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Longitudinal respiratory subphenotypes in patients with COVID-19-related acute respiratory distress syndrome: results from three observational cohorts

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Summary

Background Patients with COVID-19-related acute respiratory distress syndrome (ARDS) have been postulated to present with distinct respiratory subphenotypes. However, most phenotyping schema have been limited by sample size, disregard for temporal dynamics, and insufficient validation. We aimed to identify respiratory subphenotypes of COVID-19-related ARDS using unbiased data-driven approaches.

Methods PRoVENT–COVID was an investigator-initiated, national, multicentre, prospective, observational cohort study at 22 intensive care units (ICUs) in the Netherlands. Consecutive patients who had received invasive mechanical ventilation for COVID-19 (aged 18 years or older) served as the derivation cohort, and similar patients from two ICUs in the USA served as the replication cohorts. COVID-19 was confirmed by positive RT-PCR. We used latent class analysis to identify subphenotypes using clinically available respiratory data cross-sectionally at baseline, and longitudinally using 8-hourly data from the first 4 days of invasive ventilation. We used group-based trajectory modelling to evaluate trajectories of individual variables and to facilitate potential clinical translation. The PRoVENT-COVID study is registered with ClinicalTrials.gov, NCT04346342.

Findings Between March 1, 2020, and May 15, 2020, 1007 patients were admitted to participating ICUs in the Netherlands, and included in the derivation cohort. Data for 288 patients were included in replication cohort 1 and 326 in replication cohort 2. Cross-sectional latent class analysis did not identify any underlying subphenotypes. Longitudinal latent class analysis identified two distinct subphenotypes. Subphenotype 2 was characterised by higher mechanical power, minute ventilation, and ventilatory ratio over the first 4 days of invasive mechanical ventilation than subphenotype 1, but PaO₂/FiO₂, pH, and compliance of the respiratory system did not differ between the two subphenotypes. 185 (28%) of 671 patients with subphenotype 1 and 109 (32%) of 336 patients with subphenotype 2 had died at day 28 (p=0.10). However, patients with subphenotype 2 had fewer ventilator-free days at day 28 (median 0, IQR 0–15 vs 5, 0–17; p=0.016) and more frequent venous thrombotic events (109 [32%] of 336 patients vs 176 [26%] of 671 patients; p=0.048) compared with subphenotype 1. Group-based trajectory modelling revealed trajectories of ventilatory ratio and mechanical power with similar dynamics to those observed in latent class analysis-derived trajectory subphenotypes. The two trajectories were: a stable value for ventilatory ratio or mechanical power over the first 4 days of invasive mechanical ventilation (trajectory A) or an upward trajectory (trajectory B). However, upward trajectories were better independent prognosticators with subphenotype 2 (trajectory B) and 28-day mortality was confirmed in the replication cohorts (OR 4·65, 95% CI 1·87–11·6 for ventilatory ratio in replication cohort 1; 1·89, 1·05–3·37 for ventilatory ratio in replication cohort 2).

Interpretation At baseline, COVID-19-related ARDS has no consistent respiratory subphenotype. Patients diverged from a fairly homogenous to a more heterogeneous population, with trajectories of ventilatory ratio and mechanical power being the most discriminatory. Modelling these parameters alone provided prognostic value for duration of mechanical ventilation and mortality.

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Introduction SARS-CoV-2 has infected 229 million individuals worldwide and caused more than 4·7 million deaths as of the end of September, 2021. Infection with SARS-CoV-2, referred to as COVID-19, frequently results in acute respiratory failure that might require intensive care unit (ICU) admission for respiratory support, including invasive ventilation, with high mortality. Since acute respiratory failure in COVID-19 is caused by a single pathogen, it can be postulated that critically ill patients with COVID-19 form a homogenous group with a single phenotype. However, multiple studies have
identified various clinical subphenotypes of COVID-19. Most studies have focused on variation in biochemical information, such as plasma creatinine, D-dimer, and IL-6. These studies typically used data obtained at a single timepoint and, to date, external validation has been rare.

