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Title

Hemoglobin trajectories in acute infections and their association with mortality and persistent anemia: a retrospective cohort study

Background

Anemia is a common complication to severe infections and is present in up to 80% of all critically ill patients at hospital discharge. (1,2) Persistent anemia after discharge has been associated with increased mortality during the first year after hospitalization, as well as increased activities of daily living (ADL) dependencies and decreased physical capacity in survivors. (2,3)

The incidence of persistent anemia has increased in recent years, in part due to increased focus on the risks associated with blood transfusions and, consequently, the adoption of a more restrictive transfusion strategy for patients with severe infections. (4–6) To counter this development and keep the use of possibly harmful blood transfusions low, we need to identify patients at risk of persistent anemia early and provide preventative treatment.

One potential therapeutic option is intravenous iron, which has been shown to increase hemoglobin levels after discharge, reduce hospital readmissions, and improve long-term survival in critically ill patients—all with minimal side effects. (7–10) To identify patients who may benefit from this treatment, it is necessary to characterize the type and cause of anemia. In critically ill patients, this is often expensive and time-consuming. The identification of subgroups at risk of developing long-term anemia could help in selecting the right patients for further diagnostics.

The average change of hemoglobin over time in patients with severe infections has previously been described. (11) Due to individual differences, however, the mean change over time may not actually represent the trajectory of any real patients, and data can in this case better be described by dividing the population into distinct subgroups with similar trajectories.

Previous studies have shown that specific trajectories of platelets (12–14) and white blood cells (15) are associated with increased mortality in patients with severe infections. Similarly, specific hemoglobin trajectories associated with complications have been identified in patients undergoing cardiac surgery. (16,17) It is not known, however, whether specific trajectories of hemoglobin during admission are associated with persistent anemia or mortality.

The aims of this study are, first, to identify trajectories of hemoglobin over the first seven days of admission in patients with acute infections, and second, to evaluate whether these trajectories are associated with the occurrence of post-discharge anemia and all-cause mortality within 30 days.

Methods

Study Design

The Database of Community-Acquired Infections Requiring Hospital Referral in Eastern Denmark (DCAIED) is a database of all adult patients referred to emergency departments in Region Zealand and the Capital Region of Denmark with suspected community-acquired infection. (18)

This is a sub-study of the DCAIED. The study is approved by the local ethical committee (R-25021073).

The study will be reported according to STROBE (19) and GRoLTS (20) recommendations.

The study is based on *growth mixture modeling (GMM)*, an extension of the traditional linear mixed model. (21,22)

Statistical Principles

P-values below 0.05 are considered statistically significant. Ninety-five percent confidence intervals are presented where feasible. No correction for multiple comparison is performed, as all study results are considered exploratory.

Study Population

The background population is the population of Region Zealand and the Capital Region of Denmark (~2.8 million inhabitants).

Eligibility
Inclusion criteria are

- age ≥18 years at the time of hospital admission,
- suspected community-acquired infection (administration of antibiotics and collection of blood cultures within 72 hours of hospital admission),
- hospital admission longer than seven days (i.e., not discharged or dead before then), and
- at least two hemoglobin measurements separated by at least 24 hours.

Exclusion criteria are

- hematologic comorbidity at baseline*,
- recorded blood loss of >500 ml during days 1-7, and

- previous hospital admission <14 days before current hospitalization.

*Hematologic comorbidity is defined as having an ICD-10 code registered in the EHR prior to admission corresponding to hematologic malignancy (C81-C96) or other diseases relating to the blood and lymphatic system (D45-D77).

Baseline Characteristics

Data on the following baseline characteristics will be extracted:

- Patient demographics (age, gender)
- Vital parameters at admission
- Triage level
- SOFA-score (days one, two and three after admission; total and per organ system)
- Sepsis and/or septic shock (based on SOFA-score, blood pressure and lactate levels)
- Comorbidities (ischemic heart disease, heart failure, cerebrovascular disease, hypertension, diabetes mellitus, liver cirrhosis, chronic kidney disease, lung disease)
- Baseline hematologic status (hemoglobin, leukocytes, platelets)
- Baseline blood gas results (pO2, pCO2, lactate)
- Baseline infectious parameters (C-reactive protein, procalcitonin)
- Admitting specialty (from emergency department)
- Initial ICD-10 diagnosis
- Antibiotic prescription immediately before current hospitalization
- Initial choice of antibiotics, including time to antibiotic administration
- Administration of glucocorticoids

Baseline is defined as the worst value during the first 24 hours of admission, unless otherwise specified.

