Research Square

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Combined inflammatory and metabolic biomarkers predict the mortality rate of patients with acute dyspnoea

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Abstract

Objective

To investigate whether plasma concentrations of inflammatory biomarkers and amino acids in patients presenting to the emergency department with acute dyspnoea predict 90-day mortality.

Background

Acute dyspnoea is a common reason for emergency department (ED) visits and has heterogeneous causes; rapid risk stratification is essential. Biomarkers may complement clinical assessment. We previously found that interleukin-8 (IL-8), growth differentiation factor-15 (GDF-15), and the amino acids glycine, phenylalanine and valine were independently associated with 90-day mortality in acute dyspnoea.

Methods

Inflammatory biomarkers and amino acids were analysed in adults admitted to the ED at Skane University Hospital, Malmo, Sweden (2013–2016), and 90-day mortality was modelled using Cox proportional hazards adjusted for age, sex, oxygen saturation, respiratory rate, C-reactive protein (CRP), estimated glomerular filtration (eGFR), lactate and the “Medical Emergency Triage and Treatment System-Adult” score. The inflammatory biomarker score (IL-8 and GDF-15) was replicated in 448 newly collected patients. The combined effect of the amino acid mortality risk score (AMRS) and the inflammatory biomarker mortality risk score (IBMRS) was tested in 637 patients, assessing discrimination (AUC), risk reclassification (Net Reclassification Improvement), and internal validation by bootstrap resampling.

Results

In the replication cohort (n=448): 57 patients (12.7%) died within 90 days. Mortality was 1.8% in quartile 1 vs 28.6% in quartile 4 of the inflammatory biomarker score; per 1 SD increment, hazard ratio was 2.01 (95% CI 1.44–2.81) after multivariable adjustment.

In the combined cohort (n=637): 90-day mortality was 12.2% (n=78), with 1.2% mortality in quartile 1 and 32% in quartile 4. After multivariable adjustment, hazard ratio was 2.83 (95% CI 2.12–3.77) per 1 SD increment and was thirteen times greater for Q4 than for Q1. Adding the combined biomarker risk score to standard clinical predictors increased the time-dependent AUC from 0.79 (95% CI 0.74–0.84) to 0.85 (95% CI 0.82–0.89); the optimism-corrected Harrell’s C-index was 0.81 and the calibration slope was 0.88.

Conclusion

Independent associations between IL-8 and GDF-15 and 90-day mortality in acute dyspnoea were replicated.

IL-8, GDF-15, glycine, valine, and phenylalanine independently predicted 90-day mortality, improving AUC by approximately seven percentage points.

Background

Dyspnoea is a commonly encountered symptom, accounting for up to one-third of presentations to the internal medicine services of the emergency department (ED) in US studies.[1] Common causes include infection, chronic obstructive pulmonary disease (COPD), pulmonary embolism, congestive heart failure (CHF) and pulmonary fibrosis—each requiring distinct diagnostic pathways and therapeutic strategies. Disease severity spans a broad spectrum, both between conditions and within individual diagnoses, and is often compounded by comorbidities. In the ED setting, rapid identification and prioritisation of patients at highest risk of mortality or severe morbidity is essential, driving the implementation of standardised triage tools and local and national protocols to enhance patient safety.

Biomarkers have long supplemented vital signs and clinical findings in the ED, providing rapid, reliable and standardised insights into underlying pathophysiology and prognosis.

Our group has previously published on the utility of inflammatory biomarkers and amino acids for prognostic assessment in acute dyspnoea.[2, 3] The current investigation seeks to replicate those earlier findings—specifically the association between selected inflammatory markers and 90-day mortality—and to assess whether combining inflammatory biomarkers with plasma amino acids further improves prognostic accuracy.

Our previous studies identified glycine, phenylalanine and valine as prognostically informative amino acids, and interleukin-8 (IL-8) and growth differentiation factor-15 (GDF-15) as prognostically informative inflammatory biomarkers, for predicting 90-day mortality in patients presenting to the ED with acute dyspnoea.[2, 3]

Amino acids

Acute illness triggers a shift from an anabolic to a catabolic state mediated by stress hormones, leading to insulin resistance and altered amino acid metabolism.[4, 5] As glucose uptake becomes impaired, peripheral tissues increasingly rely on certain amino acids, such as valine, for energy.[6] The breakdown of peripheral tissue, particularly skeletal muscle, releases phenylalanine, while insulin-dependent hepatic uptake is impaired, thereby modifying plasma amino acid profiles.[7–9] Glycine also exerts anti-inflammatory effects, including inhibition of pro-inflammatory cytokine production. In a pilot randomised, double-blind, cross-over trial in cystic fibrosis, oral glycine improved clinical symptoms, spirometric measures, and inflammatory markers compared with placebo.[10, 11]

Inflammatory biomarkers

Inflammatory cytokines complement metabolic biomarkers by reflecting disease severity and underlying pathology. Interleukin-8 (IL-8), produced by macrophages, monocytes, fibroblasts, and epithelial and

endothelial cells, induces neutrophil activation, T-cell chemotaxis and angiogenesis. IL-8 plays a pivotal role in septicaemia, COPD, asthma, cancer and inflammatory bowel disease.[12–14] Elevated IL-8 levels have been associated with more severe influenza pneumonia, while decreased IL-8 levels have been linked to improved respiratory symptoms in post-acute COVID-19.[15, 16] Growth differentiation factor-15 (GDF-15) is an immunomodulatory cytokine that is elevated in malignancy, tissue injury and placental tissue, where it may shield the foetus from maternal immune attack and protect tumour cells from immune clearance.[17, 18]