A classification of critically ill patients with COVID-19 based on respiratory system compliance was postulated to present a treatable trait and to be dynamic in nature, with patients progressing from normal compliance to decreased compliance, although this trend was not observed in a meta-analysis. These classifications received criticism because they were based on retrospective inspection of small patient cohorts, mostly obtained in a single centre, without the use of statistical methods that are typically used to identify subgroups. Unbiased identification of respiratory subphenotypes of critically ill patients with COVID-19 infection requires analysis of large, multicentre cohorts with longitudinal data using appropriate methodological approaches.

In this study, using a derivation and two replication cohorts, we aimed to assess the existence of respiratory subphenotypes in critically ill patients with COVID-19. We hypothesised that using cross-sectional data, based on respiratory variables, no distinct subphenotypes of COVID-19 would be observed. Furthermore, we hypothesised that the richness of using longitudinal data for partitioning variables would be more informative in identifying respiratory phenotypes with distinct characteristics. Finally, we aimed to determine the extent to which trajectories of single variables can be used as prognostic indicators, because single variable trajectories are more clinically implementable than longitudinal multivariate subphenotyping approaches.

### Methods

**Derivation cohort**

PRoVENT–COVID was an investigator-initiated, national, multicentre, prospective, observational cohort study at 22 ICUs in the Netherlands. Consecutive patients aged 18 years or older were eligible for participation in the PRoVENT–COVID study if they were admitted to a participating ICU between March 1, 2020, and May 15, 2020, and had received invasive ventilation for respiratory failure related to COVID-19. COVID-19 was confirmed by positive RT-PCR. Patients who received non-invasive ventilation only, and patients who were transferred to a non-participating ICU within 1 h after intubation and start of invasive ventilation, were excluded from the current analysis.

A detailed description of study procedures in the derivation cohort has been reported elsewhere. Day 0 was defined as the first calendar day that a patient received invasive ventilation in a participating ICU.
irrespective of hospital or ICU admission date. In the first hour of invasive ventilation and thereafter every 8 h at fixed timepoints up to day 3, ventilator settings and parameters and vital signs were entered into the database. ARDS was defined by the Berlin definition. Outcomes were duration of invasive mechanical ventilation, ICU mortality, hospital mortality, overall 28-day mortality, overall 90-day mortality, development of venous thrombotic events during ICU stay, acute kidney injury, need for renal replacement therapy during ICU stay, and length of ICU stay. All data were entered into a password-secured, internet-based, electronic case report form (Castor EDC; Amsterdam, Netherlands). Before analysis, the study coordinators screened all data for potentially erroneous or incomplete recordings and verified and corrected information as appropriate with the help of local doctors and data collectors. After cleaning, the database was closed for analysis.

The study protocol has been previously published. The institutional review boards of the participating centres approved the study protocol and the need for individual informed consent was waived. The ethics boards of the participating hospitals approved the collection of data for the study purposes. The study coordinators and trained data collectors assisted local doctors and monitored the study according to the International Conference on Harmonization Good Clinical Practice guidelines. Study coordinators ensured integrity and timely completion of data collection.

Replciation cohorts
The two replication cohorts included patients 18 years or older who required invasive mechanical ventilation for COVID-19 pneumonia between March 1, 2020, and Dec 31, 2020, at Michigan Medicine (Ann Arbor, MI, USA) or at one of ten BJC HealthCare Hospitals (St Louis, MO, USA) in or around the St Louis metropolitan area. For both cohorts, patients transferred from outside hospitals were excluded if they were initiated on invasive ventilation before the date of transfer, or if they received less than 48 h of mechanical ventilation. COVID-19 was confirmed by positive RT-PCR. All data used in the replication cohorts were collected retrospectively and extracted from electronic health records. Analyses in the replication cohorts were done locally, such that no protected health information was exchanged between study sites.

