Statistical Analysis plan for: 'Phenotypes of endotheliopathy in critically ill patients with acute respiratory failure – a cohort study'

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Introduction

Background and Rationale

Acute respiratory failure is a common reason for admission to the Intensive Care Unit (ICU), and it is estimated that 13-20 million patients are mechanically ventilated worldwide annually[1] with more than a third dying during the hospital admission, although studies suggest that the mortality have improved in recent years.[2–45]

There is a growing understanding that syndromes such as ARDS, sepsis or acute kidney injury are best understood as consisting of several sub-phenotypes with distinct clinical characteristics, treatment responses, and outcomes[6–8]. The classification into sub-phenotypes has often relied on clinical data at baseline [7,8] but kinetics of markers with repeated measurements such as temperature, creatinine or endothelial biomarkers have been employed [6,9,10]. In a small prospective cohort-study (n = 28), Juneja et al. applied longitudinal k-means clustering to the trajectories of several endotheliopathy biomarkers (among which Soluble Thrombomodulin [sTM] was one) in COVID-19 patients and identified a high and a low severity group based on their analysis[10]. In ARDS, a hyper- and a hypoinflammatory subphenotype with different prognosis and suspected different response to treatment have been identified in multiple cohorts [7,11,12]. Recently, these subgroups have also been identified in patients with respiratory failure without ARDS [13,14], suggesting that undifferentiated patients with acute respiratory failure can be successfully divided into subgroups based on baseline biomarkers of inflammation.

One method for finding common patterns in longitudinal data is latent class linear mixed modelling[15]. An extension of the linear mixed model originally developed for research in psychometric scales, it is based on the assumption that the variation in the observations is caused from sampling from two or more latent classes or groups of subjects, each group with its own latent class-specific mean trajectory and intercept of the longitudinally measured biomarker and possibly with class-specific random effects also[15].

Endotheliopathy has been proposed as a plausible link between critical illness and multiorgan failure[16]. The endothelium comprises a vital part of the blood-air barrier and there is evidence that the endothelium is affected by treatments such as positive-pressure ventilation[17,18] and fluid therapy [19(p1),20]. We therefore

hypothesize that latent-class analysis of the trajectory of endotheliopathy biomarkers can identify clinically important subgroups in patients with acute respiratory failure.

Objectives

The objective of the study is twofold: 1) to estimate the kinetics and possible latent classes of endothelial biomarkers – Syndecan-1, Soluble Thrombomodulin (sTM) and Platelet Endothelial Cell Adhesion Molecule-1 – during the first 3 ICU-days in adult mechanically ventilated patients, and 2) investigate if the obtained latent class-based subgroups associates with clinical outcomes of acute respiratory failure and how the latent class approach compares to other approaches for capturing the development over time of endotheliopathy biomarkers.

Methods

Study design

The Metabolomics study is a single-centre prospective cohort study investigating the role of the endothelium in patient-important outcomes in a cohort of critically ill adults admitted to the ICU.

We present here an analysis of a sub-population of the Metabolomics study.

The study is reported in accordance with the STROBE-recommendations[21]

Sample size

As previous data on the trajectories of sTM, Syndecan-1 and PECAM-1 in a critically ill population of patients with acute respiratory failure are very scarce, formal power calculations were not possible to perform and the results should be considered exploratory.

Outcome Assessment

Patients were followed for 30 days after inclusion for collection of clinical data from their ICU-stay including timing of liberation from mechanical ventilation and were subsequently followed for a year for the occurrence of death from any cause.

Statistical Principles

We will consider a p-value below 0.05 significant. We will present 95% Confidence Intervals (95% CI) along with point estimates where feasible.

Results from both the univariable and multivariable analysis will be presented where relevant.

No correction for multiple comparisons will be performed, as all study results will be regarded as exploratory.

Trial Population

Screening data

The ICU at Copenhagen University Hospital – North Zealand, Copenhagen, Denmark is a 12 bed, mixed surgical/medical ICU with around 900 admissions per year.

Between November 2016 and June 2019 all patients presenting to the ICU were screened for inclusion in the Metabolomics Study.

Eligibility

Patients were eligible for inclusion if they were >18 years old and acutely admitted to the ICU with a presumed stay > 24 hours. Patients were excluded if active treatment was deemed futile by the treating clinician or if informed consent was unobtainable.

Included patients had study blood samples drawn daily for the first 5 days of their ICU-stay or until death or discharge, whichever came first.