Statistical Analysis

The statistical analysis plan is split into a first part, regarding the identification of trajectories of hemoglobin, and a second part, regarding the association between these trajectories and anemia, blood transfusion, and mortality.

Sample Size
Due to the exploratory nature of the study, and the first part in particular, formal power calculations cannot be performed.

First Part: Identification of Hemoglobin Trajectories

The primary outcome in the first part is the optimal number of latent classes and the smallest number of days of observation needed for acceptable class assignment.

Variables
Hemoglobin is the primary variable of interest. It is recorded and presented in mmol/L. Hemoglobin will be presented as the mean value for each day from day one to day seven. The trajectory of hemoglobin is the dependent variable in the first part, and the trajectory of hemoglobin will be modelled as a function of time.

Descriptive Statistics
Hemoglobin will be presented as daily mean with standard deviation.

The individual trajectories of a sample of patients will be illustrated in a spaghetti plot with a LOWESS tracing.

Analysis
The trajectory of hemoglobin will be modelled using the lcmm() function from the lcmm package. Data will be split into a training (70%) and a test (30%) data set.

Following the stepwise approach detailed by Lennon and colleagues (23), we will construct a scoping model based on a plausible number of classes, which will guide the final model development.

The optimal number of classes will be chosen based on the lowest Bayesian Information Criterion (BIC). Model adequacy will then be assessed by average posterior probability of assignments (APPA), which should be above 70% for all classes, odds of correct classification and entropy. If these measurements are violated, a class count with higher BIC will be chosen.

The optimal model will then be assessed on clinical meaningfulness and plausibility by a specialist physician. A clinically irrelevant model will be rejected.

The final model will be used to predict the class assignments for the patients in the test dataset. Here the APPA will be used for evaluation.

The percentage of patients classified to their final latent class (i.e., the class predicted at day seven), for each day from admission will be presented, to evaluate how well the model can classify patients at earlier time points.

Missing Data
The lcmm package can handle missing data under the assumption that data is missing at random. In the current setting, this hypothesis seems unlikely, and data will therefore be imputed. To maximize clinical interpretability, data will be imputed according to last observation carried forward, as this is how clinicians see hemoglobin levels.

Subgroup Analyses
Subgroup analyses will be performed in the following populations:

1. Patients who have not received any blood transfusion from admission to day seven.

Second Part: Association with Anemia and Mortality

The primary outcome in the second part is clinically important anemia within 7-30 days of admission, defined by the occurrence of

- anemia workup within 30 days of admission,
- any outpatient visit with a primary ICD-10 code corresponding to anemia within 30 days after admission, or
- blood transfusion within 30 days after admission.

The secondary outcome is all-cause mortality within 30 days of admission.

Variables
The latent classes identified in the first part will be considered the primary explanatory variables. Known predictors of poor outcome will be included in multivariate models, including age, gender, sepsis at baseline, highest SOFA-score (see Appendix) during the first 72 hours of admission, history of heart failure, history of liver cirrhosis and history of chronic kidney disease. Sepsis and septic shock are defined in accordance with the criteria proposed by Singer et.al. in 2016 (“Sepsis-3”).

Analysis
The association between the predicted latent classes and the primary outcome will be assessed using logistic regression, adjusted for age, sex and comorbidites. The association between the latent classes and mortality after 30 days will be assessed using a Cox proportional hazards model, adjusted for age, sex and comorbidities.

Statistical Programming Software
All analyses are performed with R version 4.4.2.

Appendix

SOFA-scores will be calculated based on clinical data from the electronic health record.