Statistical analysis
Subphenotypes were studied cross-sectionally at the start of invasive ventilation in the intensive care unit and every 24 h after, and longitudinally using time-dependent analysis with 8-hourly data from the first 4 days of invasive ventilation. Subphenotypes were identified using latent class analysis and using group-based trajectory modelling. Figure 1A shows a visual representation of the differences in the data used in each model. We used group-based trajectory modelling to allow for external validation of our results as no other dataset with granular multivariate data could be identified, which is indicative for the fact that group-based trajectory modelling is more clinically implementable than longitudinal latent class analysis. A stepwise approach was used that included: data setup, model estimation, model evaluation, and interpretation of the optimal model.

The following readily available clinical variables were used as input for the identification of latent classes: driving pressure, compliance of the respiratory system (tidal volume over driving pressure), minute volume ventilation, pH, ventilatory ratio (VE measurement × PaCO2 measurement ÷ VE predicted × PaCO2 predicted)—where VE is the expired minute ventilation and PaCO2 is the arterial carbon dioxide tension—difference between arterial and end-tidal CO2, and mechanical power of ventilation (0.098 × VI [in L] × RR × [Pmax – PdRV] ÷ 2)—where VI is the tidal volume, RR is the respiratory rate, Pmax is the maximum airway pressure on pressure-controlled ventilation, and PdRV is the driving pressure. Variables with mathematical coupling were not considered (eg, not including respiratory rate, tidal volume, and minute volume ventilation).

Latent class analysis was done using data obtained directly after the start of mechanical ventilation to identify cross-sectional subphenotypes. This analysis was repeated for data collected every 24 h. Latent class analysis was repeated using 8-hourly data from the first 4 days of invasive ventilation to identify time-dependent subphenotypes that emerged during the first 96 h of ICU admission. The specific model parameters are given in the appendix (p 2).
The optimal number of latent classes was selected using the lowest Bayesian information criterion, integrated complete likelihood, and Akaike information criterion values indicating more precise assignment of individuals to latent profiles. Five models, comprising 1 to 5 classes, were fitted and if one or more models had similar goodness of fit, the model with the lowest number of classes was selected.

The prognostic value of the subphenotypes was evaluated by comparing differences in ventilator-free days, 28-day mortality, occurrence of venous thrombotic events, and use of renal replacement therapy for acute kidney injury. Venous thrombotic event diagnosis was based on venous Doppler ultrasound or CT angiography.

We used group-based trajectory monitoring to assess if the trajectory of a single variable could be used to identify trajectory subphenotypes with similar dynamics to those identified by time-dependent latent class analysis, since this method was straightforward enough to allow ease of analysis in the replication datasets. We applied group-based trajectory measurement on 8-hourly data from the first 4 days to identify the trajectory subphenotypes for PaO₂/FiO₂, ventilatory ratio, mechanical power, and respiratory system compliance. Group-based trajectory monitoring is a finite mixture model used to identify clusters of patients following similar trajectories of a variable of interest and is easier to apply to a smaller dataset than longitudinal latent class analysis. The group-based trajectory monitoring algorithm computes a unique equation of the variable of interest as a function of time for each of the subphenotypes. Patients are classified into the trajectory subphenotype whose function most closely matches their measurements. This approach has previously successfully been applied to temperature trajectories.25

The fit of the group-based trajectory model from the derivation cohort was applied to the replication cohorts and the association with outcomes was evaluated.

To study the influence of treatment strategies on the prevalence of the identified subphenotype, we compared the following interventions before or at the day of initiation of invasive mechanical ventilation between the groups: high or low positive end-expiratory pressure strategy based on the selected positive end-expiratory pressure/FiO₂ table as extensively described,24 prone positioning, and use of remdesivir, corticosteroids, and tociluzimab.

Patient data are presented as mean and SD for normally distributed continuous variables, median and IQR for variables that are not normally distributed, and numbers with percentages for categorical data. Differences between identified subphenotypes were tested using t-test, Mann-Whitney U test, and Fisher’s exact test. The dynamics of each variable per subphenotype were fitted using a second-degree polynomial to show the trajectory over time between the groups. Differences between subphenotypes at baseline and differences in dynamic change over time were assessed by linear mixed effect model analysis using hours since intubation, subphenotype, and an interaction term between the two as fixed effects and a random intercept per patient. We used logistic and linear regression to assess the association between subphenotype classification and binary or continuous

| Age, years | All (n=1007) | Time-dependent subphenotypes* | p value |
|------------|-------------|------------------------------|---------|
|            | 1 (n=671)   | 2 (n=336)                    |         |
| Sex        |             |                              |         |
| Female     | 63·7 (10·8) | 63·9 (11·0)                  | 0·35    |
| Male       | 728 (73%)   | 445 (66%)                    | 283 (84%) |
| BMI, kg/m² | 28·6 (5·6)  | 28·3 (4·5)                   | 0·054   |