Methods

This article consists of two main studies. The first study, hereafter referred to as the “Replication Study”, is an internal replication study of a previous study on inflammatory biomarkers in patients with acute dyspnoea. The second study, hereafter referred to as the “Combined Inflammatory Biomarkers and Amino Acids study,” analyses the performance of a combination of inflammatory biomarkers and amino acids in predicting the prognosis of patients with acute dyspnoea. The methods have previously been described by Wiklund et al. (2016, 2021).[2, 3]

Study population

The study was conducted at the adult emergency department (ED) of Skane University Hospital in Malmo, a tertiary university hospital serving the local urban population and acting as a regional referral centre for specialised care within Region Skane, southern Sweden (approximately 1.5–1.7 million inhabitants). Patients presenting to the ED represent an unselected ED population spanning a broad range of medical conditions and disease severity.

Between March 6, 2013, and January 20, 2016, research nurses at the Emergency Department (ED) of Skane University Hospital (Malmo, Sweden) enrolled adult patients (≥18 years) presenting with acute dyspnoea as the primary symptom. Patients with acute dyspnoea were defined by having the main symptom “dyspnoea” “acute dyspnoea” or similar expressions i.e. “breathing difficulties” written in the ED electronic logbook after triage. Triage was performed by a triage nurse or the ambulance service in a regular manner without the involvement of research nurses. Identification and enrolment of patients in the study were performed during research-nurse working hours (06:45 AM–4:30 PM). Eligible patients provided written informed consent; patients unable to provide informed consent were not included.

Research nurses recorded clinical characteristics and routine blood-chemistry results using the same standardized procedures as in our previous studies.[2, 3]

A total of 999 patients were identified in the study during the specified period; 36 patients withdrew consent or left the ER before the research protocol was finished. A total of 963 patients had their blood examined for the inflammatory biomarkers IL-8 and GDF-15, and 788 patients had their blood examined for amino acids.

For the replication study of the analysis of inflammatory biomarkers, 442 of the 963 patients were excluded because they had been part of the previous study published in 2016, leaving 521 patients for the independent replication analysis of IL-8 and GDF-15. An additional 73 (14%) patients were excluded because of missing values, resulting in 448 patients eligible for analysis.

For the combined inflammatory biomarker and amino acid study, 151 (19.2%) of the 788 patients were excluded because of missing values, resulting in 637 patients for the analysis of the combination of inflammatory biomarkers and amino acids. A comparison of complete-case and incomplete-case participants is provided in Supplementary Table 6, and the number of missing observations by variable is summarised in Supplementary Table 7.

A flow chart displaying the final selection of patients can be seen in Figure 1.

Position of Figure 1

Clinical parameters

Clinical parameters were obtained as described previously.[2, 3]

Research nurses recorded vital signs assessed by ED or ambulance staff: respiratory rate was manually counted, while blood pressure, oxygen saturation and heart rate were measured using a CARESCAPE Monitor B850 or B650 (GE Healthcare).[19, 20] Consciousness level was graded via the Reaction Level Scale (RLS).[21] After enrolment, patients reported smoking status (never, former, or current), existing dyspnoea-related comorbidities (e.g., CHF, COPD, coronary artery disease (CAD), atrial fibrillation, restrictive lung disease, cancer, thromboembolic and rheumatic disorders), and current medications. Research nurses then reviewed medical records to verify prior diagnoses and medication use. Blood samples were collected during the patient's ED stay; no fixed time window from presentation was specified, but samples were frozen within 4 hours of collection.

Triage was performed using the Medical Emergency Triage and Treatment System–Adult (METTS-A), a five-level triage tool that integrates presenting symptoms with physiological parameters (oxygen saturation, respiratory rate, heart rate, blood pressure, level of consciousness and temperature). [22] METTS-A priorities range from Priority 5 (blue; no emergency care required) and Priority 4 (green; lowest urgency/stable), through Priority 3 (yellow; moderate urgency) and Priority 2 (orange; high urgency/rapid assessment), to Priority 1 (red; immediate life-threatening condition). No patients in the Priority 5 category were identified for this study, and this category is therefore excluded from further analysis.

National Early Warning Score (NEWS), as defined by Royal College of Physicians was calculated retrospectively by summing component scores for recorded physiological parameters at presentation (respiratory rate, oxygen saturation, systolic blood pressure, heart rate, temperature and level of consciousness), with supplemental oxygen scored when available. NEWS was categorised as low (0–4),

medium (5–6) or high (≥7), and the presence of any single-parameter score of 3 (“red score”) was reported (Supplementary Tables 4 and 5).[23]

The endpoint was all-cause mortality within 90 days from presentation to the ED, hence the final endpoint being 90 days from when the last patient was enrolled. Survival data were retrieved from the national civil registry, which is linked to the personal identification number of each patient. If a patient died, the date of death was recorded.