For the present study in the Metabolomics cohort, only patients that presented with acute respiratory failure are included. Acute respiratory failure was defined as patients receiving either Invasive (IV) or Non-Invasive Ventilation (NIV) on the first day of ICU-admission.

Withdrawal

Reasons for withdrawal were patients erroneously included (i.e. not fulfilling eligibility criteria or already enrolled at a previous ICU-stay) or withdrawn consent.

Baseline Patient Characteristics

The characteristics collected at baseline include:

- Patient demographics (age and gender)
- Chronic comorbidities (a history of heart failure, hypertension, diabetes, stroke, prior MI etc.)
- Primary reason for ICU-admission (as per SAPS 3 categories)
- Category of ICU-admission (medical, surgical, acute, elective)
- Timeline parameters (time of inclusion, time of ICU-admission, time of hospital admission etc.)
- Infection status at admission
- ICU-admission source (operating theatre, ward, emergency department etc.)

- Physiology at baseline (PAF-ratio, blood pressure, pulse etc.)
- Therapy at baseline (noradrenaline, insulin etc.)
- Respiratory therapy at baseline

Analysis

Part one

Outcome Definitions

The primary outcome is the latent class assignments for each patient, based om the trajectories of Syndecan-1, sTM and PECAM-1 alone and in conjunction.

Variables

The variables of interest are Syndecan-1, sTM and PECAM-1. These are continuous and presented in ng/ml. They are measured once daily for the first three days of the study in all patients alive and still admitted to the ICU. The trajectories of Syndecan-1, sTM and PECAM-1 are considered dependent variables and their development is modelled as a function of time to obtain latent classes of patients based on these trajectories.

Analysis methods

We will summarize the kinetics of Syndecan-1, sTM and PECAM-1 with daily mean and standard deviation, or median and interquartile range as appropriate.

We will model the trajectories over time for Syndecan-1, sTM and PECAM-1 separately using latent class linear mixed modelling with the R-package lcmm[15], specifically using the function lcmm(), that handles continuous non-gaussian outcomes.

We will assume that the trajectories of the three biomarkers are explained by time at the population level and allow the effect of time as well as the intercept to vary among individual patients. We will use non-linear link functions on the biomarkers of endothelial dysfunction as well as transformations of the time-variable if model fit is improved as expressed by the Akaike Information Criteria (AIC).

As time variable we will use both time since enrollment measured in days as well as the number of the ICU-day (with the possible values 1,2 and 3), and choose the one that provides the best model fit as expressed by the AIC.

We will group patients into latent classes based on their trajectories of Syndecan-1, sTM and PECAM-1 respectively and incrementally increase the number of assumed latent classes until the optimal number of classes is found.

We will choose between latent-class models with an increasing number of latent classes using a combination of the AIC, the Sample Adjusted Bayes Information Criterion (SABIC), entropy (with values closer to one indicating better discriminatory ability) and the proportion of patients assigned to each class, as we will avoid models with classes containing < 5 % of patients.

To avoid local maxima we will run models for 30 iterations from 100 random vectors of initial values as recommended by the authors of the lcmm-package[22].

We will calculate the posterior probability of class-assignment and reject models with a mean posterior probability < 0.7 in any class.

We will also model the joint trajectories during the three first ICU-days of Syndecan-1, sTM and PECAM-1, specifically using the function multi-lcmm() from the lcmm-R-package [15]. Multi-lcmm assumes an underlying disease process measured with multiple biomarkers or tests - in this study the common process of endotheliopathy measured with Syndecan-1, sTM and PECAM-1. We will perform the modelling and latent class analysis in the same way as described above.

To test the stability of the findings, we will randomly split the dataset 90%/10% 100 times and for each of the three biomarkers we will run the lcmm-function with optimal settings and number of latent classes found in the main analysis on the whole dataset. We will plot the predicted values of the biomarkers from the models obtained from the split dataset to investigate if the algorithm would find similar groups in terms of predicted trajectories in different datasets.

Further, we will assess the discriminatory ability of the models. We will perform bootstrap sampling with replacement to produce 1000 new datasets. We will classify patients in each dataset using the models obtained from analysis on the full dataset and calculate the total mean posterior probability for each class of patients. We will also calculate the fraction of times each patient is assigned to a particular group over how many times the patient appears in the datasets.

As a further control we will apply longitudinal k-means clustering by means of the R-package kml3d to classify patients. The kml3d algorithm works only on complete trajectories and we therefore will use multiple imputation as recommended by the authors of the kml3d-package. We will then compare the obtained classification from k-means clustering with that obtained from the primary analysis using latent class mixed modelling by means of graphical representation as well as cross-tabulation of the groupings.