Day of admission†

| Comorbidities                          | All (n=1007) | Time-dependent subphenotypes* | p value |
|----------------------------------------|-------------|------------------------------|---------|
| Arterial hypertension                  | 342 (34%)   | 229 (34%)                    | 113 (34%) | 0·030 |
| Heart failure                          | 42 (4%)     | 28 (4%)                      | 14 (4%) | 1·000 |
| Diabetes                               | 225 (22%)   | 163 (24%)                    | 62 (18%) | 0·044 |
| Chronic kidney disease                 | 45 (4%)     | 163 (24%)                    | 62 (18%) | 0·015 |
| Chronic obstructive pulmonary disease  | 83 (8%)     | 59 (9%)                      | 23 (7%) | 0·350 |
| Immunosuppression                      | 24 (2%)     | 15 (2%)                      | 9 (3%) | 0·830 |

Day of admission†

| Tidal volume per kg predicted bodyweight, mL/kg | All (n=1007) | Time-dependent subphenotypes* | p value |
|------------------------------------------------|-------------|------------------------------|---------|
| Respiratory rate, breaths/min                  | 21·00 (4·60) | 20·95 (4·54)                  | 21·96 (4·84) | 0·001 |
| Minute ventilation, L/min                      | 9·40 (2·70)  | 8·97 (2·40)                   | 10·25 (3·12) | <0·001 |
| Positive end-expiratory pressure, cmH₂O        | 12·6 (2·9)   | 12·4 (2·8)                    | 13·2 (2·9) | <0·001 |
| High positive end-expiratory pressure strategy | 269 (27%)   | 167 (25%)                    | 102 (30%) | 0·130 |
| Plateau pressure, cmH₂O                       | 27·3 (5·2)   | 26·9 (5·1)                    | 28·0 (5·3) | 0·003 |
| Driving pressure, cmH₂O                       | 14·9 (4·4)   | 14·8 (4·3)                    | 15·2 (4·5) | 0·000 |
| Compliance respiratory system, mL/cmH₂O       | 32·3 (12·0)  | 31·8 (11·5)                   | 33·3 (13·0) | 0·082 |
| PaO₂, mm Hg                                   | 93 (43)     | 91 (43)                      | 98 (43) | 0·026 |
| PaO₂/FiO₂, mm Hg                              | 148 (75)    | 143 (77)                     | 153 (73) | 0·182 |
| Mild                                           | 262 (26%)   | 162 (24%)                    | 100 (30%) | 0·110 |
| Moderate                                       | 520 (52%)   | 350 (52%)                    | 170 (51%) | -     |
| Severe                                         | 225 (22%)   | 159 (24%)                    | 66 (20%) | -     |
| Mechanical power, J/min                       | 18·4 (6·7)  | 17·3 (6·1)                    | 20·6 (7·3) | <0·001 |
| PaCO₂, mm Hg                                   | 45·2 (11·8) | 43·5 (10·7)                   | 48·8 (13·1) | <0·001 |
| Ventilatory ratio                             | 1·64 (0·68) | 1·52 (0·49)                   | 1·89 (0·91) | <0·001 |
| Difference between arterial and end-tidal CO₂ | 7·72 (11·14)| 6·71 (10·40)                  | 9·79 (12·31) | <0·001 |
| pH                                             | 7·36 (0·10) | 7·36 (0·09)                   | 7·34 (0·10) | <0·001 |
| Creatinine, μmol/L/L                          | 74 (61-96)  | 73 (58-93)                    | 77 (65-97) | 0·015 |
| Urine output, mL/day                          | 705 (370-1148) | 670 (350-1125) | 725 (415-1202) | 0·071 |
| Fluid balance, mL/day                         | 608 (29-1418) | 539 (13-1268) | 712 (71-1587) | 0·048 |
| Prone position in first ICU day               | 300 (30%)   | 199 (30%)                    | 101 (30%) | 0·520 |
| Vasopressor in first ICU day                  | 792 (73%)   | 520 (77%)                    | 271 (81%) | 0·340 |