Assays

Plasma C-reactive protein, lactate and creatinine were measured at presentation using a Radiometer ABL800 Flex or an Afinion AS100 Analyzer.[24, 25] Estimated glomerular filtration rate was calculated with the Lund–Malmo creatinine-based equation—chosen over MDRD for its validation in laboratory assays and superior accuracy at eGFR <30 and 30–59 ml/min/1.73 m².[26] Blood samples were immediately processed in the ED, separated into serum and plasma, then frozen at –80 °C for later biomarker analysis.

Biomarker measurement

Frozen plasma samples were analysed for the inflammatory biomarkers interleukin-8 (IL-8) and growth differentiation factor-15 (GDF-15) using the Proseek Multiplex CVD I biomarker panel at Olink Bioscience (Uppsala, Sweden); samples were shipped in September 2014 and September 2016. The amino acids glycine, phenylalanine and valine were analysed using a proton nuclear magnetic resonance (NMR) platform (Bruker AVANCE III 500 MHz spectrometer) at Nightingale Health Plc (Helsinki, Finland); samples were shipped in September 2015, as previously described.[14, 15, 21-23] [19, 20, 27-29]Laboratory analyses were performed blinded, and laboratory personnel were unaware of patient characteristics, clinical outcomes, and the study hypotheses.

Statistical analysis

Statistical analyses were performed as previously described by Wiklund et al.[2, 3] The methods are briefly summarised below. No a priori data partitioning (e.g., training/test split) was undertaken; analyses were conducted in the prespecified complete-case cohorts, and model performance was evaluated in the full dataset, with internal validation performed using bootstrap resampling. In addition, an annotated and clarified version of the SPSS syntax used for the main analyses is provided in the Supplementary Appendix.

The previously published models were developed in earlier cohorts of adult patients presenting with acute dyspnoea at the same ED and using comparable eligibility criteria, outcome definition (all-cause 90-day mortality), and biomarker assays.[2, 3] The present Replication Study evaluated the inflammatory

biomarker score in an independent patient sample from the same healthcare setting, explicitly excluding individuals included in the earlier study. The Combined study evaluated an expanded predictor set by adding amino acids to the inflammatory biomarker score, while retaining the same clinical predictors and outcome definition. The distributions of key demographic characteristics, predictors, and outcomes in the evaluation cohorts are presented in Table 1 and Supplementary Table 1 and can be compared descriptively with the corresponding distributions reported for the development cohorts by Wiklund et al. [2, 3]

Replication Study

Inflammatory biomarker values (and C-reactive protein (CRP)) were log-transformed to normalise skewed distributions and standardised per 1 SD increment. We then used Cox proportional-hazards models to assess each biomarker's association with 90-day mortality, with follow-up from ED presentation to death or 90 days. Model 1 adjusted for age and sex, while Model 2 additionally adjusted for age, sex, the Medical Emergency Triage and Treatment System–Adult (METTS-A) triage score, oxygen saturation, respiratory rate and CRP, entering all biomarkers simultaneously. Biomarkers that remained significant in Model 2 were combined into an inflammatory biomarker mortality risk score (IBMRS), weighted by their β-coefficients and expressed as a z-score. The IBMRS was then analysed both as a continuous variable and by quartiles in Cox models—adjusted as above—to determine its relationship with 90-day mortality.

Combined Inflammatory Biomarkers and Amino Acids study

Skewed distributions of amino acids, inflammatory biomarkers, CRP and lactate were normalised by natural-log transformation and standardised per 1 SD. Cox proportional-hazards models were used—with follow-up from ED presentation to death or 90 days—to examine each biomarker's association with 90-day mortality. Biomarkers showing significance when adjusted for covariables (see Tables 2 and 3) were combined into two weighted scores: the amino acid mortality risk score (AMRS) and the inflammatory biomarker mortality risk score (IBMRS), each calculated by summing β-coefficients multiplied by biomarker z-scores.

Each score was entered into Cox models adjusted for age and sex. A combined biomarker risk score (AMRS+IBMRS) was then created (expressed as a z-score and quartiles) and assessed for its relationship with 90-day mortality via Cox models adjusted for sex, age, METTS-A category, oxygen saturation, respiratory rate, CRP, lactate and estimated glomerular filtration rate (eGFR). Additional statistical analyses included evaluating prognostic improvement in discrimination using time-dependent ROC analysis (area under the curve (AUC)) when adding amino acids and inflammatory biomarkers to traditional risk factors, and Net Reclassification Improvement (NRI), with significance set at $p<0.05$.[30] Model discrimination was assessed using Harrell's concordance index (C-index). Internal validation was

performed using bootstrap resampling (1000 repetitions), and optimism-corrected estimates were calculated. In addition, shrinkage was evaluated using the bootstrap-corrected calibration slope.

Model performance was evaluated by (i) discrimination, quantified using receiver operating characteristic (ROC) analysis and the area under the curve (AUC), and compared between the clinical model and the clinical-plus-biomarker model; (ii) risk reclassification, quantified using the continuous Net Reclassification Improvement (NRI); and (iii) visual assessment of prognostic separation across risk strata using Kaplan–Meier curves by quartiles of the combined biomarker risk score (Figures 2–3). Calibration was assessed using the bootstrap-corrected calibration slope.