The scores for kidney function, liver function, platelets and level of consciousness will be calculated based on the value corresponding to the highest SOFA-score from each admission day.

The score for circulation will be calculated based on recorded values for blood pressure and administrations of vasoactive medications. Medication data recorded in other units than

µg/kg/min will be converted to µg/kg/min if possible, and otherwise recorded as the lowest possible dose of the medication. The data corresponding to the highest SOFA-score for each admission day will be recorded.

The score for respiratory function will be calculated based on recorded values for the ratio between PaO2 and FiO2, when available. Values within six hours from one another will be used to calculate a ratio. The lowest ratio for each day will be used to calculate a SOFA-score. If one or both are missing, an estimation based on the method presented and validated by Valik et.al. will be used (i.e., assigning patients a respiratory SOFA-score of 1 if peripheral saturation is 91-94% and a SOFA-score of 2 if it is <91%). (24) Data on mechanical ventilation will be extracted from the electronic health record. CPAP, NIV and invasive ventilation will be considered mechanical ventilation.

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Tables and Figures

Tables

Table 1

	Overall (n = ###)
n	###
Age, years (mean, SD)	###
Male sex, n (%)	###
Triage level, n (%)	
Orange	###
Red	###
Highest SOFA score day 1 (median, IQR)	###
Sepsis, n (%)	###
Septic shock, n (%)	###
Comorbidities	
Ischemic heart disease, n (%)	###
Heart failure, n (%)	###
Cerebrovascular disease, n (%)	###
Hypertension, n (%)	###
Diabetes mellitus, n (%)	###
Liver cirrhosis, n (%)	###
Chronic kidney disease, n (%)	###
Lung disease, n (%)	###
Laboratory values at baseline	
Hemoglobin (mmol/L, mean, SD)	###

	Overall (n = ###)
Leukocytes (10^9/L, mean, SD)	###
Platelets (10^9/L, mean, SD)	###
Arterial pO_2 (kPa, mean, SD)	###
Arterial pCO_2 (kPa, mean, SD)	###
Lactate (mmol/L, mean, SD)	###
CRP (mg/L, mean, SD)	###
Procalcitonin (ng/mL, mean, SD)	###
Admitting specialty, n (%)	
Internal Medicine	###
Neurology	###
Surgery	###
Other	###
Antibiotics prior to admission, n (%)	###
Initial antibiotic choice, n (%)	
Ampicillin + Gentamicin	###
Cefuroxime	###
Meropenem	###
Piperacillin-tazobactam	###
Other	###
Time to antibiotics (hours, mean, SD)	###
Glucocorticoids administered, n (%)	###

Table 2

	Multivariate model	

Outcome	HR (95% CI)	P
Anemia	###	###
All-cause mortality within 30 days	###	###

Figures (based on mock data)