(Table 1 continues on next page)
outcomes, respectively. Odds ratios (ORs) with 95% CIs for the association between subphenotype and 28-day mortality, adjusted for age, gender, and BMI were calculated. A sensitivity analysis was done on data collected from patients who remained on invasive mechanical ventilation for more than 96 h. Missing data were imputed for the latent class analysis using multivariate imputation by chained equations; the results from the first dataset are presented and the additional datasets were evaluated for consistency. Data analysis was done in R version 4.0.3 through the R studio interface, with the exception of group-based trajectory monitoring, which applied using the `traj` package command in Stata MP16. The PRoVENT-COVID study is registered with is registered with ClinicalTrials.gov, NCT04346342.

**Role of the funding source**

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

**Results**

Between March 1, 2020, and May 15, 2020, 1122 patients were screened for study entry, of whom 115 were excluded because they previously received invasive ventilation, leaving 1007 patients included in the derivation cohort (appendix p 4). We recorded patient characteristics (table 1). Included patients were mostly men (814 [73%] of 1007 patients), with a mean age of 63·7 years (SD 10·8) and a mean BMI of 28·6 kg/m² (SD 5·6). 294 (29%) of 1007 patients), with a mean age of 63·7 years (SD 10·8) and a mean BMI of 34·0 kg/m² (SD 18·6) in replication cohort 1 and 326 from replication cohort 2 (appendix p 4). We recorded patient characteristics at the start of mechanical ventilation (ie, day 0) and outcomes in the derivation cohort (appendix p 9).

The PRoVENT-COVID study is registered with is registered with ClinicalTrials.gov, NCT04346342.

### Table 1: Patient characteristics at the start of mechanical ventilation (ie, day 0) and outcomes in the derivation cohort

| Outcome                                      | All (n=1007) | Time-dependent subphenotypes* | p value |
|----------------------------------------------|-------------|-----------------------------|---------|
| | 1 (n=671) | 2 (n=336) |
| Outcomes                                     |             |                             |         |
| Acute kidney injury§                          | 447 (44%)   | 270 (40%)                   | 0·001   |
| Renal replacement§                           | 180 (18%)   | 109 (16%)                   | 0·088   |
| Venous thrombotic event§                     | 285 (28%)   | 176 (26%)                   | 0·048   |
| ICU length of stay, days                     | 15 (9–27)   | 15 (9–25)                   | 0·36    |
| Duration of invasive mechanical ventilation, days | 13 (8–23)   | 13 (8–23)                   | 0·41    |
| Ventilator-free days and alive at day 28, days | 2 (0–16)    | 5 (0–17)                    | 0·016   |
| Day 7 mortality                              | 112 (11%)   | 72 (11%)                    | 0·83    |
| Day 28 mortality                             | 294 (29%)   | 185 (28%)                   | 0·10    |
| Day 90 mortality                             | 349 (35%)   | 223 (33%)                   | 0·24    |

Data are mean (SD), n (%), or median (IQR). *Latent class analysis using 8-hourly data from the first 4 days of invasive ventilation was used to identify time-dependent subphenotypes that emerged during the first 96 h of ICU admission. †Date of ICU admission was the same as the day of start of mechanical ventilation in this cohort. ‡In blood samples. §During ICU stay.

Using the longitudinal data from the first 4 days of invasive ventilation (figure 1C), a two-class model best fit the derivation cohort (appendix p 6). Entropy was 82% and the probability of class membership was good (appendix p 7). Sensitivity analysis including only the 826 (82%) patients who remained on invasive mechanical ventilation for more than 96 h showed similar results (appendix p 8), with only 22 (3%) patients changing class membership. We recorded standardised mean differences between classes over time (figure 2). Subphenotype 2 (336 [33%] of 1007 patients) was characterised by increasing minute ventilation, mechanical power, and ventilatory ratio over the first 4 days of invasive mechanical ventilation (appendix p 9).