Missing data

The lcmm-package handles missing data as Missing At Random (MAR). MAR is defined as missingness being related to observed data-points.

In the present dataset, biomarkers of endothelial dysfunction were measured in patients still alive and admitted to the ICU. As biomarkers of endothelial dysfunction is associated with disease severity, we expect that missing values of endothelial biomarkers will be more frequent in patients with very high (because they die) or very low (because they are discharged from the ICU) initial values of the biomarkers of endothelial dysfunction. We therefore conclude that the MAR-description fits with the METABOLOMICS dataset and no imputations will be performed. As a sensitivity analysis, we will analyse the trajectories of the endotheliopathy biomarkers in a joint-model, that also models the dropout of patients due to death or discharge with time-to-event modelling [15]. If these models provide superior fit to the data based on the same criteria as above (AIC, SABIC, entropy) they will be chosen over the models assuming data is MAR.

Part two

Outcome Definitions

The primary outcome is liberation from mechanical ventilation. We define liberation from mechanical ventilation to occur either on the second of two consecutive ICU-days without the need for IV or NIV, or on the day of ICU discharge if cessation of mechanical ventilation occurs on the same day as ICU-discharge in patients that are not readmitted to the ICU within two days. We define the competing risk of death on mechanical ventilation as patients dying before or within 24 hours from liberation from mechanical ventilation. Patients are censored if they were moved to a different hospital while still receiving mechanical ventilation or at 30 days if still receiving mechanical ventilation.

The secondary outcome for part two of the study is 30-day all-cause mortality.

Variables

We will consider the latent class-based subgroups obtained in part one of the study as the primary explanatory variables. We will obtain the latent class-based subphenotype of each patient by assigning each patient to the class with the highest posterior probability for each of the chosen latent class models.

To inform the choice of confounders to be controlled for in the multivariable Cox regression analysis, we drew a directed acyclic graph (DAG) using the web based tool Dagitty[23]. Based on the DAG of the expected causal relationships, we decided upon controlling for the following confounders at baseline: age, acute kidney injury (AKI), known chronic obstructive pulmonary disorder (COPD), mental status, pulmonary or non-pulmonary infection, and surgery prior to ICU admission.

