Circulation: Heart Failure

ORIGINAL ARTICLE

Plasma Proteome Analysis Identifies Vascular Endothelial Growth Factor Receptor 1 as a Prognostic Biomarker in Cardiogenic Shock

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BACKGROUND: Cardiogenic shock (CS) is a severe complication of acute myocardial infarction (AMI) leading to poor outcomes. Specific biomarkers, with subsequent validation of their prognostic relevance in CS, are urgently needed to improve therapies and outcomes. Accordingly, the present study investigated the plasma proteome using proximity extension assay technology to identify novel specific biomarkers with subsequent validation of their prognostic relevance in CS.

METHODS: Using proximity extension assay (Olink Explore, 2942 proteins), the proteomic signature in the plasma of 9 AMI patients without shock and 8 AMI patients with CS (AMICS; exploration cohort) at admission was analyzed. Candidate biomarkers were measured in the plasma of 421 patients with AMICS from the CULPRIT-SHOCK cohort (REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT01927549, validation cohort). Their prognostic relevance was assessed for 180-day survival as the primary end point.

RESULTS: Proteome profiling was successful for 2925 proteins and identified VEGFR1 (vascular endothelial growth factor receptor 1, also known as Flt1) as elevated in AMICS compared with nonshock AMI in the exploration cohort ($P<0.001$). In patients from the independent validation cohort, nonsurvivors had markedly higher VEGFR1 levels (6.8 versus 3.8 ng/L; $P<0.001$). In Cox regression, VEGFR1 levels were independently associated with a higher 180-day mortality risk even after adjusting for the Simplified Acute Physiology Score II (per ng/L; adjusted hazard ratio, 1.06 [95% CI, 1.03–1.09]; $P<0.001$) and yielded incremental prognostic information in addition to serum lactate levels ($P<0.001$). The levels of VEGFR1 in surviving (30 days; n=29) and nonsurviving (n=21) patients with AMICS were determined at different time points (days 0, 1, and 5) in a third cohort, showing continuously higher levels in nonsurvivors.

CONCLUSIONS: Plasma proteomic screening identified VEGFR1 as an early biomarker in patients with AMICS that provided independent prognostic information in a large cohort of well-defined patients with AMICS.

Key Words: biomarkers ■ humans ■ lactates ■ prognosis ■ shock

Cardiogenic shock (CS) is a severe complication in 5% to 10% of patients with acute myocardial infarction (AMI) that leads to the death of almost every second patient due to reduced organ perfusion with subsequent multiorgan failure.[1–3] Despite extensive research and considerable advances, mortality rates remain unacceptably high. Percutaneous coronary intervention (PCI) targeting the infarct-related artery has emerged as the most pivotal evidence-supported intervention for AMI-related CS (AMICS). However, most early interventions aiming to modify the outcome of AMICS have yielded unsatisfactory outcomes.[4]

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WHAT IS NEW?

- The present study aimed to identify novel specific, and predictive biomarkers in patients with acute myocardial infarction-associated cardiogenic shock (AMICS).
- Initially, the plasma proteome of a small number of ST-segment–elevation myocardial infarction patients with and without cardiogenic shock was screened by high-plex proximity extension assay (Olink Explore; ≈3000 proteins).
- The screen demonstrated VEGFR1 (vascular endothelial growth factor receptor 1, also known as Flt1) highly elevated in AMICS compared with nonshock patients with acute myocardial infarction.
- In the large CULPRIT-SHOCK cohort, VEGFR1 was confirmed as a novel independent prognostic marker in AMICS.

WHAT ARE THE CLINICAL IMPLICATIONS?

- Cardiogenic shock is a severe complication of acute myocardial infarction leading to poor outcomes, yet biomarkers with prognostic value are scarce.
- Novel proteomic technologies offer unprecedented scope and sensitivity for detecting novel biomarkers for identifying patients at risk of clinical deterioration.
- VEGFR1 is a novel biomarker that independently confers prognostic accuracy in patients with AMICS.
- The combination of VEGFR1 and lactate yields higher accuracy in predicting 180-day mortality than each marker independently.
- Early and easily accessible biomarkers such as VEGFR1 will allow to identify patients at risk for AMICS and allow to improve therapies and outcomes.