Statistical analyses were performed using IBM SPSS Statistics 27 (SPSS Inc., Chicago, IL, USA) and R: A Language and Environment for Statistical Computing, version 2025 (R Core Team, Vienna, Austria), including the packages survival, timeROC, nricens, and rms.[31]

Results

Replication study

In the replication of the previous study, 448 new patients were included, 57 (13%) of whom died within 90 days of admission to the emergency department (ED).[3] The clinical characteristics of the study population are presented in Supplementary Table 1. Eighty-one patients were admitted to a ward with continuous monitoring of vital signs, 159 to a ward with intermittent monitoring, 208 were discharged from the ED, four died during their stay in the ED, and 15 died in a hospital ward. In Cox proportional-hazards models adjusted for age and sex, GDF-15 and IL-8 were both significantly associated with mortality ($p<0.05$) (Supplementary Table 2). The inflammatory biomarker mortality risk score (IBMRS) was ranked into quartiles and entered into a Cox proportional-hazards model adjusted for age, sex, METTS-A, oxygen saturation, respiratory rate and CRP (hazard ratio (HR) 2.01 (95% CI 1.44–2.81), $p<0.05$) (Supplementary Table 3). The IBMRS was significantly associated with 90-day mortality, with a six-fold higher risk in quartile 4 than in quartile 1. Baseline National Early Warning Score (NEWS) distributions for the replication cohort are shown in Supplementary Table 5.

Combined Inflammatory Biomarkers and Amino Acids study

In the combined inflammatory biomarker and amino acid study, 637 patients were included, of whom 78 (12%) died within 90 days of admission to the ED. The clinical characteristics of the study population are presented in Table 1. A total of 131 patients were admitted to a ward with continuous monitoring of vital signs, 247 were admitted to a ward with intermittent monitoring, 248 were discharged from the ED, five died during their stay in the ED, and 12 died in a hospital ward. The results of Model 1 (adjusted for sex and age) are presented in Table 2. All selected biomarkers and amino acids were associated with increased mortality ($p<0.05$). The combined biomarker risk score (AMRS+IBMRS) was ranked and divided into quartiles from lowest to highest. The data were then entered into a Cox proportional-hazards model together with the covariates age, sex, METTS-A, oxygen saturation, respiratory rate, CRP, lactate

and eGFR. The combined biomarker risk score was significantly associated with 90-day mortality (HR 2.83 (95% CI 2.12–3.77), p<0.05) (Table 3). Mortality was 1.3% in quartile 1 and 32% in quartile 4 (Figure 2). Discrimination improved with the addition of the combined biomarker risk score to standard clinical predictors, with the 90-day time-dependent AUC increasing from 0.78 (95% CI 0.73–0.83) to 0.85 (95% CI 0.81–0.89), with a statistically significant difference between models (p = 0.00224). (Figure 3). The model using standard clinical predictors showed a Harrell's C-index of 0.76, with an optimism-corrected Harrell's C-index of 0.738 (95% CI: 0.736–0.740) and a calibration slope of 0.85. The new model, adding the combined biomarker risk score to the standard clinical predictors, demonstrated improved discrimination (Harrell's C-index 0.825), with an optimism-corrected Harrell's C-index of 0.808 (95% CI: 0.806–0.809) and a calibration slope of 0.88. The difference in corrected Harrell's C-index was 0.070 (95% CI: 0.067–0.072) (p < 0.001).

Baseline National Early Warning Score (NEWS) distributions for the combined cohort are shown in Supplementary Tables 4.

The proportional hazards assumption was satisfied for all entered biomarkers, biomarker scores, and score quartiles, as indicated by non-significant global Schoenfeld residual tests.

The addition of the combined biomarker risk score improved risk classification for mortality in patients with acute dyspnoea (NRI 0.68, 95% CI 0.49–0.91), driven by correct upward reclassification in 65% of patients who died and correct downward reclassification in 69% of survivors.

Position of Table 1, Table 2, Table 3, Figure 2, Figure 3

Discussion

Replication study

The results of this study are reassuring, replicating and confirming the results of a previous study, which demonstrated that a strong and independent relationship exists between the levels of interleukin-8 and growth differentiation factor 15 and the risk of 90-day mortality in patients with acute dyspnoea.[3]

Combined Inflammatory Biomarkers and Amino Acids study
The combined analysis of the plasma concentrations of three amino acids and two inflammatory biomarkers in this study demonstrated a strong and independent relationship with 90-day mortality in a cohort of patients with acute dyspnoea presenting at the ED, irrespective of the underlying pathology.

We argue that a patient presenting with acute dyspnoea can have measurable changes in both metabolism and/or increased inflammatory activity, which, if measured, can indicate the severity of illness and prognosis.

Inflammation and metabolic changes in critical illness share some similarities but also have notable differences. Different disease syndromes affect various bodily systems differently; therefore, the ability

to measure both the level of inflammatory and metabolic changes provides greater assurance in making critical decisions than when only one of them is used.

Metabolic changes that partake in severe illness are measurable and have been demonstrated in several studies, including studies concerning heart failure, COPD and septicaemia.[32-34] These changes may be evident when the patient enters a catabolic state as a reaction to severe illness, and stress hormones are released, resulting in increased proteolysis and insulin resistance and decreased lipolysis.[35, 36] This results in a change in the levels of amino acids associated with a worse outcome, which can be observed in this study.