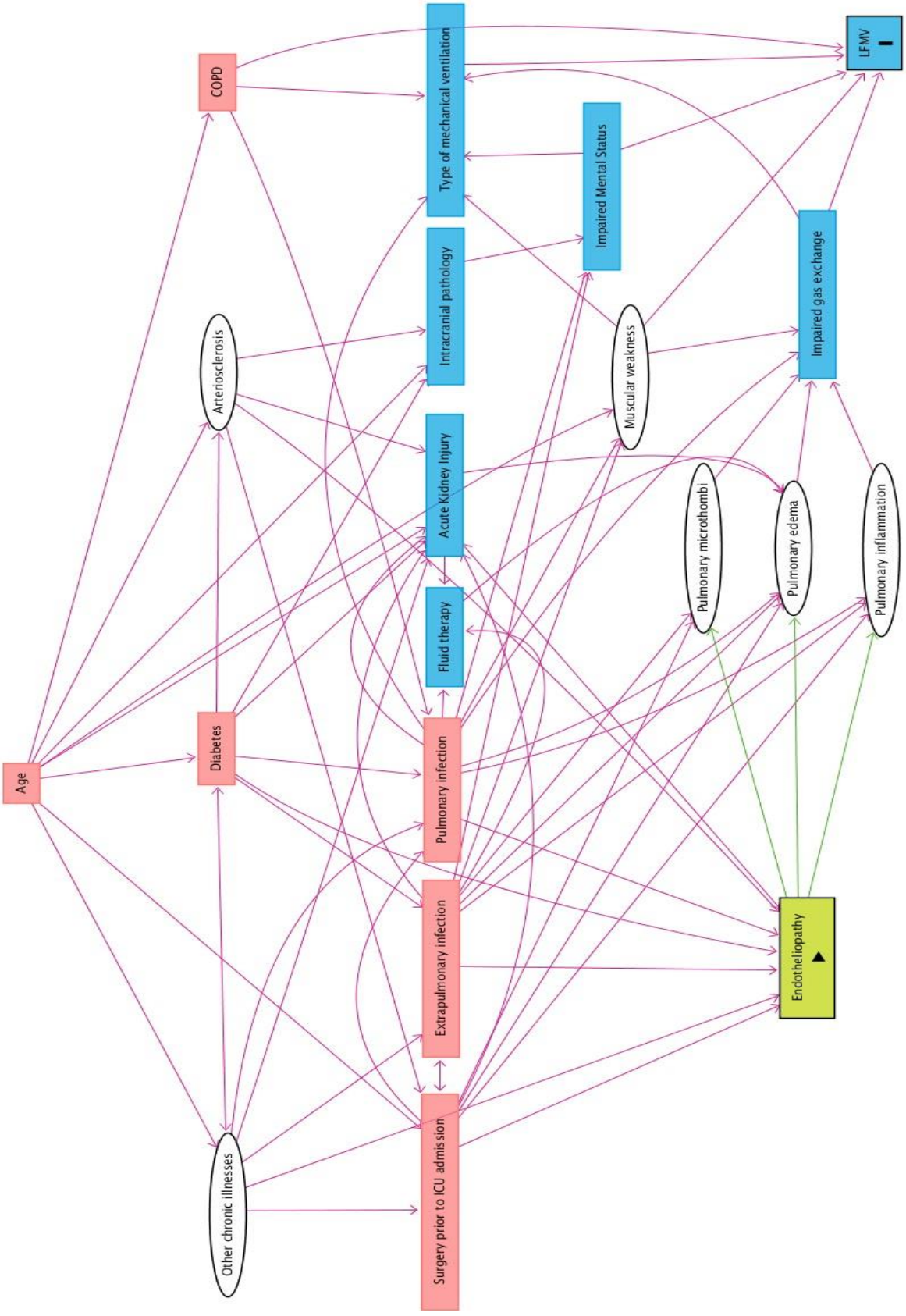

Figure 1: Directed Acyclic Graph depicting the expected causal relationships between endotheliopathy and liberation from mechanical ventilation (LFMV)

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COPD is adjudicated by one author (MSL) based on information from the EMR without knowledge of the results of the primary outcome or of the values of sTM, Syndecan-1 or PECAM-1.

AKI is defined as stage 2 or more from the KDIGO guidelines and calculated based on the worst creatinine during the first day of ICU-admission.[24] Baseline creatinine is defined as the median of all creatinine levels from 180 to 8 days prior to ICU-admission. In patients without a baseline creatinine, the creatinine levels is estimated with revised Lund-Malmö formula assuming a normal GFR of 70 ml/min/1,73m2.[25]

Mental status is represented by the Glascow coma scale (GCS) that is adjudicated by one of the authors (MSL) based on information from the EMR without knowledge of the results of the primary outcome or of the values of sTM, Syndecan-1 or PECAM-1.

Pulmonary or non-pulmonary infection is adjudicated by one of the authors (MSL) based on information from the EMR without knowledge of the results of the primary outcome or of the values of sTM, Syndecan-1 or PECAM-1.

Analysis Methods

The association of the endotheliopathy subgroups with the rate of liberation from mechanical ventilation will be analysed using cox regression and reported as cause-specific hazards using the method of Ozenne et al.[26] Each model is controlled for the abovementioned variables. This analysis is done only in patients still receiving mechanical ventilation after three days, as including patients being liberated from mechanical ventilation or dying before day 3 would violate the general assumption of Cox-regression that information from the future cannot be used to predict the past.

The association of the endotheliopathy subgroups with 30-day all-cause mortality will be modelled with cox regression and controlled for the same variables as the analysis of the primary outcome. This analysis is done only in patients still alive after three days, for the same reasons as mentioned above.

We will also perform a Cox regression analysis allowing the endotheliopathy biomarker to vary from day to day during the first three days using liberation from mechanical ventilation as well as 30-day all-cause mortality as outcomes[27].

We will model the effects of continuous variables with restricted cubic splines with 2 knots[28] if the introduction of splines significantly improves model fit as evaluated by the Wald Statistic.

All models will be examined for standard measures of model fit and assumptions and the included variables will be transformed as appropriately. By introducing interaction terms to the models, we will test if the

associations between the endotheliopathy subgroups and the primary outcome as well as 30-day all-cause mortality are different between: patients with and without septic shock at baseline; patients receiving invasive or non-invasive ventilation at baseline; patients with or without a history of COPD.

Missing Data

Missing data will be handled as follows: if less than 2,5% of an outcome or variable is missing, missing data is handled by case-wise deletion; if more than 2,5% is missing, multiple imputation will be performed using chained equations with predictive mean matching and logistic regression for numerical and categorical variables respectively.[29] All variables and outcomes included in the models will be used to inform the imputations. 10 datasets will be imputed.

Statistical programming software

All analyses are performed with R version 4.1.1 (The R Foundation for Statistical Computing).

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