Strategies to optimise the treatment of community-acquired pneumonia

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CHAPTER 4

Community-acquired pneumonia subgroups and differential response to corticosteroids: a secondary analysis of controlled studies

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ABSTRACT

Background

Latent class analysis (LCA) has identified subgroups with meaningful treatment implications in acute respiratory distress syndrome. We performed a secondary analysis of three studies to assess whether LCA can identify clinically distinct subgroups in community-acquired pneumonia (CAP) and whether the treatment effect of adjunctive corticosteroids differs between subgroups.

Methods

LCA was performed on baseline clinical and biomarker data from the Ovidius trial (n=304) and the Steroids in Pneumonia (STEP) trial (n=727), both randomised controlled trials investigating adjunctive corticosteroid treatment in CAP, and the observational TripleP cohort (n=201). Analyses were conducted independently in two cohorts (Ovidius–TripleP combined and the STEP trial). In both cohorts, differences in clinical outcomes and response to adjunctive corticosteroid treatment were examined between subgroups identified through LCA.

Results

A two-class model fitted both cohorts best. Class 2 patients had more signs of systemic inflammation compared to class 1. In both cohorts, length of stay was longer and in-hospital mortality rate was higher in class 2. In the Ovidius trial, corticosteroids reduced the median length of stay in class 2 (6.5 versus 9.5 days) but not in class 1 (p-value for interaction=0.02). In the STEP trial, there was no significant interaction for length of stay. We found no significant interaction between class assignment and adjunctive corticosteroid treatment for secondary outcomes.

Conclusions

In two independent cohorts, LCA identified two classes of CAP patients with different clinical characteristics and outcomes. Given the different response to adjunctive corticosteroids in the Ovidius trial, LCA might provide a useful basis to improve patient selection for future trials.

INTRODUCTION

Treatment of community-acquired pneumonia (CAP) is based on early diagnosis and prompt initiation of antibiotic therapy.[1] Despite effective treatment, CAP remains a leading cause of mortality and morbidity worldwide.[2] Adjunctive treatment with corticosteroids might improve clinical outcomes in patients with CAP.[3]

A local immune response is crucial to contain and eliminate the primary infection in CAP.[4] However, an uncontrolled or excessive local immune response could result in systemic inflammation and subsequent multi-organ dysfunction.[5]

Adjunctive treatment with corticosteroids, a potent inhibitor of the immune response, has shown to reduce length of stay (LOS) and time to clinical stability in hospitalised patients with CAP.[3] However, corticosteroids did not lower the mortality rate, and increased the incidence of hospital readmission and hyperglycaemia requiring insulin therapy.[3] Therefore, treatment guidelines do not recommend routine use of corticosteroids in patients with CAP.[1]

In a clinically heterogeneous condition as CAP, it is likely that a subgroup of patients does benefit from corticosteroid treatment.[6] It has been hypothesised that corticosteroid treatment should be given to the subgroup with an excessive systemic inflammation response, whereas patients with a local and controlled immune response should not receive corticosteroid treatment.[7] So far, patients with CAP have been stratified by pneumonia severity index (PSI), initial C-reactive protein concentration, and inflammatory status, but stratification did not result in an unequivocal definition of a subgroup benefiting from corticosteroid therapy and therefore did not result in adjustment of clinical guidelines.[3,8–10]

In other heterogeneous conditions, such as sepsis or acute respiratory distress syndrome, substantial efforts have been made to identify subgroups characterised by different prognoses and responses to treatment.[11] In patients with acute respiratory distress syndrome, a latent class analysis (LCA) was used to identify subgroups with different treatment responses to ventilator and fluid management .[12,13] The identification of patients that are likely to respond to (corticosteroid) treatment, i.e. predictive enrichment, is a step towards personalised medicine and improved patient selection for future clinical trials.[14]

In this secondary analysis of three controlled studies, we attempted to identify CAP subgroups through LCA of baseline clinical and biomarker data from two randomised controlled trials and one prospective cohort study. In addition, we examined whether LCA-based subgroups were associated with different clinical outcomes and a different response to adjunctive corticosteroids.

METHODS

Study population and study design

This is a secondary analysis of demographic, clinical and biomarker data obtained at baseline from patients enrolled in the observational TripleP cohort[15], and two multicentre randomised controlled trials: the Ovidius trial (NCT00471640)[16] and the Steroids in Pneumonia (STEP) trial (NCT00973154).[17] All studies included hospitalised adult patients with CAP (see supplementary material).

In the Ovidius trial, patients with CAP were randomly allocated to receive intravenous dexamethasone 5 mg daily or placebo for 4 days following hospital admission.[16] The STEP trial randomised 727 patients with CAP to either placebo or oral prednisolone 50 mg daily for 7 days in the per protocol analysis.[17] LOS, the primary endpoint in the Ovidius trial and main secondary endpoint in the STEP trial, was significantly reduced in patients assigned to adjunctive treatment with corticosteroids. Details of the original studies are published elsewhere.[16,17]

The Ovidius trial and TripleP study were approved by the Medical Ethics Committee at the St Antonius Hospital. The ethical committees of all participating hospitals and Swissmedic approved the STEP trial.

Methods

Two separate LCAs were performed for the identification of subgroups: one in a combined cohort of TripleP and the Ovidius trial, and one in the STEP trial. The observational TripleP cohort (n=201) and the Ovidius trial (n=304) were combined to obtain a larger sample size. We chose to combine these cohorts as the TripleP cohort preceded the Ovidius trial and reported similar clinical and biomarker data. The Ovidius trial and TripleP study are two mutually exclusive cohorts. The STEP trial (n=727) was analysed independently as different clinical and biomarker data were recorded.

After identification of subgroups by LCA, differences in clinical outcomes between these subgroups and the presence of interaction between treatment allocation and LCA-defined subgroups were assessed separately in both cohorts (Ovidius–TripleP combined and STEP). For the Ovidius–TripleP cohort, only patients who participated in the Ovidius trial were included in the analysis of the interaction between adjunctive corticosteroids. The primary outcome was LOS and secondary outcomes were intensive care unit (ICU) admission, in-hospital mortality, 30-day mortality and 30-day hospital readmission.

Statistical analysis

Baseline characteristics of the Ovidius–TripleP combined and STEP cohorts were presented as count (%) for categorical variables, and mean (standard deviation) or median (interquartile range, IQR) for continuous variables, after testing for normal distribution. Baseline characteristics of both cohorts were compared using an independent samples t-test, Mann–Whitney U test or Chi-squared test, as appropriate.

The DepmixS4 package in R 4.0.0 (R core team, 2020) was used to conduct the LCA. Baseline clinical and biomarker data obtained at hospital admission were used as class-defining variables in the LCA. A full list of class-defining variables included in the LCA for each cohort is shown in the supplementary material. Assignment of patients to classes was performed independently of clinical outcomes. LCA was first conducted in the Ovidius–TripleP cohort, and was repeated independently in the STEP cohort. Missing data were accommodated by estimating model parameters based on the full information maximum likelihood.[18]

We fitted models with latent classes ranging from two to five classes. To determine the best-fitting model, we used the following criteria: 1) clinical interpretability, i.e. whether identified classes corresponded to clinically coherent clusters of clinical and biomarker data; 2) the number of patients assigned to the smallest class, where a model with small class size is statistically less meaningful; and 3) the Bayesian information criterion, where a lower number corresponds with improved model fit. For clinical interpretability, all continuous variables in the LCA were rescaled to a z-scale with a mean of zero and standard deviation of 1. Subsequently, clinical interpretability was assessed by two authors independently (PZ and HE). Discrepancies were resolved by consensus and, if necessary, a third author was consulted.

Once the number of classes was determined, patients were assigned to the class with maximum probability of class assignment based on the LCA model. The probability of a patient being assigned to a specific class is a weighted average of the N class-specific probabilities in LCA, so each patient has probabilities assigned to all classes, respectively. For example, a patient with a probability of 90% to be assigned to class 1 and 10% probability to be assigned to class 2 was assigned to class 1. Subsequently, the association between class assignment and baseline characteristics or clinical outcomes was tested using Chi-squared, Mann–Whitney U or independent samples t-test, as appropriate. Finally, for the Ovidius trial and STEP cohorts, we tested the interaction between randomly assigned treatment and class on clinical outcomes with the Poisson regression model for LOS and Chi-squared test for categorical outcomes. A p-value <0.05 was deemed statistically significant.

RESULTS

Baseline characteristics

Baseline characteristics of both cohorts are presented in Table 1 and Supplemental Table E1. In short, patients in the Ovidius–TripleP cohort were younger, had fewer comorbidities and had higher levels of inflammatory biomarkers as compared to patients in the STEP cohort. LOS was longer in the Ovidius–TripleP cohort as compared to the STEP cohort (8.5; 6.0–13.0 days versus 7.0; 4.0–10.0 days, p-value <0.001). Secondary outcomes were similar between both cohorts.

