Statistical analysis plan (SAP)

Title: Predicting 90-day survival in septic ICU patients: a *post-hoc* study of longitudinal Heparin-binding protein measures from the FINNAKI cohort

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Roles of SAP contributors
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Note: There is no study protocol since this is a non-interventional exploratory *post-hoc* study.

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Study background and rationale

Heparin binding protein (HBP) is a neutrophil-derived, pro-inflammatory and vascular permeability-inducing protein and a promising sepsis biomarker (1). Several studies of single measurements of HBP concentration in plasma have reported high prognostic accuracy on survival and development of sepsis and septic shock (2-4). However, studies on repeated measures of HBP are few and investigations on the predictive performance of longitudinal HBP are lacking (5-7). We hypothesize that longitudinal HBP hold added predictive value, similar to that of lactate and procalcitonin (8, 9). Furthermore, previous studies on HBP and survival prediction lack adjustments for potential confounders (2-4). Our primary aim is to investigate longitudinal HBPs added predictive value in a contemporary survival model including clinically relevant predictors.

Null hypotheses

Plasma HBP concentration does not add predictive value to clinically available predictors regarding the number of days alive within 90 days from ICU admission (90-day survival) among patients with severe sepsis and septic shock when measured repeatedly during ICU stay (hour 0, 12, 24, 36, 48 hours, 3 days and 5 days from ICU admission, hereafter referred to as “longitudinal HBP” (ng/ml)).

Study population and design

This is an exploratory *post-hoc* study of predictive biomarker performance using a cohort from the prospective, observational, multicentre FINNAKI study (10). The analysis will include patients with severe sepsis or septic shock diagnosed on the day of ICU admission and who had at least one plasma sample available from the first five days of ICU stay. This totals 652 patients (22%) out of the total 2901 patients included in the original FINNAKI cohort (figure 1). Due to the exploratory nature of the study no power calculation was made.

Original inclusion and exclusion criteria

The FINNAKI study consecutively included all emergency ICU admission and the elective admissions with an ICU stay of above 24 hours from seventeen Finnish ICUs during a five-month period (1 September 2011 to 1 February 2012). In brief, exclusion criteria for the FINNAKI study were patients who 1) had end-stage renal disease requiring maintenance dialysis, 2) were organ donors, 3) received intermediate care, 4) had received renal replacement therapy (RRT) while enrolled in the study during a previous ICU admission, 5) were transferred from another ICU where the data collection for the study was fulfilled, or 6) were not permanently living in Finland or were unable to give consent due to insufficient language skills.

Primary (exploratory) objective

A. To investigate if longitudinal HBP adds predictive value to clinically available predictors 90-day survival

Secondary (exploratory) objectives

B. To evaluate if longitudinal HBP alone predicts 90-day survival
C. To evaluate if plasma HBP measured at ICU admission predicts 90-day survival
D. To investigate if longitudinal HBP adds predictive value to a single HBP measure at ICU admission regarding 90-day survival
E. To investigate if plasma HBP measured at ICU admission adds predictive value to clinically available predictors of 90-day survival

Overall statistical approach

We will evaluate added predictive value through fitting univariate and multivariate proportional hazard regression models (Cox) using clinically available predictors of 90-day survival and compare model performance using likelihood ratio chi-square (LR) tests pre versus post addition of single or longitudinal biomarker data. The longitudinal part will be a linear mixed-effects model with a random intercept and random slope which will be joined into the base Cox model (joint modelling). We will fit five models in total and perform three LR tests.

Included Covariates

Six clinically available candidate predictors will be included in the full survival model. They have been selected by availability and former known association with the outcome: Age (years, continuous), sex (2 categories), functional performance pre-ICU (four-level ordinal scale from 1, best, to 4, worst (i.e. cannot care for him/herself)), lactate on admission (mmol/L, continuous), sequential organ failure assessment (SOFA) score on admission (points, continuous) and pre-existing chronic health conditions (included separately as 0/1 or as a summarised score 0 to 5 depending on Wald χ^2 and df: any malignancy, chronic heart failure (CHF), chronic kidney disease (CKD), chronic liver failure (CLF), chronic obstructive pulmonary disease (COPD)).

Level of statistical significance and confidence intervals

P values below 0.05 in two-sided tests will be considered statistically significant. 95% confidence intervals (95% CI) will be reported.

Timing of final analysis

The final analysis is planned for the first half of 2021.

Statistical software used
Stata/MP 16.1 and R/R-studio

Stepwise analysis plan

1. Determine outcome. Survival time up to 90 days from ICU admission. Cases with missing outcome will be discarded.
2. Crude analysis. All variables (except HBP) will be analysed univariately against the primary outcome using the Cox model, on their original scale, using complete case analysis.
3. Missing data. All variables will be screened for frequency and type of missingness (i.e. missing at random or not). Multiple imputation will be used if missingness exceeds 5% (excl. HBP).
4. Variable transformations. Variables will be tested for a non-linear relationship with the outcome using restricted cubic splines and transformed accordingly (incl. HBP). Extreme outliers will be checked for data entry errors and possibly truncated or winsorized (excl. HBP). Categorical variables will be checked for small categories (<5%) or and excluded if violated.
5. Fitting three Cox models.