The choice of 90-day mortality was made considering that it was a long enough period to include as many events as possible while still being short enough for it to be linked to the acute incident that brought the patient to the ED.

The findings in this analysis have several clinical implications, especially in the acute setting of the ED. To provide value in physicians' decision-making, there must be a clear difference in mortality between the lowest and highest quartiles of the population. In this analysis, there was a mortality of 29% in quartile 4 compared with 1.8% in quartile 1. The notably high mortality rate of 12% within the relatively short-term follow-up time could indicate that the evaluation of the changes in biomarker values provides a good proxy of the severity of disease and risk of mortality at the time of presentation to the ED. In addition to being used as a tool to evaluate patients at risk of high mortality, the analysis of biomarkers can also be used as a tool to differentiate between the need for diagnostics and interventions at the time of presentation and when the patient can be referred to out-of-hospital facilities for further diagnostics and treatment.

If implemented clinically, use of the combined biomarker risk score would not require interactive handling of input data beyond routine acquisition of vital signs and standard blood sampling. Score calculation could be automated within the laboratory information system or electronic health record once the relevant biomarker results are available. The intended end users are clinicians responsible for acute assessment in the ED, and appropriate interpretation requires clinical expertise; the score is intended to support—rather than replace—clinical judgement and existing triage protocols.

Biomarkers are attractive candidates in the primary assessment of patient conditions, as they are relatively cheap, quick, and pose little or no risk for the patient.

Limitations

This study could not be used to differentiate between different diagnoses in patients presenting with dyspnoea, in part owing to a lack of dependable information regarding diagnoses in deceased patients. Further studies are needed to evaluate the links between changes in biomarkers in different diseases/syndromes. The mean age of the study population was relatively high, which may limit the

generalisability of the findings; therefore, additional studies are warranted to confirm applicability across age groups.

By nature, observational studies can only imply correlations between markers and disease outcomes. The only way to deduce if analysis of these biomarkers has value as a clinical tool is by conducting a randomized clinical trial (RCT). In future research, the results from this study may be used to decide which biomarkers to include when designing an RCT. In such an RCT, the differences in the clinical outcomes of patients between physicians with or without knowledge of the studied biomarkers may be evaluated.

Diagnosis-stratified analyses were limited because the primary discharge diagnosis was frequently not established with certainty. In addition, diagnostic classification relied on ICD-coded discharge diagnoses in the electronic health record, which may be subject to coding bias and provides limited clinical granularity (e.g., variation in coding practices between clinicians, use of non-specific symptom-based codes such as “dyspnoea” rather than an aetiological diagnosis, or preferential coding of comorbidities or complications over the primary cause of presentation).

The most critically ill patients admitted through the ED were not included in this study, as they were unable to provide consent. Similarly, the least severely affected patients are more likely to seek primary care and do not require further referral to the ED; therefore, these patients were not included in this study. These selection biases may interfere with the generalisability of the results. Consequently, the present findings cannot be extrapolated to patients admitted to the intensive care unit (ICU); however, this study may represent an important step towards obtaining ethical approval to include patients who are unable to provide informed consent at presentation in the ED.

The high continuous NRI should be interpreted with caution, as it is highly sensitive and may produce large values even for small, potentially clinically irrelevant changes in predicted risk. Moreover, NRI does not account for calibration or directly reflect clinical usefulness and should therefore be considered alongside complementary performance measures.

The combined model included 78 events and approximately nine predictors (EPV ~8–9), which may increase the risk of overfitting. This is supported by wide confidence intervals in some subgroup analyses, reflecting limited precision due to few events per stratum.

Bootstrap validation yielded calibration slopes below 1, indicating some overfitting, although not extreme. However, this does not fully eliminate optimism, and external validation in independent cohorts is required before clinical implementation.

Abbreviations

AMRS Amino acid mortality risk score

AUC Area under the curve

IBMRS Inflammatory biomarker mortality risk score

ICD International Classification of Diseases

ICU Intensive care unit

IL-8 Interleukin-8

ED Emergency Department

eGFR Estimated glomerular filtration rate

CAD Coronary artery disease

CHF Congestive heart failure

CI Confidence interval

CRP C-reactive protein

COPD Chronic obstructive pulmonary disease

GDF-15 Growth differentiation factor-15

HR Hazard ratio

LM Lund–Malmo creatinine-based glomerular filtration rate prediction equation

MDRD Modification of Diet in Renal Disease

METTS-A Medical Emergency Triage and Treatment System-Adult

NMR Proton nuclear magnetic resonance

NEWS National Early Warning Score

ROC Receiver operating characteristic

C-index Harrell’s concordance index

NRI Net Reclassification Index/Net Reclassification Improvement

Q Quartile

RCT Randomized clinical trial

RLS Reaction Level Scale

SD Standard deviation

Declarations

Ethics approval and consent to participate

The study protocol no 2011/537 with the addition 2012/762 was approved by the Regional Ethics Review Board in Lund, Swedish Ethical Review Authority, Sweden, and conducted in accordance with the Declaration of Helsinki. All participants received both oral and written information about the study and provided written informed consent prior to enrolment.