Table 1 Baseline characteristics

	Ovidius–TripleP cohort (n=505)	**STEP cohort (n=727)**
Demographic data		
Age (years)	67 (51–78)	73 (60–83)
Male	295 (58.4)	452 (62.2)
Caucasian	491 (97.2)	712 (97.9)
Duration of symptoms (days)	4 (2–7)	4 (2–7)
Antibiotics at home	130 (25.7)	164 (22.6)
Corticosteroids at home	34 (6.7)	14 (1.9)
Comorbidities		
Nursing home resident	19 (3.8)	0 (0.0)
Cerebrovascular accident	46 (9.1)	67 (9.2)
Malignancy	45 (8.9)	70 (9.6)
Liver disease	2 (0.4)	28 (3.9)
Renal disease	40 (7.9)	218 (30.0)
Congestive heart failure	68 (13.5)	134 (18.4)
Chronic obstructive pulmonary disease	98 (19.4)	122 (16.8)
Diabetes mellitus	77 (15.2)	139 (19.1)
Current smoker	81 (16.0)	188 (25.9)
Pneumonia severity index score	87 (63–114)	90 (64–113)
Outcome		
Length of stay (days)	8.5 (6.0–13.0)	7.0 (4.0–10.0)
ICU admission	38 (7.5)	39 (5.4)
In-hospital mortality	24 (4.8)	24 (3.3)
30-day mortality	26 (5.1)	28 (3.9)
Readmission	37 (7.3)	39 (5.4)

Data are n (%), mean (standard deviation) or median (interquartile range). ICU: intensive care unit; STEP: Steroids in Pneumonia.

Latent class modelling: identification of number of classes

We fitted latent class models ranging from two to five classes (Table 2). First, we examined clinical interpretability by plotting class-defining variables for all models and assessed whether identified classes corresponded to clinically coherent subgroups (Figure 1 and Supplemental Figure E1). In both the Ovidius–TripleP cohort and the STEP cohort, a two-class model resulted in two coherent and distinct clinical classes.

Addition of a third, fourth or fifth class resulted in further subdivision of patients assigned to class 2 in the two-class model, without adding an additional coherent or distinct clinical class. Subsequently, we explored the number of patients per subgroup in all models (Table 2). The addition of a third class to the two-class model resulted in a smaller third class of 58 patients in the Ovidius–TripleP cohort and 72 patients in the STEP cohort. We observed a further decline in the number of patients in the smallest class in a four- or five-class model. Lastly, the Bayesian information criterion was lowest in the five-class model in both the Ovidius–TripleP cohort and the STEP cohort, suggesting a better fit for the five-class model. Even though a data-driven approach suggested more than two classes, a three-class model did not result in an evident third clinical entity. Thus, clinical interpretability of the two-class models in conjunction with the relatively small number of patients in the three-, four- or five-class models led us to proceed with the two-class models for both cohorts. We will refer to the classes as class 1 and class 2 in the remainder of the manuscript. For the three-class model we show clinical characteristics for each class in the supplementary material.

Table 2 Fit statistics for latent class models from two to five class models

Number of classes	BIC	Number of patients per class				
		1	2	3	4	5
Ovidius-TripleP cohort						
2	124 577.2	411	94			
3	120 741.9	153	58	294		
4	120 507.3	61	112	296	36	
5	118 372.7	33	25	94	108	245
STEP cohort						
2	116 815.7	574	153			
3	106 770.5	99	556	72		
4	71 445.1	24	125	466	112	
5	70 684.5	132	18	44	434	99

BIC: Bayesian information criterion; STEP: Steroids in Pneumonia.

Patients were assigned to the class for which the probability of belonging to that class was the highest. Thus, all patients in both cohorts were assigned to either class 1 or class 2. In the Ovidius–TripleP cohort, 411 patients were assigned to class 1 and 94 to class 2. In the STEP cohort, 574 and 153 patients were assigned to class 1 and class 2, respectively. Probabilities of class assignment for the two-class model are presented in supplemental Figure E2. The average probability of a patient belonging to the class to which it was assigned was 99.4% for class 1 and 98.6% class 2 in the Ovidius–TripleP cohort, and 98.7% for class 1 and 99.1% for class 2 in the STEP cohort. This indicated a good model fit and robust class assignment.

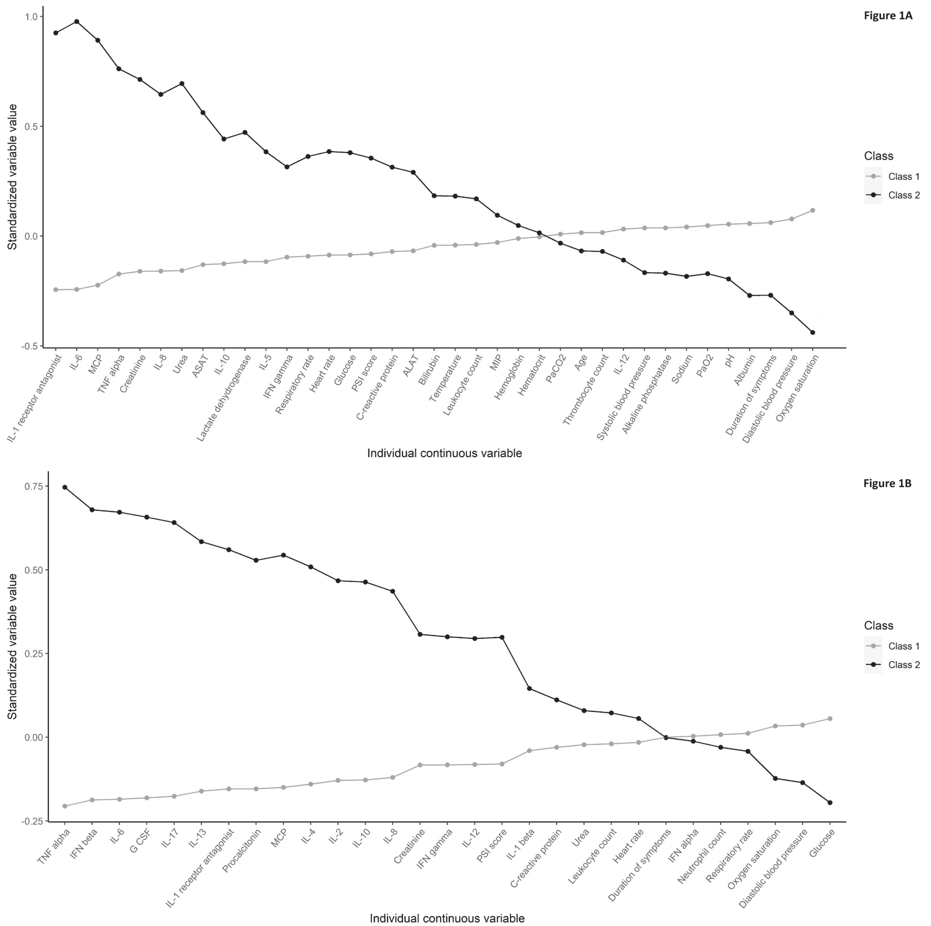

Figure 1 Continuous variables (standardised) by class assignment for the a) Ovidius–TripleP cohort and b) Steroids in Pneumonia (STEP) cohort. Differences between the standardised values of each variable by class (y-axis) for the variable shown on the x-axis. The variables are sorted by degree of separation between classes: from the maximum positive separation on the left (where the standardised value of class 2 is higher than the standardised value of class 1) to the maximum negative separation on the right (where the standardised value of class 2 to is lower than the standardised value of class 1). The crossover of the lines indicates that the standardised value for this variable was the same for classes 1 and 2 (i.e. no difference between class 1 and class 2 for this variable). Therefore, variables near the intersection of both lines are similar in both classes and thus are not class-defining. The method of variable standardisation is described in the methods section. If the standardised value of a certain variable is 1 for a class, it means that the mean value for that variable within that class was one standard deviation higher than the mean value for that variable in the whole cohort. ALAT: alanine transaminase; ASAT: aspartate transaminase; G-CSF: granulocyte colony-stimulating factor; IFN: interferon; IL: interleukin; MCP: monocyte chemoattractant protein; MIP: macrophage inflammatory protein; PaCO2: arterial carbon dioxide tension; PaO2: arterial oxygen tension; PSI: pneumonia severity index; TNF: tumour necrosis factor.

Class characteristics

Differences between class 1 and class 2 in the Ovidius–TripleP cohort are shown in Figure 1a and Table 3. The most noteworthy and clinically relevant differences were that patients in class 2 had higher plasma concentration of interleukin (IL)-1 receptor antagonist, IL-6, monocyte chemoattractant protein and tumour necrosis factor-α (TNF-α) compared to class 1. Furthermore, patients assigned to class 2 seemed to have more severe illness seeing as they had lower oxygen saturation, lower diastolic blood pressure and had a higher PSI score at admission.