Model 1: Fit full Cox model using candidate predictors
Model 2: Fit full Cox model using candidate predictors and HBP on admission
Model 3: Fit univariate Cox model using HBP on admission (objective C)

6. Correlated data. We will screen for multicollinearity in the multivariate models, using variance inflation factor (VIF). We will examine variables with high VIF (>4) and for variables that correlate above 0.8 one from the pair will be excluded from the model.
7. Interaction. We will screen for interaction effects with pooled interaction tests. If the test signals that interaction exist, we will test specific interactions within the model.
8. Influential observations. We will test for influential observations with dfBeta > 0.2 and consider sensitivity analyses without these cases.
9. Assumptions 1. We will test proportional hazards assumption using a global test, Schoenfeld residuals and a log-log plot of survival.
10. Refit models. If needed, we will refit the multivariate model in its final form according to 6-9. If assumptions are still not met, we will consider incorporating a time-interaction-variable in the Cox model.
11. Longitudinal model. We will fit a linear mixed-effects model of HBP with a random intercept at the individual patient level and a random slope for time, using an unstructured covariance matrix.
12. Assumptions 2. We will check linear assumptions using a residual-fitted plot, residual histogram and qq-normal plot. If assumptions are violated, we will try fitting a generalized linear model using an appropriate distribution family (e.g. gamma) or consider log-transforming HBP after matching zero-values to next sample minimum.
13. Refit model. Refit longitudinal model according to 12 if needed.
14. Fitting two joint models.

Model 4: Fit a joint model using an unadjusted Cox model and a longitudinal HBP model (objective B)
Model 5: Fit a joint model using the full Cox model including candidate predictors and a longitudinal HBP model.

15. Internal validation. We will perform bootstrapping for all models to determine the amount of overfitting.
16. Model performance. We will present HR and 95% CI for all included variables and any interaction terms in all five models, respectively, as well as measures of model

performance, calibration and discriminatory ability (i.e. LR statistic, R^2 estimate, C statistic and risk variance).

17. Test for added value. We will test for a significant difference in model performance using three LR tests to compare:
    1) Model 1 vs 2 (objective E),
    2) Model 3 vs 4 (objective D) and
    3) Model 1 vs 5 (objective A)
18. Added information size. We will quantify the fraction of new predictive information by comparing the relative explained variance between models. We will also consider estimating increase in net benefit if plausible.
19. Graphic presentation. We will present a calibration plot and further visualise added predictive information graphically, e.g. by using a histogram of predicted risks between models (pre vs post addition of HBP). We will also present a dynamic prediction diagram for a single individual regarding the variation in mortality risk associated with longitudinal changes in HBP.
20. Multiplicity. We are not planning to adjustment for multiple comparisons.
21. Subgroup analyses. None planned.

References

1. Fisher J, Linder A. Heparin-binding protein: a key player in the pathophysiology of organ dysfunction in sepsis. J Intern Med. 2017;281(6):562-74.
2. Linder A, Christensson B, Herwald H, Bjorck L, Akesson P. Heparin-binding protein: an early marker of circulatory failure in sepsis. Clin Infect Dis. 2009;49(7):1044-50.
3. Linder A, Arnold R, Boyd JH, Zindovic M, Zindovic I, Lange A, et al. Heparin-Binding Protein Measurement Improves the Prediction of Severe Infection With Organ Dysfunction in the Emergency Department. Crit Care Med. 2015;43(11):2378-86.
4. Kahn F, Tverring J, Mellhammar L, Wetterberg N, Blackberg A, Studahl E, et al. Heparin-Binding Protein As A Prognostic Biomarker of Sepsis and Disease Severity at The Emergency Department. Shock. 2019.
5. Tverring J, Nielsen N, Dankiewicz J, Linder A, Kahn F, Akesson P. Repeated measures of Heparin-binding protein (HBP) and procalcitonin during septic shock: biomarker kinetics and association with cardiovascular organ dysfunction. Intensive Care Med Exp. 2020;8(1):51.
6. Johansson J, Lindbom L, Herwald H, Sjoberg F. Neutrophil-derived heparin binding protein--a mediator of increased vascular permeability after burns? Burns. 2009;35(8):1185-7.
7. Linder A, Akesson P, Inghammar M, Treutiger CJ, Linner A, Sunden-Cullberg J. Elevated plasma levels of heparin-binding protein in intensive care unit patients with severe sepsis and septic shock. Crit Care. 2012;16(3):R90.
8. Schuetz P, Maurer P, Punjabi V, Desai A, Amin DN, Gluck E. Procalcitonin decrease over 72 hours in US critical care units predicts fatal outcome in sepsis patients. Crit Care. 2013;17(3):R115.
9. Nguyen HB, Rivers EP, Knoblich BP, Jacobsen G, Muzzin A, Ressler JA, et al. Early lactate clearance is associated with improved outcome in severe sepsis and septic shock. Crit Care Med. 2004;32(8):1637-42.
10. Nisula S, Kaukonen KM, Vaara ST, Korhonen AM, Poukkanen M, Karlsson S, et al. Incidence, risk factors and 90-day mortality of patients with acute kidney injury in Finnish intensive care units: the FINNAKI study. Intensive Care Med. 2013;39(3):420-8.