Consent for publication

Not applicable

Availability of data and materials

The data supporting the findings of this study are available from the corresponding author (K.W.) upon reasonable request. The dataset required to reproduce the main analyses comprises a de-identified, participant-level file including: cohort indicator (replication/combined); follow-up time and 90-day mortality status; the clinical covariates used for adjustment (age, sex, METTS-A category, oxygen saturation, respiratory rate, CRP, lactate, and eGFR); and the biomarker measurements (IL-8, GDF-15, glycine, phenylalanine, and valine).

Competing interests

No conflicts of interest

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Patient and public involvement

Patients and/or the public were not involved in the design, conduct, reporting, interpretation, or dissemination of this study.

Statement on AI-usage

AI-assisted tools were used to support language refinement and editing. All scientific content, study design, data analysis, and interpretation were conducted by the authors, who take full responsibility for the integrity and accuracy of the work.

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Tables

Table 1 Clinical characteristics of the study population in the combined study (n=637)

Age (years) mean ± SD	71.2 ± 17.8
Sex Female (%)	338 (53.1)
Oxygen saturation (%) median (IQR)	95 (90–98)
Respiratory Rate median (IQR)	24 (20–28)
CRP (mg/dl) median (IQR)	10.0 (3.5–36.0)
Congestive Heart Failure no. (%)	221 (34.7)
Chronic Obstructive Pulmonary Disease no. (%)	191 (30.1)
Lactate (mmol/L) median (IQR)	1.5 (1.2–2.1)
eGFR (ml/min/1.73 m²) median (IQR)	62.1 (43.0–78.6)
Smoking (16 missing)*	
Nonsmoker no. (%)	184 (29.6)
Former smoker no. (%)	323 (52.0)
Active Smoker no. (%)	114 (18.4)
*METTS-A***	
Priority 4 (green) no. (%)	43 (6.8)
Priority 3 (yellow) no. (%)	295 (46.3)
Priority 2 (orange) no. (%)	227 (35.6)
Priority 1 (red) no. (%)	72 (11.3)

*Smoking categories: non-smoker/former smoker/current smoker.
**METTS-A priority (4-level triage; least to most urgent): Priority 4 (green) lowest urgency/stable; Priority 3 (yellow) moderate urgency; Priority 2 (orange) high urgency/rapid assessment; Priority 1 (red) immediate life-threatening.[22]
CRP: C-reactive protein.
eGFR: estimated glomerular filtration rate.

Table 2 Relationships between biomarkers and 90-day mortality risk in the combined study (Cox proportional-hazards model)

Biomarker	Hazard ratio (95% CI)*	p
Inflammatory biomarkers		
Interleukin-8	1.99 (1.63–2.43)	$9.71x10^{-12}$
Growth differentiation factor-15	2.30 (1.69–3.13)	$1.32x10^{-7}$
Score of inflammatory biomarkers (IBMRS)**	2.54 (1.99–3.24)	$4.59x10^{-14}$
Amino acids		
Glycine	1.25 (1.01–1.54)	0.039
Phenylalanine	1.47 (1.19–1.81)	$3.8x10^{-4}$
Valine	0.61 (0.48–0.76)	$1.7x10^{-5}$
Score of amino acids (AMRS)***	2.46 (1.92–3.15)	$8.12x10^{-13}$
Combined biomarker risk score		
Combined biomarker risk score (AMRS+IBMRS)	2.79 (2.20–3.53)	$1.30x10^{-17}$

*Adjusted for age and sex.

**IBMRS is the inflammatory biomarker mortality risk score (interleukin-8 and growth differentiation factor-15).

***AMRS is the amino acid mortality risk score (glycine, phenylalanine and valine).

Table 3 Multivariable-adjusted hazard ratios for the combined biomarker risk score (AMRS+IBMRS) in relation to 90-day mortality (Cox proportional-hazards model)

	N/N Events	Hazard Ratio (95% CI)
All patients	637/78	2.83 (2.12–3.77)
Quartile 1	159/2	1 (ref)
Quartile 2	159/7	2.33 (0.48–11.36)
Quartile 3	160/18	6.0 (1.36–26.5)
Quartile 4	159/51	13.68 (3.10–60.46)

$p=1.80\times10^{-12}$; p-trend=7.0×10^{-6}

*Adjusted for age, sex, METTS-A, oxygen saturation, respiratory rate, CRP, lactate and eGFR. Quartiles were defined by ranking the combined biomarker risk score from Q1 (lowest) to Q4 (highest).

AMRS: amino acid mortality risk score (glycine, phenylalanine and valine). IBMRS: inflammatory biomarker mortality risk score (interleukin-8 and growth differentiation factor-15).