Table 3 Values of variables at baseline stratified by class in the Ovidius–TripleP cohort

Variable	Class 1 (n=411)	Class 2 (n=94)	Missing n (%)
Age (years)	67 (51–79)	67 (53–76)	0 (0)
Alanine transaminase (U/L)	28 (16–44)	28 (19–55)	152 (30.1)
Albumin (g/L)	37 (33–40)	36 (33–38)	339 (67.0)
Alkaline phosphatase (U/L)	90 (70–130)	90 (61–113)	167 (33.1)
Altered mental status ¶	47 (11.4)	10 (10.6)	11 (2.2)
Aspartate transaminase (U/L)	34 (23–51)	38 (25–78)#	153 (30.3)
Bilirubin (μmol/L)	12 (9–16)	16 (12–24)#	199 (39.4)
C-reactive protein (mg/L)	196 (94–300)	294 (107–389)#	9 (1.8)
Cortisol (nmol/L)¶	226.0 (148.0–159.1)	446.8 (322.4–691.4)#	23 (4.6)
Corticosteroids at home¶	30 (7.5)	4 (4.4)	15 (3.0)
Creatinine (μmol/L)	84 (70–106)	111 (91–157)#	10 (2.0)
Diastolic blood pressure (mmHg)	75 (68–83)	70 (60– 80)#	11 (2.2)
Duration of symptoms (days)	4 (3–7)	3 (2–5)#	16 (3.2)
Glucose (mmol/L)	7.0 (6.0–8.3)	7.5 (6.2–9.8)#	39 (7.7)
Heart rate (beats/min)	95 (82–109)	110 (87–118)#	9 (1.8)
Haematocrit (L/L)	0.40 (0.36–0.43)	0.39 (0.37–0.43)	17 (3.4)
Haemoglobin (mmol/L)	8.3 (7.6–9.0)	8.3 (7.8–9.0)	10 (2.0)
Interferon-γ (pg/mL)	202.1 (16.8–288.3)	217.8 (10.0–354.9)	213 (42.2)
Interleukin-1 receptor antagonist (pg/mL)	102.8 (18.0–448.4)	1042.5 (204.2–4309.2)#	79 (15.6)
Interleukin-6 (pg/mL)	51.0 (18.0–156.3)	749.7 (101.2–2209.7)#	63 (12.5)
Interleukin-5 (pg/mL)	0.54 (0.24–0.77)	0.46 (0.26–0.61)	333 (65.9)
Interleukin-8 (p/mL)	14.8 (8.1–29.3)	59.5 (32.1–152.2)#	56 (11.1)
Interleukin-10 (pg/mL)	3.4 (1.4–9.0)	15.9 (5.8–79.7)#	94 (18.6)
Interleukin-12 (pg/mL)	7.3 (4.1–10.5)	8.3 (5.6–11.5)	337 (66.7)
Lactate dehydrogenase (U/L)	328 (252–480)	435 (313–604)#	212 (42.0)
Legionella species¶	14 (3.4)	6 (6.4)	0 (0)

Table 3 Continued

Variable	Class 1 (n=411)	Class 2 (n=94)	Missing n (%)
Leukocyte count (10^9 cells/L)	13.5 (9.5–17.7)	14.9 (10.8–20.1)	9 (1.8)
Macrophage inflammatory protein (pg/mL)	6.1 (3.7–8.5)	6.8 (4.6–10.4)	236 (47)
Male¶	236 (57.4)	59 (62.8)	0 (0)
Monocyte chemoattractant protein (pg/mL)	274.2 (74.7–536.6)	918.4 (242.9–2463.3)#	46 (9.1)
Oxygen saturation (%)	94 (92–97)	94 (88–96)#	107 (21.2)
Oxygen therapy¶	70 (17.0)	30 (31.9)#	312 (61.8)
P_{aO2} (kPa)	8.80 (7.80–10.38)	8.40 (7.10–9.90)#	124 (24.6)
P_{aCO2} (kPa)	4.40 (4.10–4.90)	4.40 (4.00–4.85)	124 (24.6)
pH	7.47 (7.44–7.50)	7.46 (7.42–7.49)	124 (24.6)
Pleural effusion¶	61 (14.8)	21 (22.3)	9 (1.8)
Pneumonia severity index score	84 (60–111)	102 (73–126)#	0 (0)
Respiratory rate (breaths/min)	22 (18–30)	25 (20–30)#	104 (20.6)
Sodium (mmol/L)	135 (132–137)	133 (129–137)#	9 (1.8)
Streptococcus pneumoniae ¶	85 (20.7)	39 (41.5)#	0 (0)
Systolic blood pressure (mmHg)	131 (120–146)	126 (112–145)	11 (2.2)
Temperature (°C)	38.2 (37.4–39.0)	38.5 (37.4–39.3)	9 (1.8)
Thrombocyte count (10^9 cells/L)	253 (200–317)	237 (177–327)	9 (1.8)
Tumour necrosis factor-α (pg/mL)	5.9 (3.1–10.2)	12.4 (6.1–29.6)#	224 (44.4)
Urea (mmol/L)	6.4 (4.6– 9.5)	9.8 (6.3–15.2)#	17 (3.4)

Data are shown as median (interquartile range) or n (%). #: statistically significant difference between class 1 and class 2. ¶: non-class-defining variables (variable not included in latent class analysis). Missing data is n (%) for the whole cohort.

Differences between class 1 and class 2 in the STEP cohort are shown in Figure 1b and Table 4. In the STEP cohort, the most noteworthy and clinically relevant differences between classes were higher plasma concentrations of TNF-α, interferon-β, IL-6, granulocyte colony stimulating factor and IL-17 in class 2 compared to class 1. Patients in class 2 also had a higher PSI score compared to class 1. However, there was no difference in oxygen saturation or diastolic blood pressure.

Table 4 Values of variables at baseline stratified by class in the STEP cohort

Variable	Class 1 (n=574)	Class 2 (n=153)	Missing n (%)
Altered mental status¶	33 (5.7)	13 (8.5)	0 (0)
C-reactive protein (mg/L)	155 (74–247)	171 (93–268)	7 (1)
Creatinine (µmol/L)	86 (68–109)	98 (72–132)#	6 (0.8)
Diastolic blood pressure (mmHg)	70 (60–78)	66 (59–75)	4 (0.6)
Duration of symptoms (days)	4 (2–7)	4 (2–7)	17 (2.3)
Glucose (mmol/L)	6.4 (5.5–7.7)	6.0 (5.5–7.3)	179 (24.6)
Granulocyte colony stimulating factor (pg/mL)	7.0 (7.0–8.7)	21.1 (9.3–59.3)#	55 (7.6)
Heart rate (beats/min)	83 (72–95)	84 (71–101)	4 (0.6)
Interferon-α (pg/mL)	0.24 (0.24–0.33)	0.56 (0.30–1.02)#	55 (7.6)
Interferon-β (pg/mL)	22.7 (14.5–34.0)	41.3 (22.0–74.1)#	55 (7.6)
Interferon-γ (pg/mL)	2.8 (2.8–2.8)	2.8 (2.8–4.6)#	55 (7.6)
Interleukin-1β (pg/mL)	1.0 (1.0–1.0)	1.0 (1.0–2.8)#	55 (7.6)
Interleukin-1 receptor antagonist (pg/mL)	33.0 (33.0–551.5)	1280.1 (33.0–6244.1)#	55 (7.6)
Interleukin-2 (pg/mL)	4.4 (4.4–4.4)	4.4 (4.4–4.4)#	55 (7.6)
Interleukin-4 (pg/mL)	5.5 (5.5–5.5)	5.5 (5.5–24.4)#	55 (7.6)
Interleukin-6 (pg/mL)	40.6 (14.6–102.5)	172.0 (59.7–748.4)#	55 (7.6)
Interleukin-8 (pg/mL)	3.9 (1.9–9.7)	19.8 (6.6–46.1)#	55 (7.6)
Interleukin-10 (pg/mL)	0.9 (0.7–1.4)	2.2 (1.3–4.8)#	55 (7.6)
Interleukin-12 (pg/mL)	1.1 (1.1–1.4)	2.2 (1.3–3.7)#	55 (7.6)
Interleukin-13 (pg/mL)	1.3 (1.3–1.3)	2.4 (1.3–8.8)#	55 (7.6)
Interleukin-17 (pg/mL)	0.57 (0.57–0.57)	0.87 (0.57–1.86)#	55 (7.6)
Legionella species¶	11 (1.9)	3 (2.0)	102 (14.0)
Leukocyte count (10^9 cells/L)	11.9 (8.7–15.6)	12.2 (9.2–15.8)	4 (0.6)
Male¶	345 (60.1)	107 (69.9)#	0 (0)
Monocyte chemoattractant protein (pg/mL)	39.8 (25.5–70.1)	66.6 (37.2–242.9)#	55 (7.6)
Neutrophil count (10^9 cells/L)	9.8 (6.9–13.2)	10.2 (7.4–13.3)	64 (9.7)
Oxygen saturation (%)	95 (92–96)	94 (92–96)	25 (3.4)
Oxygen therapy¶	298 (51.9)	79 (51.6)	6 (0.8)
Pleural effusion¶	65 (11.3)	18 (11.8)	0 (0)
Pneumonia severity index score	88 (63–111)	98 (74–131)#	0 (0)
Procalcitonin (ng/mL)	0.39 (0.16–1.68)	1.14 (0.28–10.35)#	133 (18.3)
Respiratory rate (breaths/min)	20 (18–24)	20 (17–24)	136 (18.7)
Streptococcus pneumoniae ¶	75 (13.1)	31 (20.3)#	104 (14.3)
Tumour necrosis factor-α (pg/mL)	1.8 (1.8–1.9)	2.7 (1.8–4.0)#	55 (7.6)
Urea (mmol/L)	6.6 (4.8–10.0)	7.9 (5.4–13.4)#	37 (5.1)

Data are shown as median (interquartile range) or n (%). #: statistically significant difference between class 1 and class 2. ¶: non-class-defining variables (variable not included in latent class analysis). Missing data is n (%) for the whole cohort.