Venous thromboembolism was more common in subphenotype 2 (109 [32%] of 336 patients) than in subphenotype 1 (176 [26%] of 671 patients; p=0·048). The median numbers of ventilator-free days and patients alive at day 28 were lower in subphenotype 2 than in...
subphenotype 1 (5, IQR 0–17 for subphenotype 1 and 0–15 for subphenotype 2; p=0·016; table 1; appendix p 10). Day 7, day 28, and day 90 mortality individually were not significantly different in subphenotype 2 compared with subphenotype 1 (table 1).

The trajectories of PaO₂/FiO₂ and respiratory compliance were distinct from those observed in the time-dependent latent class analysis-derived subphenotypes and were not considered further. The group-based trajectory model of ventilatory ratio and mechanical power showed overlapping trajectories with the time-dependent latent class analysis-derived subphenotypes (figures 1D, 3). Two trajectories were observed, as follows: a stable value for ventilatory ratio or mechanical power over the first 4 days of invasive mechanical ventilation (trajectory A overlapping with subphenotype 1) or an upward trajectory (trajectory B overlapping with subphenotype 2). 28-day mortality and the rate of venous thrombotic events were higher in patients categorised to ventilatory ratio and mechanical power trajectory B than in their trajectory A counterparts (table 2). Trajectory B was independently associated with 28-day mortality for ventilatory ratio (OR 1·64, 95% CI 1·17–2·29) and mechanical power (1·82, 1·24–2·66) after adjusting for sex, age, and BMI. Thus, group-based trajectory model-derived trajectories for ventilatory ratio and mechanical power had prognostic potential in the derivation cohort and were evaluated in the replication cohorts.

After adjusting for sex, age, and BMI, ventilatory ratio trajectory B was independently associated with 28-day mortality in replication cohort 1 (OR 4·65, 95% CI 1·87–11·6) and replication cohort 2 (1·89, 1·05–3·37). Mechanical power trajectory B was independently associated with mortality in replication cohort 1 (OR 2·98, 95% CI 1·51–5·87), but not in replication cohort 2 (0·96, 0·54–1·72). Patients with mechanical power trajectory B had a longer duration of mechanical

Figure 3: Comparison of dynamic changes of time dependent latent class analysis subphenotypes and trajectory analysis
At each 8-hourly timepoint the median and IQR is plotted. The line shows second-degree polynomial regression.

| All (n=1007) | Ventilatory ratio trajectories | p value | Mechanical power trajectories | p value |
|-------------|-------------------------------|---------|-------------------------------|---------|
|              | A (n=777)                     | B (n=230) |                               |         |
| Subphenotype 2 (%) | 336 (33%)                   | 390 (24%)  | 146 (63%)                    | <0·001  |
| Age, years   | 63 (10 8)                    | 63 (10 9)  | 63 (10 10)                   | 0·750    |
| Male sex     | 728 (72%)                    | 573 (74%)  | 155 (67%)                    | 0·071    |
| Body-mass index, kg/m² | 28 (5 6)           | 28 (4 3)   | 29 (8 7)                     | <0·001   |
| High positive end-expiratory pressure strategy | 269 (27%)           | 211 (27%) | 58 (25%)                     | 0·010    |

| Outcomes                                      | p value | p value |
|-----------------------------------------------|---------|---------|
| Acute kidney injury                           | 0·001   | 0·001   |
| Renal replacement                             | 0·001   | 0·001   |
| Venous thrombotic event                       | 0·001   | 0·001   |
| ICU length of stay, days                      | 0·007   | 0·007   |
| Duration of invasive mechanical ventilation, days | 0·008  | 0·008   |
| Ventilator-free days and alive at day 28, days | 0·001   | 0·001   |
| Day 7 mortality                               | 0·011   | 0·011   |
| Day 28 mortality                              | 0·018   | 0·018   |
| Day 90 mortality                              | 0·002   | 0·002   |

Data are n (%) or mean (SD). Trajectory A is a stable trajectory and trajectory B is an increasing trajectory.

