outcome.



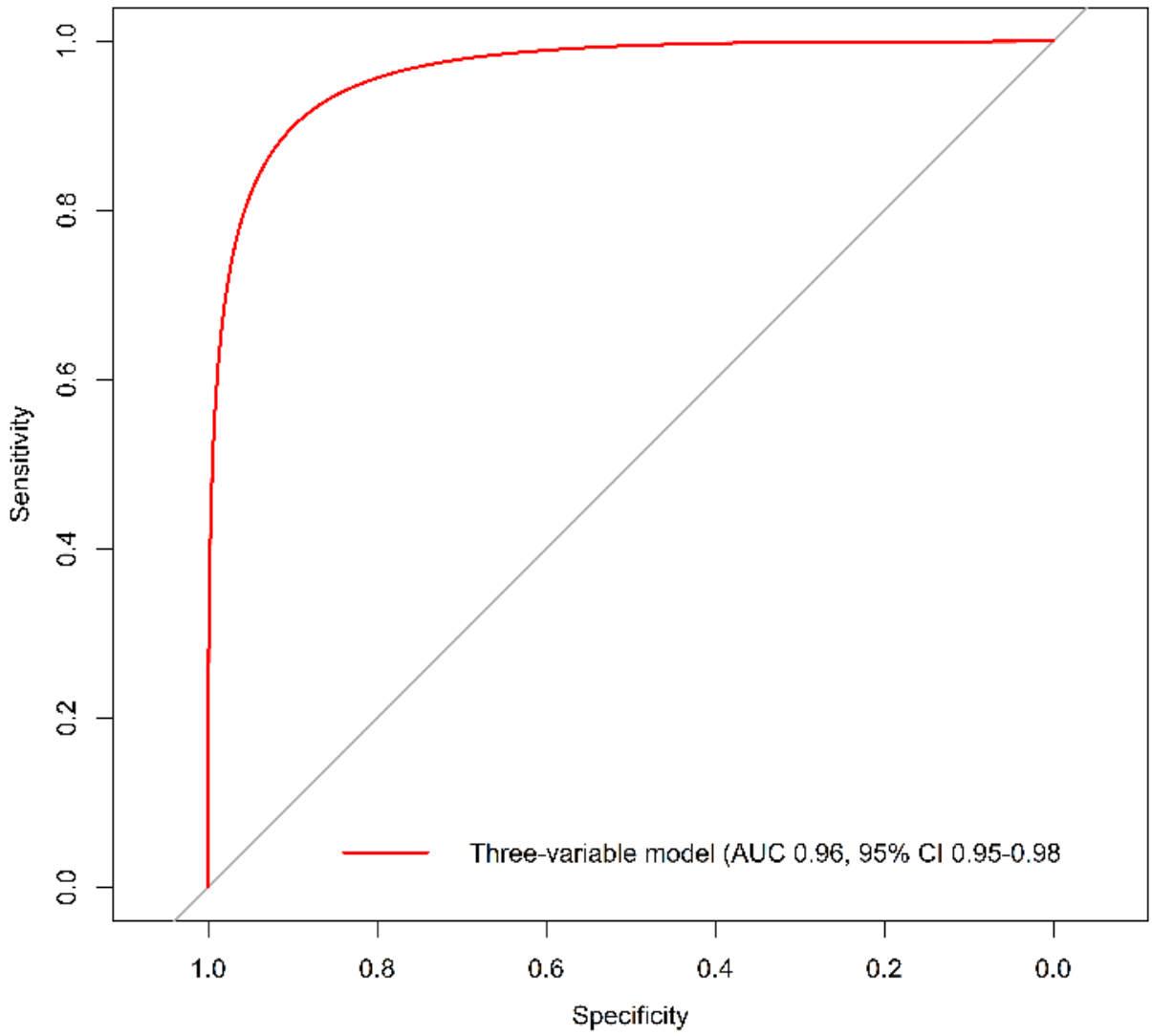
e-Figure 2. Weights distribution plot for the inverse probability weights that were used to adjust for confounding in overall group (A), patients with hypoinflammatory phenotype (B) and patients with hyperinflammatory phenotype (C).



e-Figure 3. Estimating the optimal number of clusters using calinsky criterion (A), gap statistics (B) and average silhouette method (C).



e-Figure 4. Difference in key variable between the hyperinflammatory and hypoinflammatory phenotypes. Figure shows the differences in High-sensitive C-reactive protein (A), White blood cell count (B), Interleukin-2R (C), Interleukin-6 (D), Interleukin-8 (E), Interleukin-10 (F), Tumour Necrosis Factor-α (G), D-dimer (H), and Neutrophil to Lymphocyte ratio (I). Differences between phenotypes were significant for all key variables.



e-Figure 5. Receiver operating characteristic curves of the three-variable model.

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