4

Class prediction with a small number of variables

In order to determine whether classes could be identified based on a reduced number of variables, we tested a three-variable model including variables available for both cohorts and differing most between classes (IL-6, TNF-α and oxygen saturation at hospital admission). An area under the curve (AUC) was calculated to evaluate this reduced model compared to the full model. The AUC was 0.78 and 0.65, respectively, for the Ovidius–TripleP cohort and the STEP cohort. Contingency tables comparing class membership between reduced and full model are shown in the supplementary material (Table E2).

Association between class and clinical outcomes

Subsequently, we assessed clinical outcomes in both classes (Table 5). In the Ovidius–TripleP cohort, patients in class 2 had a significantly longer LOS (10.5; 6.5–16.0 days versus 8.0; 6.0–12.0 days, p-value <0.01) and higher rate of ICU admissions. In-hospital mortality and 30-day mortality rates were significantly higher in class 2. Similar results were observed in the STEP cohort, as patients in class 2 had a longer LOS (7.0; 5.0–12.0 days versus 7.0; 4.0–10.0 days, p-value <0.01), and a higher in-hospital mortality rate (Table 5).

Effect of corticosteroids on outcome stratified by class

Lastly, we used the data from the Ovidius trial and the STEP cohort to determine whether classes responded differently to randomly assigned adjunctive treatment with corticosteroids (Table 6). In the Ovidius trial, dexamethasone reduced LOS in patients assigned to class 2 (6.5; 5.5–10.0 days versus 9.5; 5.0–14.5 days), whereas LOS was similar between treatment groups in class 1 (p-value for interaction 0.02). In the STEP cohort, there was no significant interaction for LOS between class assignment and adjunctive treatment with corticosteroids. In both cohorts, we found no significant interaction for secondary outcomes between class assignment and adjunctive treatment with corticosteroids.

Table 5 Association between class assignment and clinical outcomes

Ovidius–TripleP cohort			
Clinical outcome	**Class 1 (n=411)**	**Class 2 (n=94)**	**p-value**
Length of stay (days)	8.0 (6.0–12.0)	10.5 (6.5–16.0)	<0.01
ICU admission	16 (3.9%)	22 (23.4%)	<0.01
In-hospital mortality	14 (3.4%)	10 (10.6%)	0.01
30-day mortality	15 (3.6%)	11 (11.7%)	<0.01
Readmission	29 (7.1%)	8 (8.5%)	0.79
STEP cohort			
Clinical outcome	**Class 1 (n=574)**	**Class 2 (n=153)**	**p-value**
Length of stay (days)	7.0 (4.0–10.0)	7.0 (5.0–12.0)	<0.01
ICU admission	28 (4.9%)	11 (7.2%)	0.35
In-hospital mortality	13 (2.3%)	11 (7.2%)	<0.01
30-day mortality	18 (3.1%)	10 (6.5%)	0.09
Readmission	30 (5.2%)	9 (5.9%)	0.91

Data are n (%) or median (interquartile range). ICU: intensive care unit.

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Table 6 Differential response to adjunctive corticosteroid treatment by latent class assignment

Ovidius trial					
	Class 1 (n=251)		**Class 2 (n=52)**		
	Corticosteroid (n=124)	**Placebo (n=128)**	**Corticosteroid (n=27)**	**Placebo (n=25)**	**P***
Length of stay (days)	6.5 (5.0–8.5)	7.5 (5.5–10.5)	6.5 (5.5–10.0)	9.5 (5.0–14.5)	0.02
ICU admission	4 (3.2)	4 (3.1)	3 (11.1)	6 (24.0)	0.64
In-hospital mortality	7 (5.6)	3 (2.3)	1 (3.7)	5 (20.0)	0.12
30-day mortality	7 (5.6)	4 (3.1)	2 (7.4)	5 (20.0)	0.33
Readmission	6 (4.8)	4 (3.1)	1 (3.7)	3 (12.0)	0.56
STEP cohort					
	Class 1 (n=574)		**Class 2 (n=153)**		
	Corticosteroid (n=285)	**Placebo (n=289)**	**Corticosteroid (n=77)**	**Placebo (n=76)**	**P***
Length of stay (days)	6.0 (4.0–9.0)	7.0 (5.0–10.0)	7.0 (4.0–11.0)	8.0 (5.0–13.3)	0.46
ICU admission	11 (3.9)	17 (5.9)	6 (7.8)	5 (6.6)	0.61
In-hospital mortality	8 (2.8)	5 (1.7)	5 (6.5)	6 (7.9)	0.71
30-day mortality	11 (3.9)	7 (2.4)	4 (5.2)	6 (7.9)	0.50
Readmission	21 (7.4)	9 (3.1)	5 (6.5)	4 (5.3)	0.69

Data are n (%) or median (interquartile range). *p-value: for interaction between class assignment and corticosteroid treatment. ICU: intensive care unit.

DISCUSSION

In this secondary analysis of three controlled studies, LCA identified two distinct classes of CAP patients with different biomarker profiles, clinical characteristics and clinical outcomes. Classes were identified in two independent cohorts, despite multiple significant differences in baseline characteristics between cohorts. In the Ovidius trial, adjunctive treatment with corticosteroids reduced LOS only in patients assigned to class 2. We found no differential treatment response for LOS in the STEP cohort or for secondary outcomes in both cohorts.

In both cohorts, class 2 was characterised by higher concentrations of inflammatory biomarkers, creatinine and higher PSI scores. Additionally, patients assigned to class 2 in the Ovidius–TripleP cohort had lower oxygen saturation, lower diastolic blood pressure and higher incidence of oxygen therapy. In contrast, patients in class 1 were characterised by lower concentrations of inflammatory plasma biomarkers and lower PSI scores. Furthermore, in the Ovidius–TripleP cohort, cortisol was also higher in class 2 compared to class 1; we assume this is explained by the that fact patients with more inflammation have a higher activation of the hypothalamic–pituitary–adrenal axis and thus higher cortisol levels than patients with lower levels of systemic inflammation because they are more severely ill. Moreover, in both cohorts, LOS was longer, and incidence of ICU admissions and mortality rates were higher in class 2. Thus, patients in class 2 had a stronger systemic inflammatory response, whereas patients in class 1 had fewer signs of systemic inflammation. Patients in class 2 were more likely to benefit from the anti-inflammatory effects of corticosteroids, whereas the patients assigned to class 1 were less likely to benefit from the anti-inflammatory effects, at a similar risk of adverse effects.

Corticosteroids reduced LOS in patients with CAP in the Ovidius trial and in the STEP trial.[16,17] An individual patient data meta-analysis enrolling data from six randomised controlled trials comparing corticosteroids with placebo in 1506 patients with CAP, including the Ovidius trial and STEP trial, confirmed that adjunctive treatment with corticosteroids reduced LOS.[3] In this meta-analysis, however, the authors could not identify patient subgroups more likely to benefit from corticosteroids based on PSI score (PSI class 1–3 versus PSI class 4–5), initial C-reactive protein concentration (cut-off 188 mg/L), initial ICU admission, or systemic inflammatory response syndrome criteria. However, in a clinically heterogeneous condition as CAP, it is unlikely that all patients benefit equally from corticosteroids.[9,14]

In the Ovidius trial, we found that patients assigned to class 2 who were treated with corticosteroids showed a significant reduction in LOS, whereas corticosteroids did not reduce LOS in patients assigned to class 1. These results suggest that the subgroup of CAP patients with signs of a systemic inflammatory response benefit from corticosteroids and patients with a less pronounced systemic inflammatory response do not. However, these results could not be verified in the STEP cohort, even though PSI score was similar between both cohorts. A possible explanation is that LCAs were performed separately in the Ovidius–TripleP cohort and the STEP cohort and included a different set of class-defining variables for each cohort (Figure 1) because available biomarkers differed between both cohorts. Thus, the LCA models were not identical in both cohorts. Furthermore, concentrations of inflammatory biomarkers were higher at baseline in the Ovidius cohort compared to the STEP cohort, indicating a more pronounced inflammatory response in the Ovidius cohort that corticosteroids could inhibit. The reduced three variable model – consisting of IL-6, TNF-α and oxygen saturation – showed that the AUC for class assignment was higher in the Ovidius–TripleP cohort as compared to the STEP cohort. This also suggests that the Ovidius–TripleP cohort relies more on inflammatory response. Adding to the above, in the STEP cohort, disease severity defined by PSI score was mainly influenced by higher age and more comorbidities, whereas in the Ovidius cohort PSI score was mainly influenced by clinical characteristics and biomarker data indicative of more severe disease. Consequently, clinical variables at baseline did not differ between class 1 and class 2 in the STEP cohort, whereas clinical variables at baseline did differ between classes in the Ovidius cohort. Other explanations might be the difference in corticosteroid therapy (dexamethasone versus prednisolone) or the shorter LOS in the STEP cohort (median 8.5; 6.0–13.0 days in Ovidius cohort versus 7.0; 4.0–10.0 days in STEP cohort) making potential differences between classes in the STEP cohort more difficult to detect.

Inflammatory biomarkers contributed more to the determination of classes than clinical data, including C-reactive protein, procalcitonin or leukocyte count. These results suggest that the inflammatory biomarkers were able to identify aspects of CAP pathophysiology that otherwise remained hidden in routinely collected clinical data.