Figure 1: Percentage of patients classified into final class per day

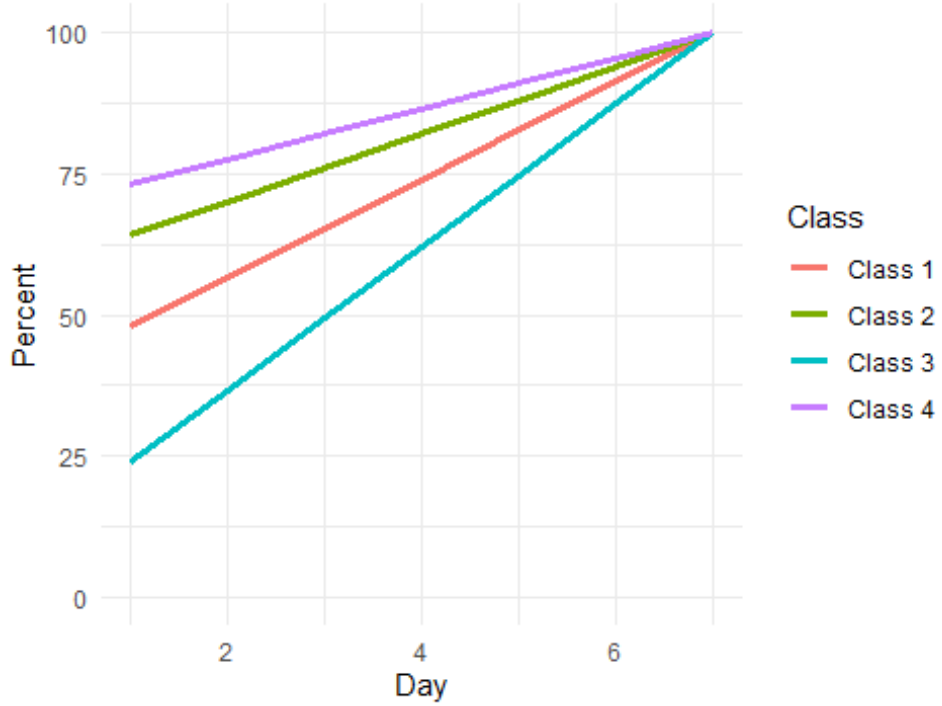
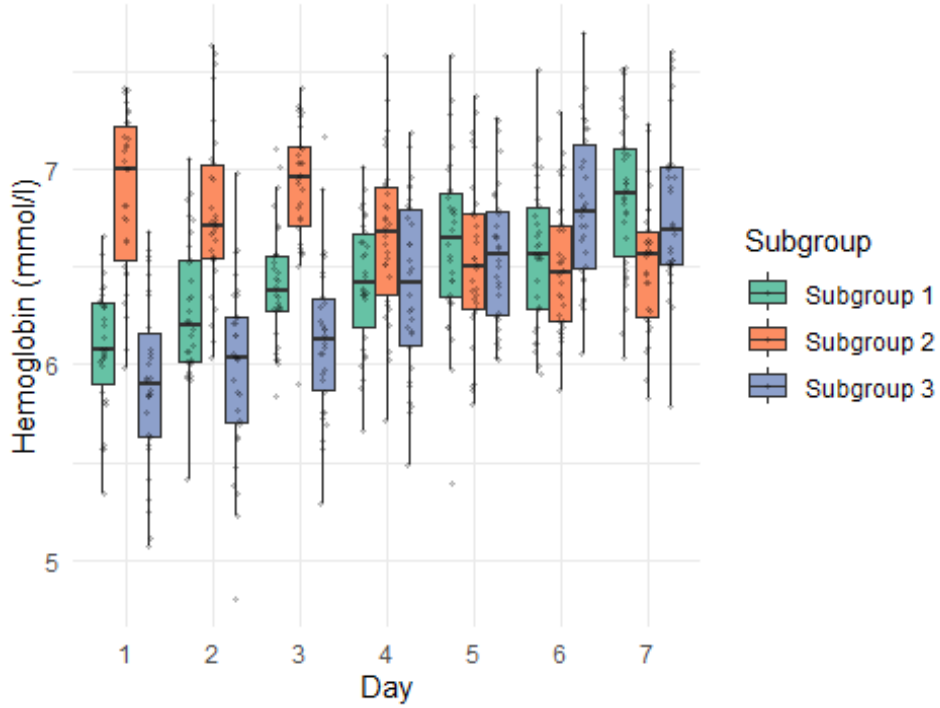


Figure 2: Mean value of hemoglobin (mmol/L) per class and day



Supplementary Tables (based on mock data)

Supplementary Table 1: Model characteristics

Model	AIC	BIC	SABIC	npm	%class 1	%class 2	%class 3	%class 4	%class 5	%class 6
1_class	1504	1317	1233	15	28.8	NA	NA	NA	NA	NA
2_class	1177	1497	1905	16	78.8	40.9	NA	NA	NA	NA
3_class	1173	1250	1244	11	88.3	94.0	4.6	NA	NA	NA
4_class	1394	1540	1610	18	52.8	89.2	55.1	45.7	NA	NA
5_class	1591	1559	1745	14	95.7	45.3	67.8	57.3	10.3	NA
6_class	1743	1686	1156	18	90.0	24.6	4.2	32.8	95.5	89