Figures

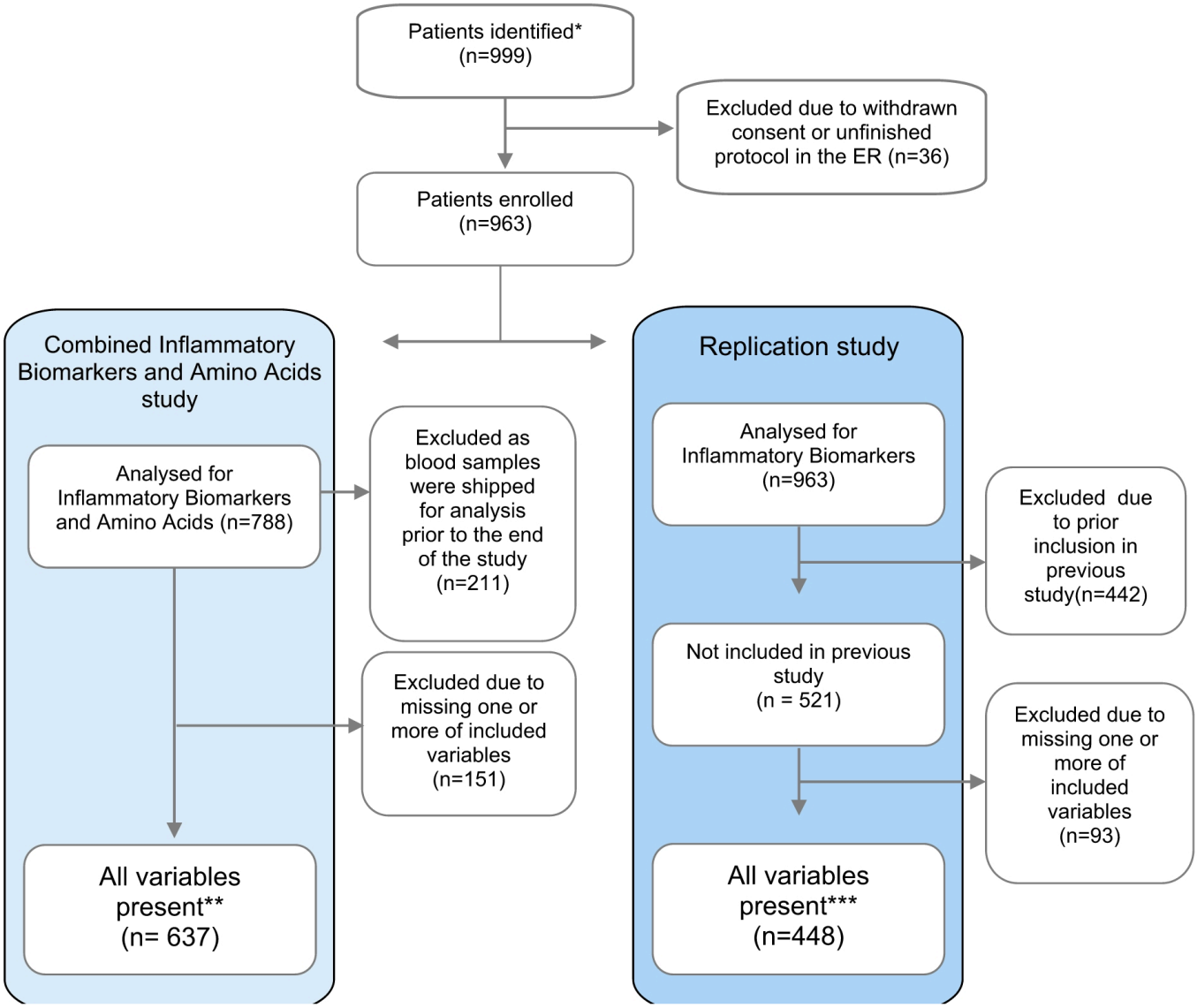


Figure 1

Study flow diagram of participant inclusion in the replication study and the combined inflammatory biomarkers and amino acids study.

*Patients aged ≥18 years presenting to the ED with acute dyspnoea as the primary complaint
** Variables in analysis: age, sex, METTS-A, oxygen saturation, respiratory rate, CRP, lactate, eGFR, IL-8, GDF-15, glycine, phenylalanine and valine.