This study has several limitations. First, LCA model selection and interpretation often involves a level of subjectivity.[19] We decided to select a two-class model instead of more classes based on clinical interpretability and the number of patients assigned to the smallest class. Hypothetically, a third class or even a fourth class could have been forced in by generating a smaller cluster of patients with a more extreme set of variables. However, a three-or-more-class model did not result in additional groups with more extreme variables, but in mixed classes without a coherent clinical pattern. Second, we assumed patients in class 2 to have a systemic inflammatory response and patients in class 1 to have a more controlled inflammatory response based on distribution of inflammatory biomarkers in plasma. We did not measure the pulmonary response and therefore do not know whether inflammation is indeed contained locally in patients assigned to class 1. We refrained from using terms as hyperinflammatory or hypoinflammatory, previously proposed in subgroups of patients with acute respiratory distress syndrome, as all patients are admitted because of CAP, which can hardly be considered a hypoinflammatory condition.[20,21] Third, this is a secondary analysis which requires prospective validation before definitive conclusions regarding patient subgroup identification and adjunctive corticosteroid treatment can be drawn. Fourth, LOS was calculated from day of hospital admission to day of discharge or day of in-hospital death. Thus, LOS was underestimated in patients that died during hospital admission. However, in both cohorts, in-hospital mortality rate was higher in class 2 as compared to class 1. If reported LOS were an underestimation, this would mainly be the case in class 2 and the difference in LOS between classes would be even larger than reported. Fifth, the clinical and biomarker data used in this analysis was limited to the data available for both cohorts and to data obtained at time of hospital admission. As the aim of data collection for the original studies was to calculate the PSI score, clinical data used in the LCA resembled the PSI score to some extent and PSI score differed significantly between class 1 and class 2 in both cohorts. However, the classes identified by LCA were largely based on biomarker data and thus captured different subgroups of patients than classes based on PSI score only. Lastly, because data was obtained at time of hospital admission, it is unknown whether identified classes remained stable later during the course of CAP.

To our knowledge, this is the first study that identified CAP subgroups through LCA. Because the present study is a proof-of-concept study, our results are not directly applicable for daily clinical practice. Future studies should include validation of our findings in a third independent cohort, after which a clinically useful model with a limited number of variables should be developed to ensure applicability. Lastly, validation of these clinical models in predicting response to treatment should be assessed in prospective studies.

In conclusion, we identified two classes of CAP patients with different clinical characteristics, inflammatory profiles and clinical outcomes in two independent cohorts. Furthermore, in the Ovidius trial, adjunctive treatment with corticosteroids reduced LOS only in the patients assigned to class 2 and not in the patients assigned to class 1. Given the different response to adjunctive treatment in subgroups in the Ovidius cohort, identification of subgroups might provide a useful basis for improved patient selection in future clinical trials.

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SUPPLEMENTARY MATERIAL

METHODS

Definition of CAP

CAP was defined as a new pulmonary infiltrate on chest x-ray, accompanied by at least one of the following criteria: cough, sputum, temperature >38°C (or <35°C), auscultatory findings consistent with pneumonia, C-reactive protein >15 mg/L, leukocyte count >$10x10^9$ cells/L or <$4x10^9$ cells/L, or >10% bands in leucocyte differentiation.[1,2]

Systemic biomarkers

Systemic concentrations of inflammatory biomarkers were measured in plasma samples obtained on the day of hospital admission before administration of any study medication. Samples were stored at -80°C. Analysis was performed using multiplex multi-analyte profiling (Millipore, Billerica, USA), as described previously.[3,4] Different biomarker panels were used in the Ovidius-TripleP cohort and the STEP cohort (Table 1).

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VARIABLES INCLUDED IN THE LCA MODEL

Class defining variables included in the LCA of the Ovidius-TripleP cohort

Age	Urea	CRP	Interleukin-6
Systolic blood pressure	Albumin	Thrombocyte count	Interleukin-8
Diastolic blood pressure	ALAT	Hemoglobin	Interleukin-10
Symptom duration	ASAT	Hematocrit	Interleukin-12
Oxygen saturation	Alkaline phosphatase (U/L)	pH	Monocyte chemoattractant protein
Body temperature	LDH	PaO2	Macrophage inflammatory protein
Heart rate	Bilirubin	PaCO2	Tumour necrosis factor α
Respiratory rate	Glucose	Interleukin-1 receptor antagonist	Interferon gamma
PSI score	Sodium	Interleukin-5	Interleukin-12
Creatinine			

Class defining variables included in the LCA of the STEP cohort

Diastolic blood pressure	Glucose	Interleukin-4	Monocyte chemoattractant protein
Symptom duration	CRP	Interleukin-6	Tumour necrosis factor alpha
Oxygen saturation	Procalcitonin	Interleukin-8	Interferon alpha
Heart rate	Neutrophil count	Interleukin-10	Interferon beta
Respiratory rate	White blood cell count	Interleukin-12	Interferon gamma
PSI score	Interleukin-1 beta	Interleukin-13	
Creatinine	Interleukin-1 receptor antagonist	Interleukin-17	
Urea	Interleukin-2	Granulocyte-colony stimulating factor	

SUPPLEMENTARY FIGURES

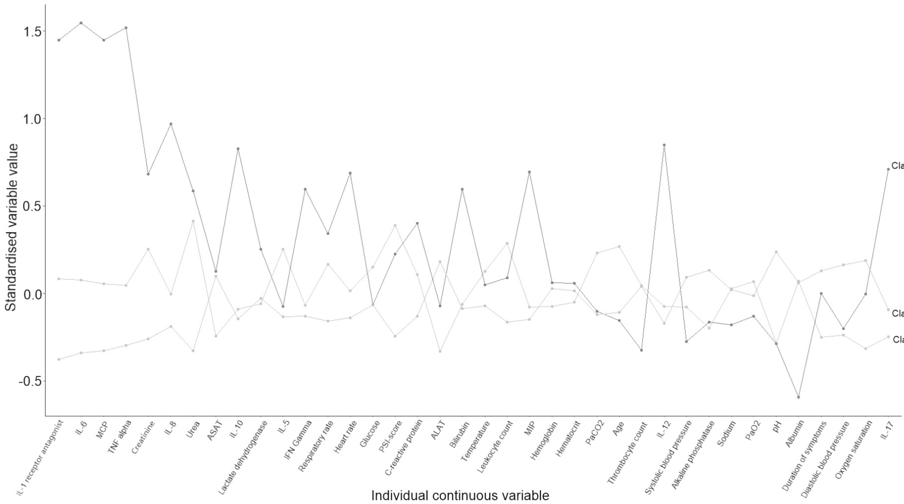

Figure E1a-1 Three-class model

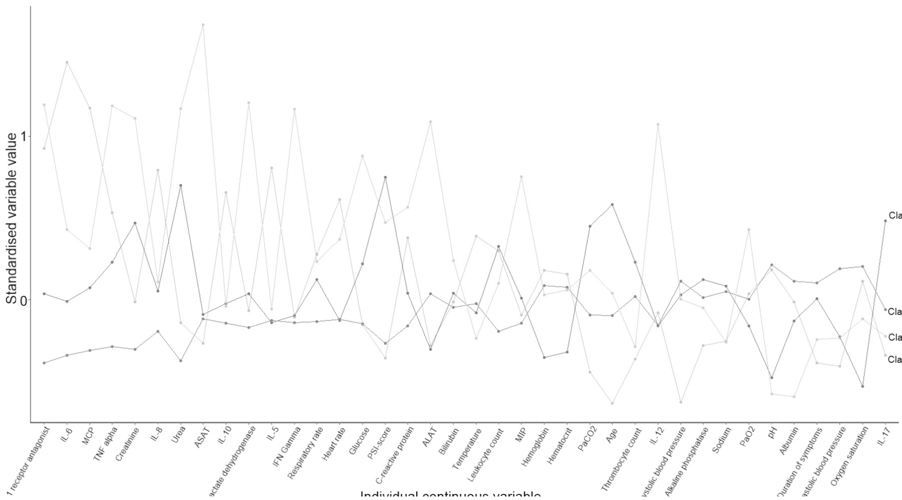

Figure E1a-2 Four-class model

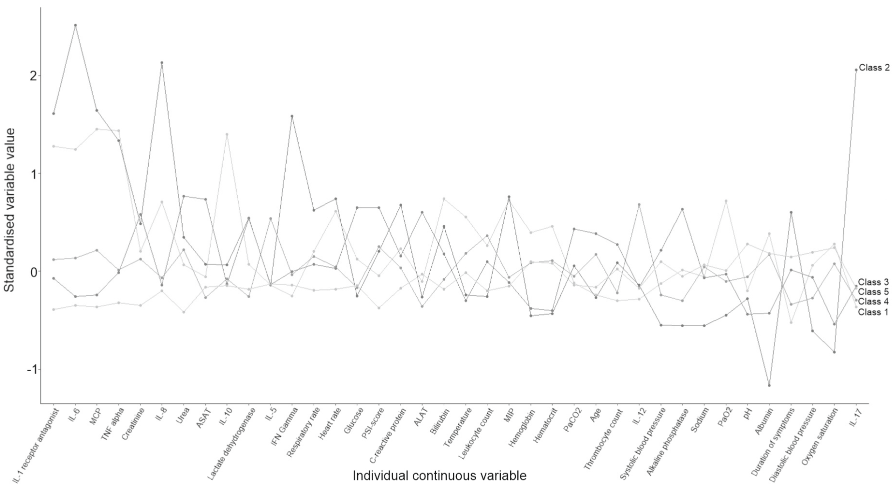

Figure E1a-3 Five-class model

Supplementary Figure E1a Continuous variables by class assignment in a three, four, or five-class model in the Ovidius-TripleP cohort.