Table 2: Outcomes for trajectories of ventilatory ratio and mechanical power in derivation cohort
ventilation than patients in mechanical power trajectory A in the derivation and replication cohorts (tables 2, 3).

We found no difference in the use of a high positive end-expiratory pressure strategy between the time-dependent latent class analysis derived subphenotypes 1 and 2 (table 1), nor for the ventilatory ratio trajectories A and B (table 3). Patients with the upward trajectory (ie, trajectory B) of mechanical power were more frequently exposed to a high positive end-expiratory pressure strategy (table 3).

We found no difference in the use of remdesivir, corticosteroids, or tociluzimab between the A and B trajectories of mechanical power and ventilatory ratio in replication cohort 1, but there were differences in replication cohort 2 (table 3). However, use of these drugs did not explain the differences in prevalence of the trajectory subphenotypes between the cohorts.

**Discussion**

In these large observational cohort studies with granular clinical data of critically ill patients with acute respiratory failure due to severe COVID-19, we found no empirical evidence for the existence of respiratory subphenotypes at the start of invasive ventilation, nor at cross-sectional analysis in the succeeding 4 days. However, using time-dependent analysis, we identified two subphenotypes that developed during the first 4 days of invasive mechanical ventilation. Trajectories of ventilatory ratio and mechanical power were most discriminatory and modelling these parameters alone provided prognostic value for duration of mechanical ventilation and mortality.

Using respiratory and ventilatory data available directly after the start of invasive mechanical ventilation in patients with COVID-19-related ARDS, we found no suggestion for the existence of latent classes. In other
words, the data were best explained by the presence of a single phenotype. This finding emphasises the importance of a data-driven approach to subphenotyping and contradicts earlier efforts that attempted to identify respiratory subphenotypes based on physiological reasoning and clinical observations. This conclusion does not necessarily mean that the physiological description of the cases that were reported were false, but rather that these fall at the extremes of a normal distribution and do not represent distinct subclasses. Insufficient evidence for latent classes based on respiratory and gas exchange variables alone is in line with a study in which classification of subphenotypes of COVID-19-related ARDS was driven by plasma biomarkers of organ failure, inflammation, and coagulation, whereas respiratory and gas exchange variables did not provide any discrimination.

Subphenotypes emerged in the derivation cohort using longitudinal data from the first 4 days of invasive ventilation. The importance of dynamic changes over time has been exemplified by the identification of temperature trajectory subphenotypes in patients with sepsis and COVID-19. Patterns in temperature trajectories showed prognostic enrichment for mortality and were associated with differences in inflammatory markers in plasma. We used group-based trajectory modelling to identify subphenotypes based on the dynamic changes in \( \text{PaO}_2/\text{FiO}_2 \), compliance of the respiratory system, ventilatory ratio, and mechanical power. The major advantage of modelling one variable with group-based trajectory modelling compared with time-dependent latent class analysis is that the former is more suitable for application in a clinical setting and across multiple cohorts. Indeed, we were unable to identify any database with sufficiently granular data of all eight variables to externally validate the time-dependent latent class analysis approach. Furthermore, in our analyses, we found that both ventilatory ratio and mechanical power trajectories were predictive of duration of mechanical ventilation in two datasets, while only ventilatory ratio trajectories were indicative of an increased likelihood of 28-day mortality.