*** Variables in analysis: age, sex, METTS-A, oxygen saturation, respiratory rate, CRP, eGFR, IL-8 and GDF-15

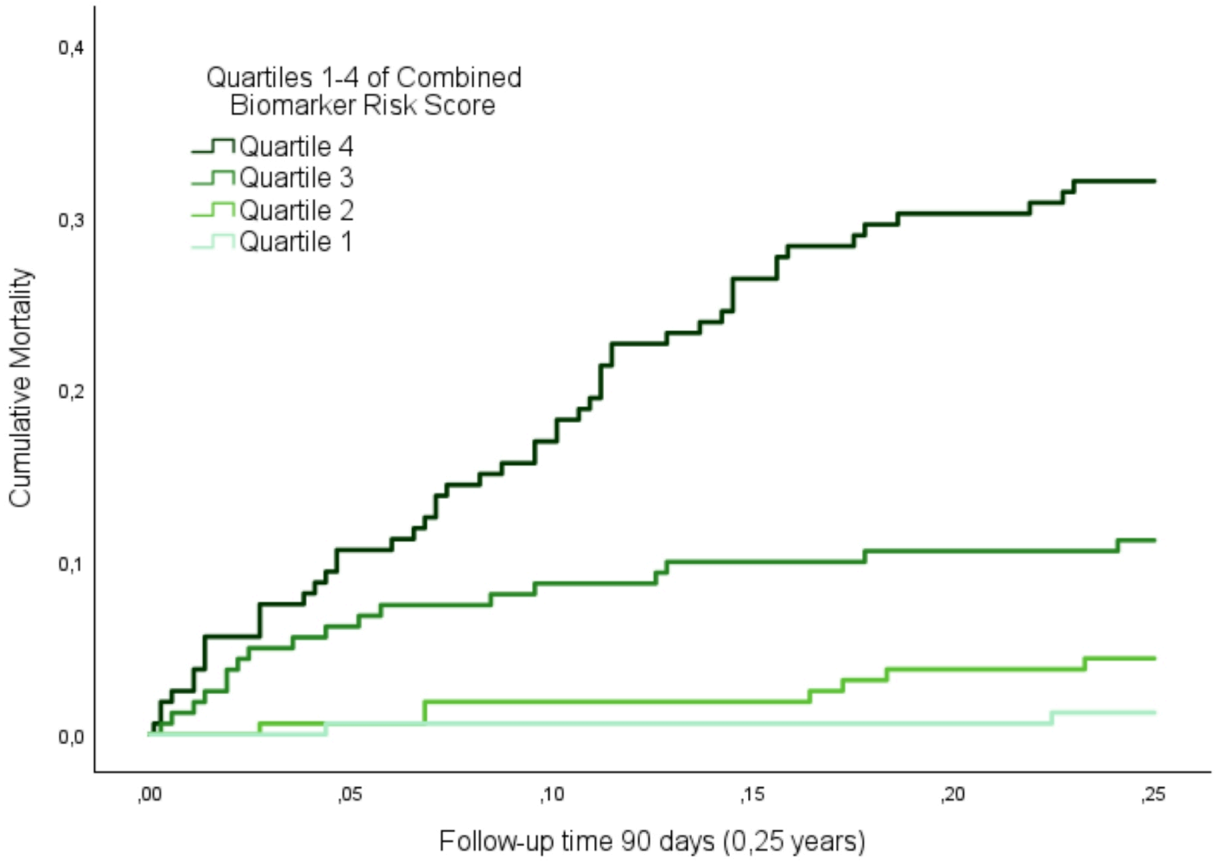


Figure 2

Kaplan–Meier plot showing cumulative mortality during 90 days of follow-up by quartiles of the combined biomarker risk score (AMRS+IBMRS). Q1 denotes the lowest quartile and Q4 the highest quartile. The y-axis has been truncated at 0.4.

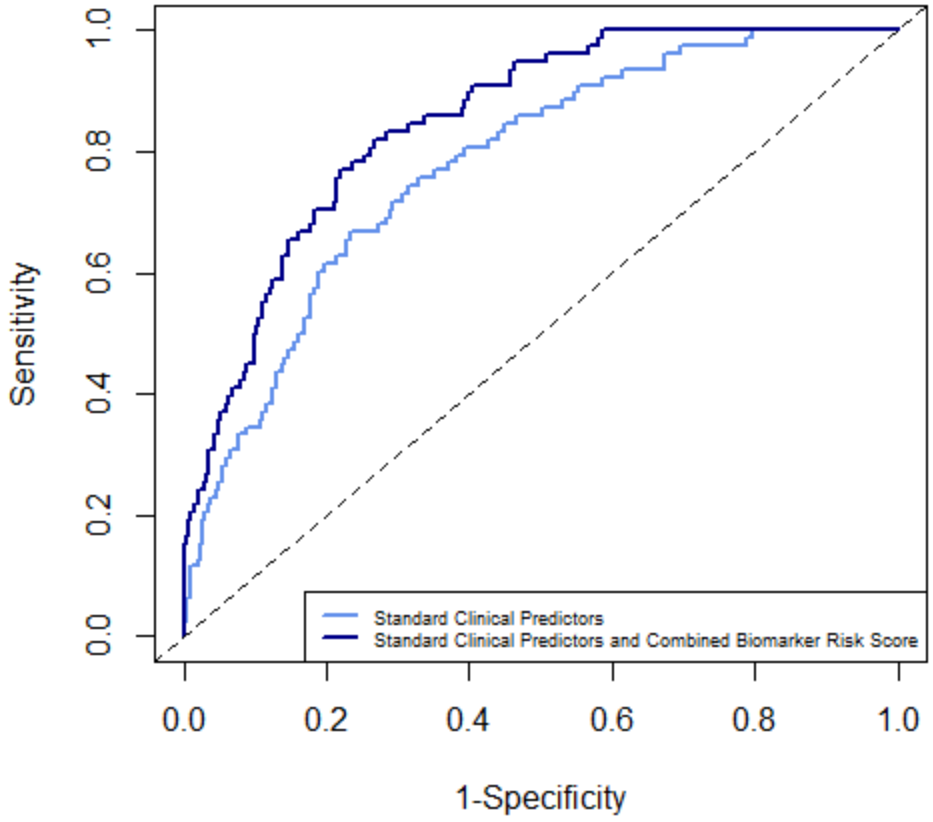


Figure 3

Time-dependent receiver operating characteristic (ROC) curves comparing the model with standard clinical predictors and the model including standard clinical predictors plus the combined biomarker risk score for prediction of 90-day mortality.

Standard clinical predictors (age, sex, METTS-A, oxygen saturation, respiratory rate, CRP, lactate and eGFR): time-dependent AUC 0.78 (95% CI 0.73–0.83).

Standard clinical predictors plus the combined biomarker risk score: time-dependent AUC 0.85 (95% CI 0.81–0.89). The difference between models was statistically significant (p = 0.00224).

Supplementary Files

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- Supplementarymaterial.docx