On the Y-axis differences in the standardised values of each variable by subgroup are shown. The individual continuous variables are shown along the x-axis. Variables are sorted by degree of separation between classes.
Abbreviations: IL= interleukin; MCP= Monocyte chemoattractant protein; TNF=Tumour necrosis factor; , ASAT= Aspartate transaminase ; IFN= Interferon; PSI= Pneumonia Severity index; ALAT=Alanine transaminase; MIP= Macrophage inflammatory protein.

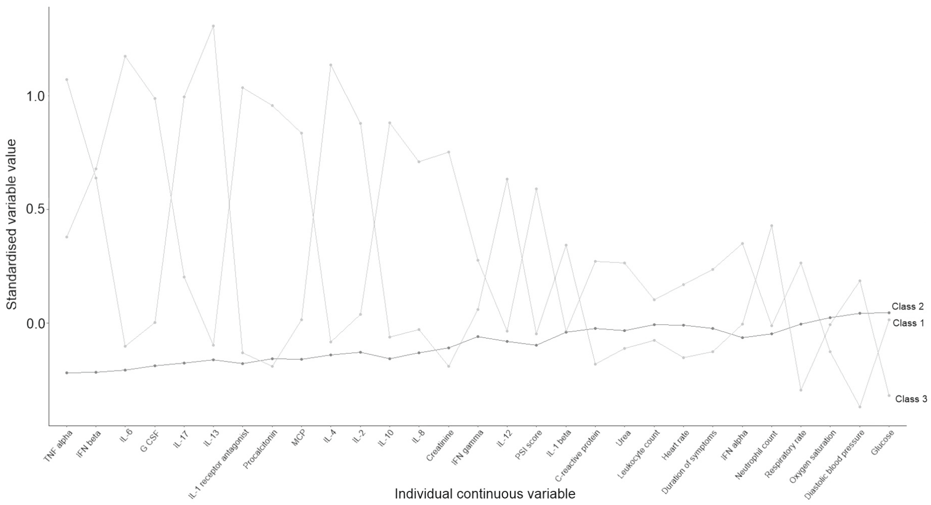

Figure E1b-1 Three-class model

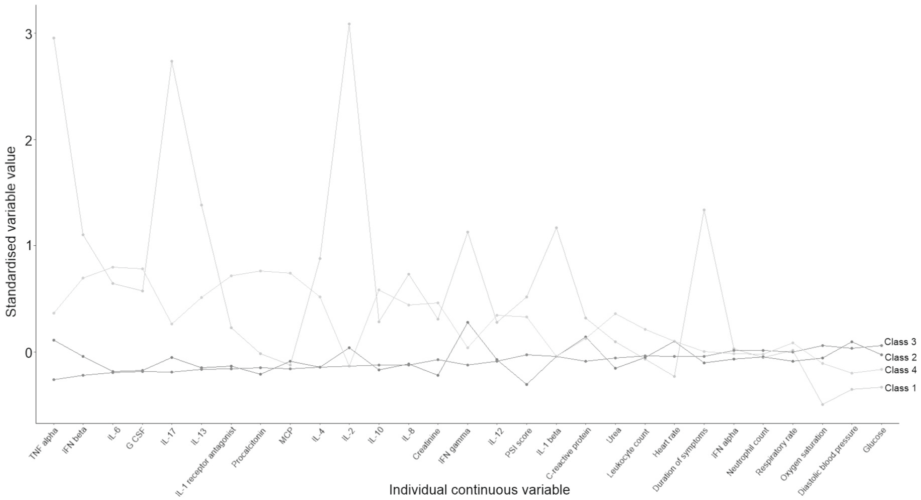

Figure E1b-2 Four-class model

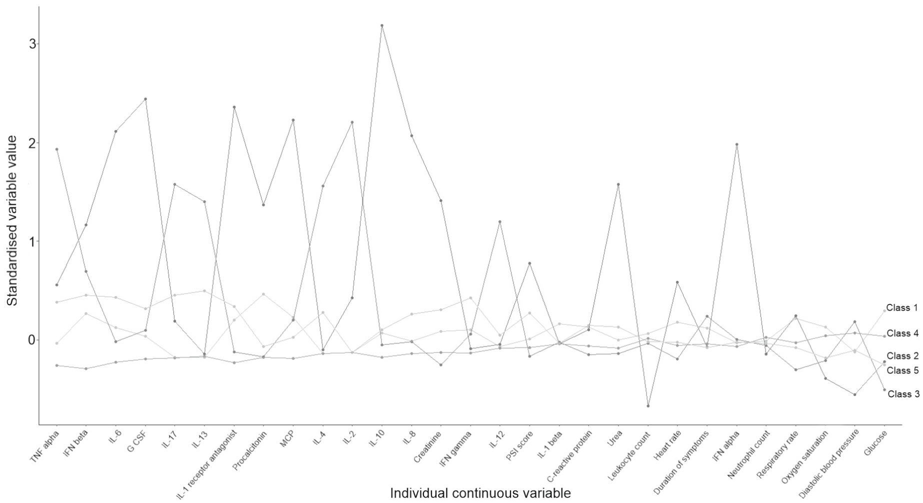

Figure E1b-3 Five-class model

Figure E1b Continuous variables by class assignment in a three, four, or five-class model in the STEP cohort.

On the Y-axis differences in the standardised values of each variable by subgroup are shown. The individual continuous variables are shown along the x-axis. Variables are sorted by degree of separation between classes.
Abbreviations: IL= interleukin; MCP= Monocyte chemoattractant protein; TNF=Tumour necrosis factor; IFN= Interferon; PSI= Pneumonia Severity index; G-CSF= Granulocyte colony-stimulating factor.

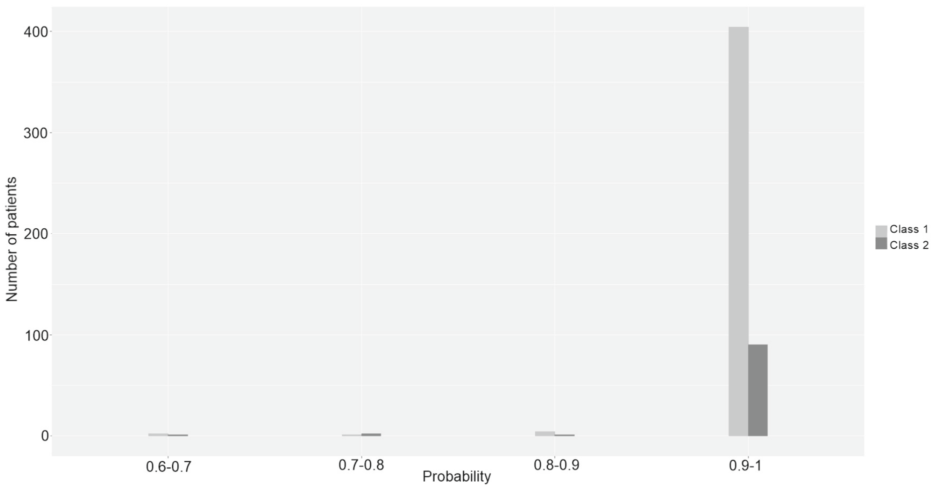

Figure E2a

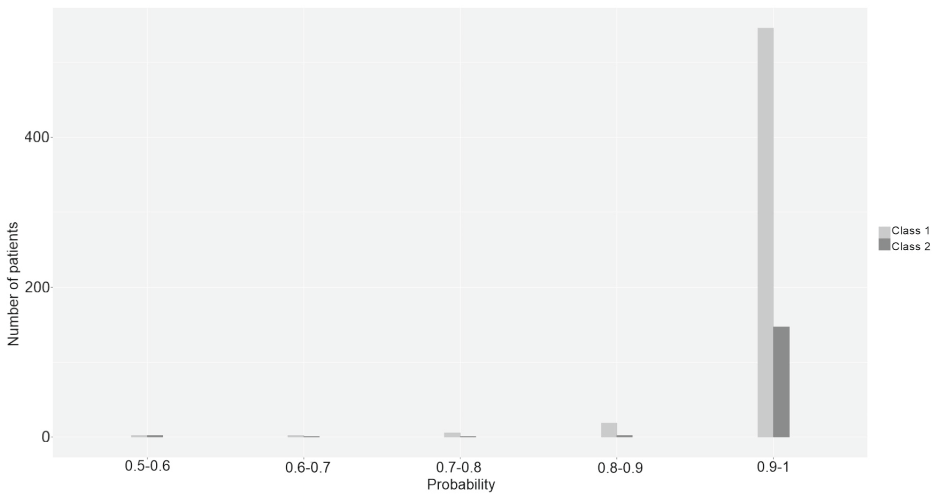

Figure E2b

Figure E2 Probability of class assignment in a two-class model for the Ovidius-TripleP cohort (Figure E2a) and the STEP cohort (Figure E2b).

In the figures above the probability of class assignment is shown on the x-axis and the number of patients on the y-axis. This figure shows that the majority of patients had a chance of 90-100% of being assigned to the correct class. For subsequent analyses, patients were assigned to the class with the highest probability of assignment.