Trajectory analysis of the ventilatory ratio seems to be a promising method for prognostication. The two trajectories had similar rates of baseline organ dysfunction but differentially developed complications such as venous thrombotic events and acute kidney injury requiring renal replacement therapy. Ventilatory ratio is easily calculated at the bedside when arterial blood gas analysis is available and is a good surrogate marker for dead space ventilation, although it can be influenced by other factors. The subphenotype with an upward ventilatory ratio trajectory showed a higher mortality independent of baseline risk factors. Patients with an upward ventilatory ratio trajectory also more frequently had venous thrombotic events, and pulmonary embolism is a likely contributor to ventilation-perfusion mismatch in this patient group, although we did not study this relationship directly in this study. We speculate that patients with an upward ventilatory ratio trajectory show more pulmonary perfusion defects and might benefit from more intensive anticoagulatory treatment, even in the absence of pulmonary embolism. Perfusion defects in patients with COVID-19 can also be driven by immune-response related in-situ thrombosis, for which anticoagulation therapy might be less effective. However, immunomodulatory therapies such as dexamethasone and tocilizumab were not associated with a decreased prevalence of the upward trajectory subphenotype in this study, suggesting that these therapies do not eliminate the increase in ventilatory ratio over time.

Ventilatory ratio and mechanical power might be influenced by treatment strategies; therefore, we studied if variation in clinical practice could explain the existence of the identified subphenotypes. A difference in positive end-expiratory pressure strategy was observed between the mechanical power trajectory subphenotypes, with a higher positive end-expiratory pressure strategy more common in patients with an upward trajectory. Given that an increase in positive end-expiratory pressure without a decrease in driving pressure can result in an increase in mechanical power, this could be suggestive for inappropriate use of high levels of positive end-expiratory pressure in this subset of patients. This finding requires further evaluation in larger cohorts of critically ill patients with COVID-19-related ARDS. However, pharmacological treatment with remdesivir, corticosteroids, and tocilizumab did not consistently explain differences in the prevalence of the trajectory subphenotypes.

This study has several important strengths. Other studies have reassessed the prognostic accuracy of mechanical power, ventilatory ratio, and \( \text{PaO}_2/\text{FiO}_2 \) after 24–48 h but, to our knowledge, this study is the first to identify subphenotypes based on respiratory and gas exchange variables in a large cohort of patients, with external validation of the findings. We combined cross-sectional latent class analysis, time-dependent latent class analysis, and group-based trajectory modelling to move from a simplistic model of two cross-sectional subphenotypes to a classification based on the individual trajectories of ventilatory ratio and mechanical power during the first 96 h of mechanical ventilation. Use of a single variable is important for clinical applicability, which is shown by the fact that we were unable to identify a replication cohort with 8-hourly data for all variables using time-dependent latent class analysis. A major limitation of this study is the observational nature of the data, which prevented us from studying the potential for predictive enrichment. Furthermore, data were carefully curated in the derivation cohort, whereas there was an automated system to extract data from electronic health records in replication cohorts. Despite this difference in data curation, the relationship between trajectories and outcomes persisted. Furthermore, even
though consecutive patients were included in all cohorts, the variation in mortality rates suggest that case mix varied. Both factors could explain the different incidence of the trajectory B of ventilatory ratio and mechanical power between cohorts.

In conclusion, patients with COVID-19-related ARDS consistently show a single respiratory phenotype at the start of invasive mechanical ventilation. The data suggest that there are at least two distinct trajectories during the first days of invasive mechanical ventilation, with one subphenotype showing increasing minute ventilation, mechanical power, and ventilatory ratio. Trajectories of mechanical power and ventilatory ratio were independently associated with outcome. COVID-19-related ARDS seems to diverge from a fairly homogenous respiratory physiology to a more heterogeneous population during the first 4 days of invasive mechanical ventilation. This finding reveals the importance of including time as a key variable in future efforts towards subphenotyping COVID-19. Anticoagulation and positive end-expiratory pressure selection should be considered as treatable traits in the identified subphenotypes.

Contributors
All authors designed the study together and were involved in collecting the data with the help of the study collaborators. LDJB, MS, PS, SVB, and PL did the analyses and drafted the manuscript. AFB, MB, AMT, ASN, MJ5, RD, and FP revised the initial draft. All authors approved the final version of the manuscript. LDJB, MS, PL, and AFB had access to the data. LDJB, MS, and PL verified the data and had access to the raw data.

Data sharing
Submitted work. All other authors declare no competing interests.

Declaration of interests
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Data sharing
Deidentified participant data with a data dictionary can be shared after approval of a proposal with a signed data access agreement and in collaboration with the study group.

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