SUPPLEMENTARY TABLES

Table E1 Baseline characteristics Ovidius-TripleP cohort and STEP cohort

	Ovidius-TripleP cohort (n = 505)	STEP cohort (n = 727)
Demographic data		
Age (years)	67 (51-78)	73 (60-83)
Male	295 (58.4)	452 (62.2)
Caucasian	491 (97.2)	712 (97.9)
Duration of symptoms (days)	4 (2-7)	4 (2-7)
Antibiotics at home	130 (25.7)	164 (22.6)
Corticosteroids at home	34 (6.7)	14 (1.9)
Comorbidities		
Nursing home resident	19 (3.8)	0 (0.0)
Cerebrovascular accident	46 (9.1)	67 (9.2)
Malignancy	45 (8.9)	70 (9.6)
Liver disease	2 (0.4)	28 (3.9)
Renal disease	40 (7.9)	218 (30.0)
Congestive heart failure	68 (13.5)	134 (18.4)
Chronic obstructive pulmonary disease	98 (19.4)	122 (16.8)
Diabetes mellitus	77 (15.2)	139 (19.1)
Current smoker	81 (16.0)	188 (25.9)
Clinical data		
Altered mental status*	57 (11.3)	46 (6.3)
Pleural effusion	82 (16.2)	86 (11.8)
Systolic blood pressure (mmHg)	130 (118-146)	124 (110-140)
Diastolic blood pressure (mmHg)	75 (66-82)	69 (60-78)
Heart rate (beats per minute)	97 (84-111)	83 (72-96)
Respiratory rate (breaths per minute)	24 (20-30)	20 (18-24)
Temperature (°C)	38.2 (37.4-39.0)	37.6 (37.0-38.2)
Oxygen saturation (%)	94 (91-97)	94 (92-96)
Oxygen therapy	100 (19.8)	377 (51.9)
Oxygen therapy (L/min)	1 (0-4)	2 (2-4)
Pneumonia severity index score	87 (63-114)	90 (64-113)
Routine laboratory data		
Leukocyte count (10^9 cells per L)	13.8 (9.7-18.4)	12.0 (8.8-15.6)
Neutrophil count (10^9 cells per L)	-	9.9 (6.9-13.3)
Thrombocyte count (10^9 cells per L)	250 (197-318)	-
C-reactive protein (mg/L)	210 (95-317)	160 (79-249)

4

Table E1 Continued

	Ovidius-TripleP cohort (n = 505)	STEP cohort (n = 727)
Procalcitonin (ng/mL)	-	0.46 (0.17-2.50)
Hematocrit (L/L)	0.40 (0.37-0.43)	-
Hemoglobin (mmol/L)	8.3 (7.6-9.0)	-
Urea (mmol/L)	6.8 (4.8-10.2)	6.9 (4.9-10.4)
Creatinine (µmol/L)	90 (71-112)	88 (69-113)
Sodium (mmol/L)	134 (131-137)	137 (134-139)
Glucose (mmol/L)	7.1 (6.0-8.6)	7.3 (6.3-8.9)
pH	7.47 (7.44-7.49)	-
PaO_2 (kPa)	8.7 (7.7-10.3)	-
$PaCO_2$ (kPa)	4.4 (4.1-4.9)	-
Alkaline phosphatase (U/L)	90 (68-127)	-
Aspartate transaminase (U/L)	35 (23-52)	-
Alanine transaminase (U/L)	28 (17-45)	-
Lactate dehydrogenase (U/L)	351 (255-518)	-
Bilirubin (µmol/L)	13 (9-17)	-
Albumin (g/L)	37 (33-39)	32 (28-36)
Biomarker data		
Interleukin-1 receptor antagonist (pg/mL)	163.8 (25.1-694.7)	33.0 (33.0-1126.5)
Interleukin-1 beta (pg/mL)	-	1.0 (1.0-1.0)
Interleukin-2 (pg/mL)	-	4.4 (4.4-4.4)
Interleukin-4 (pg/mL)	-	5.5 (5.5-5.5)
Interleukin-5 (pg/mL)	0.5 (0.3-0.7)	-
Interleukin-6 (pg/mL)	72.0 (22.5-248.7)	52.0 (19.0-142.8)
Interleukin-8 (pg/mL)	18.9 (9.1-42.6)	5.0 (2.0-13.0)
Interleukin-10 (pg/mL)	4.5 (1.6-14.2)	1.0 (0.7-1.9)
Interleukin-12 (pg/mL)	7.4 (4.3-10.8)	1.2 (1.1-1.7)
Interleukin-13 (pg/mL)	-	1.3 (1.3-1.3)
Interleukin-17 (pg/mL)	-	0.6 (0.6-0.6)
Tumor necrosis factor alpha (pg/mL)	6.7 (3.6-12.4)	1.7 (1.7-2.3)
Interferon alpha (pg/mL)	-	0.3 (0.3-0.4)
Interferon beta (pg/mL)	-	24.0 (15.0-41.0)
Interferon gamma (pg/mL)	205.9 (12.8-298.6)	2.8 (2.8-2.8)
Monocyte chemoattractant protein (pg/mL)	317.6 (88.5-654.2)	43.0 (27.0-84.8)
Macrophage inflammatory protein (pg/mL)	6.3 (3.9-8.8)	-
Granulocyte colony stimulating factor (pg/mL)	-	7.0 (7.0-13.0)

Table E1 Continued

	Ovidius-TripleP cohort (n = 505)	STEP cohort (n = 727)
Causative microorganism		
S. pneumoniae	124 (24.6)	106 (14.6)
H. influenzae	27 (5.3)	-
Legionella species	20 (4.0)	13 (1.8)
C. burnetii	28 (5.5)	-
Other	96 (19.0)	-
None identified	210 (41.6)	-
Outcome		
Length of stay (days)	8.5 (6.0-13.0)	7.0 (4.0-10.0)
ICU admission	38 (7.5)	39 (5.4)
In-hospital mortality	24 (4.8)	24 (3.3)
30-day mortality	26 (5.1)	28 (3.9)
Readmission	37 (7.3)	39 (5.4)

Data are n (%), mean (SD), or median (IQR). * Defined as a state of awareness that differed from the normal awareness of a conscious person, scored by the attending physician.

Table E2 Contingency tables comparing class membership in the reduced model and the full model for Ovidius-TripleP cohort and STEP cohort

		Full model	
Ovidius-TripleP		**Class 1**	**Class 2**
Reduced model	Class 1	343	26
	Class 2	68	68
		Full model	
STEP		**Class 1**	**Class 2**
Reduced model	Class 1	515	90
	Class 2	59	63

Data are n.

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Table E3 Values of variables at baseline stratified by class in the Ovidius-TripleP cohort for a three-class model

Variable	Class 1 (n=153)	Class 2 (n=58)	Class 3 (n=294)
Temperature (ºC)	38.4 [37.4 - 39.1]	38.3 [37.4 - 39.2]	38.1 [37.4 - 39.0]
Leukocyte count (10^9 cells per L)	15.7 [11.1- 20.6]	13.6 [9.2- 18.5]	12.6 [9.4- 16.6]
C-reactive protein (mg/L)	235 [90 - 352]	297 [110- 428]	190 [97 - 271]
Age (years)	72 [60- 81]	66 [41- 76]	63 [50 - 76]
Systolic blood pressure (mmHg)	126 [112- 146]	127 [112 - 143]	134 [120 - 147]
Diastolic blood pressure (mmHg)	70 [62 - 79]	70 [60 - 80]	77 [70 - 85]
Heart rate (beats per minute)	100 [84 - 113]	110 [99 - 126]	94 [82 - 107]
Respiratory rate (breaths per minute)	25 [20 - 30]	25 [20 - 30]	20 [18 - 30]
Hematocrit (L/L)	0.39 [0.36- 0.43]	0.40 [0.37- 0.43]	0.40 [0.37- 0.43]
Urea (mmol/L)	9.0 [6.3 – 13.7]	9.8 [6.4- 15.3]	5.7 [4.3 - 8.4]
Sodium (mmol/L)	134 [131 - 137]	133 [130 - 137]	135 [132 - 137]
Glucose (mmol/L)	7.3 [6.1 - 9.1]	7.4 [6.2- 8.6]	7.0 [6.0 - 8.3]
PaO_2 (kPa)	8.70 [7.50 - 10.80]	8.40 [7.68- 9.50]	8.90 [7.90-10.22]
$PaCO_2$ (kPa)	4.40 [4.10 - 5.10]	4.55 [4.00 - 4.93]	4.40 [4.00 - 4.73]
Creatinine (µmol/L)	99 [81 - 134]	107 [83 - 139]	82 [68 - 100]
Alkaline phosphatase (U/L)	86 [64 - 115]	80 [61 - 110]	96 [71 - 137]
Aspartate transaminase (U/L)	32 [24- 43]	47 [24 - 81]	35 [23 - 60]
Alanine transaminase (U/L)	22 [15 - 33]	28 [20 - 45]	32 [18 - 58]
Lactate dehydrogenase (U/L)	370 [265 - 489]	435 [304 - 547]	326 [248- 502]
Bilirubin (µmol/L)	13 [9 - 16]	18 [14 - 26]	12 [9 - 17]
Albumin (g/L)	37 [33 - 40]	35 [31 - 37]	37 [34 - 39]
Hemoglobin (mmol/L)	8.2 [7.5- 9.0]	8.3 [7.8 - 9.0]	8.4 [7.6 - 9.1]
Thrombocyte count (10^9 cells per L)	261 [197 - 315]	228 [177 - 292]	250 [201 - 324]
Oxygen saturation (%)	93 [90 - 97]	94 [91 - 96]	95 [92 - 97]
Duration of symptoms (days)	3 [2 - 5]	4 [2 - 6]	5 [3 - 7]
Interleukin-1 receptor antagonist (pg/mL)	387.9 [72.9- 1538.6]	1937.5 [628.4- 5823.8]	56.4 [11.4- 242.2]
Interleukin-6 (pg/mL)	220.6 [73.1 - 697.7]	1427.2 [258.1 - 2922.7]	35.6 [15.0 - 81.7]
Interleukin-8 (pg/mL)	37.2 [19.5 - 60.9]	113.6 [42.6 - 267.0]	11.5 [6.6 - 19.1]
Interleukin-10 (pg/mL)	11.1 [3.8- 28.9]	55.6 [10.9- 179.6]	2.2 [1.1- 4.8]
Pneumonia severity index score	106 [76 - 129]	95 [70 - 123]	77 [56 - 102]

Table E3 Continued

Variable	Class 1 (n=153)	Class 2 (n=58)	Class 3 (n=294)
Tumor necrosis factor alpha (pg/mL)	9.9 [6.5- 16.2]	32.2 [11.1- 49.0]	5.1 [2.6- 7.7]
Interferon gamma (pg/mL)	239.1 [21.2- 312.5]	195.0 [8.5- 406.7]	182.9 [17.1- 266.9]
Monocyte chemoattractant protein (pg/mL)	462.4 [143.9- 1122.0]	1957.5 [327.3- 3124.5]	226.9 [56.3- 425.0]
Macrophage inflammatory protein (pg/mL)	7.2 [4.9- 9.3]	7.2 [5.2- 12.2]	5.4 [3.4- 7.2]
Interleukin-12 (pg/mL)	9.3 [5.1 - 12.3]	8.5 [5.6 - 11.7]	6.5 [3.8- 10.0]
Interleukin-5 (pg/mL)	0.54 [0.32- 0.81]	0.42 [0.22- 0.60]	0.52 [0.23- 0.67]
pH	7.45 [7.42 - 7.48]	7.45 [7.42 - 7.48]	7.48 [7.45 - 7.50]
Cortisol (nmol/L)	328.6 [225.7 - 540.3]	526.7 [339.3 - 774.7]	195.8 [133.6 - 305.2]
Altered mental status	26 (17.0)	4 (6.9)	27 (9.2)
Pleural effusion	29 (19.0)	15 (25.9)	38 (12.9)
Oxygen therapy	43 (28.1)	18 (31.0)	39 (13.3)
Female	67 (43.8)	23 (39.7)	120 (40.8)

Data are n (%) or mean (SD).

Table E4 Values of variables at baseline stratified by class in the STEP cohort for a three-class model

Variable	Class 1 (n=99)	Class 2 (n=556)	Class 3 (n=72)
C-reactive protein (mg/L)	190 [72 - 294]	168 [81 - 250]	127 [67 - 210]
Diastolic blood pressure (mmHg)	65 [57 - 72]	70 [60 - 78]	69 [60 - 80]
Heart rate (beats per minute)	88 [72 - 104]	84 [73 - 95]	82 [70 - 95]
Respiratory rate (breaths per minute)	22 [18 - 26]	20 [18 - 24]	20 [16 - 24]
Urea (mmol/L)	9.3 [6.4 - 14.8]	6.6 [4.8 - 9.8]	7.0 [4.5 - 9.9]
Glucose (mmol/L)	6.5 [5.6 - 7.7]	6.5 [5.7 - 7.8]	5.8 [5.2 - 6.5]
Creatinine (μmol/L)	109 [85 - 177]	86 [67 - 108]	84 [70 - 106]
Leukocyte count (10^9 cells per L)	11.5 [7.4 - 17.1]	12.0 [8.7 - 15.9]	12.1 [9.3 - 14.6]
Oxygen saturation (%)	94 [92 - 97]	95 [92 - 96]	94 [92 - 96]
Pneumonia severity index score	106 [78 - 141]	89 [63 - 111]	82 [63 - 105]
Duration of symptoms (days)	4 [2 - 7]	4 [2 - 7]	4 [2 - 6]
Granulocyte colony stimulating factor (pg/mL)	33.0 [13.0 – 114.3]	7.0 [7.0 – 8.0]	14.0 [7.0 – 22.5]
Interferon alpha (pg/mL)	0.67 [0.39 - 1.24]	0.25 [0.25 - 0.30]	0.51 [0.27 - 1.10]
Interferon beta (pg/mL)	58.0 [34.0 - 106.5]	22.0 [14.0 – 33.0]	30.0 [17.0 – 55.0]
Interferon gamma (pg/mL)	2.8 [2.8 - 3.8]	2.8 [2.8 - 2.8]	2.8 [2.8 - 4.2]
Interleukin-1 beta (pg/mL)	1.0 [1.0 - 1.3]	1.0 [1.0 – 1.0]	1.0 [1.0 - 3.5]

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Table E4 Continued

Variable	Class 1 (n=99)	Class 2 (n=556)	Class 3 (n=72)
Interleukin-1 receptor antagonist (pg/mL)	5375.0 [1466.0 - 11687.3]	33.0 [33.0 – 495.0]	33.0 [33.0 – 733.0]
Interleukin-10 (pg/mL)	3.2 [2.1 - 13.1]	0.9 [0.6 - 1.3]	1.5 [1.0 - 2.7]
Interleukin-12 (pg/mL)	1.8 [1.1 - 2.8]	1.1 [1.1 - 1.4]	2.0 [1.2 - 4.5]
Interleukin-13 (pg/mL)	1.3 [1.3 - 2.5]	1.3 [1.3 - 1.3]	4.0 [1.3 - 13.3]
Interleukin-17 (pg/mL)	0.6 [0.6 - 1.4]	0.6 [0.6 - 0.6]	0.8 [0.6 - 1.7]
Interleukin-2 (pg/mL)	4.4 [4.4 - 4.4]	4.4 [4.4 - 4.4]	4.4 [4.4 - 4.4]
Interleukin-4 (pg/mL)	5.5 [5.5 - 6.9]	5.5 [5.5 - 5.5]	9.0 [5.5 – 32.6]
Interleukin-6 (pg/mL)	540.5 [125.5 - 1422.5]	41.0 [15.0 – 97.0]	73.0 [28.5 - 170.5]
Interleukin-8 (pg/mL)	39.0 [17.8 – 81.0]	4.0 [2.0 – 9.0]	7.0 [4.0 - 16.5]
Monocyte chemoattractant protein (pg/mL)	168.0 [71.3 - 400.3]	39.0 [25.0 – 66.0]	45.0 [27.0 - 74.5]
Tumor necrosis factor alpha (pg/mL)	2.8 [1.7 - 3.9]	1.7 [1.7 - 1.8]	2.5 [1.7 - 3.5]
Procalcitonin (ng/mL)	3.00 [0.60 - 26.36]	0.38 [0.16 - 1.88]	0.39 [0.16 - 1.14]
Neutrophil count (10^9 cells per L)	10.8 [6.6 - 15.4]	9.8 [6.9 - 13.3]	10.1 [7.6 - 12.1]
Altered mental status	8 (8.1)	31 (5.6)	7 (9.7)
Pleural effusion	8 (8.1)	58 (10.4)	17 (23.6)
Oxygen therapy	60 (60.6)	264 (47.5)	53 (73.6)
Female	31 (31.3)	206 (37.1)	38 (52.8)

Data are n (%) or mean (SD).

Table E5 Association between class assignment and clinical outcomes for a three-class model for both cohorts

Ovidius-TripleP cohort				
Clinical outcome	**Class 1 (n = 153)**	**Class 2 (n = 58)**	**Class 3 (n = 294)**	**p-value**
Length of stay (days)	9.0 (7.0-14.0)	10.3 (6.0-23.8)	8.0 (5.5-11.5)	<0.01
ICU admission	12 (7.8)	14 (24.1)	12 (4.1)	<0.01
In-hospital mortality	11 (2.7)	6 (10.3)	7 (2.4)	<0.01
30-day mortality	13 (8.5)	6 (10.3)	7 (2.4)	<0.01
Readmission	11 (7.2)	4 (6.9)	22 (7.5)	0.98
STEP cohort				
Clinical outcome	**Class 1 (n = 99)**	**Class 2 (n = 556)**	**Class 3 (n = 72)**	**p-value**
Length of stay (days)	8.0 (5.0-13.0)	7.0 (4.0-10.0)	7.0 (5.0-10.3)	<0.01
ICU admission	12 (12.1)	26 (4.7)	1 (1.4)	<0.01
In-hospital mortality	11 (11.1)	11 (2.0)	2 (2.8)	<0.01
30-day mortality	10 (10.1)	16 (2.9)	2 (2.8)	<0.01
Readmission	8 (8.1)	27 (4.9)	4 (5.6)	0.42

Data are N (%) or median (IQR). ICU intensive care unit.