Nonstandard Abbreviations and Acronyms

AIC	Akaike Information Criterion
AMI	acute myocardial infarction
AMICS	acute myocardial infarction complicated by cardiogenic shock
CS	cardiogenic shock
DOREMI	Dobutamine Compared to Milrinone in the Treatment of Cardiogenic Shock
Flt1	fms related tyrosine kinase 1
HR	hazard ratio
IABP-SHOCK II	Intraaortic Balloon Pump in Cardiogenic Shock II
NT-proBNP	N-terminal pro-B-type natriuretic peptide
PCI	percutaneous coronary intervention
PEA	proximity extension assay
SAPS	Simplified Acute Physiology Score
SYSTEMI	Systemic Organ Communication in ST-Segment–Elevation Myocardial Infarction
VEGFR1	vascular endothelial growth factor receptor 1

Thus, additional strategies to identify patients at highest risk are of great interest, to guide therapies. Newer AMICS classifications focus increasingly on hypoperfusion. The clinically used Society for Cardiovascular Angiography and Interventions shock classification divides patients into 5 classes, starting from no signs of shock up to end-stage shock, followed by circulatory collapse.[5] One major determinant of the Society for Cardiovascular Angiography and Interventions classification is plasma lactate, a nonspecific biomarker which might indicate that the organs' needs for adequate perfusion are not met.[6] In critically ill patients following resuscitation and in different forms of shock, lactate concentration and its initial kinetics are associated with mortality.[7] Lactate is also part of risk scores such as the cystatin C, lactate, interleukin-6, and NT-proBNP (N-terminal pro-B-type natriuretic peptide; CLIP) score that can predict short-term survival in patients with AMICS.[8] Other augmentative biomarkers might improve the accuracy of early diagnosis and assessment of reduced organ perfusion in CS.[9] To date, a number of biomarkers including NT-proBNP, soluble (s)ST2, troponin, dipeptidyl dipeptidase 3, adrenomedullin, angiopoietin 2, and cardiogenic shock 4 protein have been proposed and reviewed in detail by Iborra-Egea et al.[10] Yet, all these biomarkers except the combination of sST2 and NT-proBNP lack incremental information regarding long-term survival. Previous studies mainly covered single markers; however, a recent screening proteomics approach using mass spectrometry linked the combination of 4 circulating proteins to short-term outcome.[11] A proteomic screening using the latest commercially available technology and fully focused on patients with AMICS has not been performed to date.

Here, we aimed to identify novel and reliable plasma biomarkers in patients with AMICS using multiparametric and highly sensitive proximity extension assay (PEA) technology, including validation in the largest available prospective AMICS biobank to investigate long-term outcomes.

METHODS

Data Availability Statement

The data of the study are available upon request sent to the corresponding author.

Study Rationale and Design

In the first step, this study combined an explorative search of plasma proteins as possible markers of AMICS as opposed to AMI without shock (exploration cohort). Second, we validated eligible candidate biomarkers in a larger cohort of patients with AMICS (validation cohort).

Exploration Cohort

The plasma proteome signature was initially analyzed in 9 non-shock AMI versus 8 AMICS patients. Patients were selected based on comprehensive clinical characterization, including cardiovascular functional assessments and the availability of additional blood samples, to ensure high data quality for exploratory analyses. All patients were enrolled in the SYSTEMI study (Systemic Organ Communication in ST-Segment–Elevation Myocardial Infarction; REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT03539133), an open-end prospective cohort study that collects data from patients with ST-segment–elevation myocardial infarction and high-risk non–ST-segment–elevation myocardial infarction treated at the University Hospital Düesseldorf.[12,13] The study has been approved by the ethics committees of the Heinrich-Heine-University in Düesseldorf, Germany (5961R), and complies with the Declaration of Helsinki. Informed consent was obtained from all enrolled patients. Shock criteria were: systolic blood pressure <90 mm Hg (>30 minutes) or catecholamines used for maintaining a systolic blood pressure >90 mm Hg in combination with signs of generalized tissue hypoperfusion (ie, cold extremities, urine output <30 mL/h) and arterial lactate levels ≥2 mmol/L. Blood used for proteome profiling was drawn following the AMI diagnosis at hospital admission before starting coronary angiography and revascularization. EDTA-anticoagulated plasma was isolated and stored at −80 °C until further use. Detailed information about the biosampling protocol was previously published.[12] Following PCI, all patients received standard medication and were treated according to the European guidelines.[14,15]

Validation in the CULPRIT-SHOCK Cohort

The identified candidate biomarkers were subsequently validated in a secondary analysis of the randomized open-label CULPRIT-SHOCK trial (NCT01927549). The detailed protocol of the original study[16] and its major findings have been published earlier.[17] Briefly, the CULPRIT-SHOCK trial compared culprit lesion PCI versus immediate multivessel PCI in patients with multivessel coronary artery disease presenting with AMICS.[17] Inclusion criteria were identical to the patients with AMICS of the exploration cohort. In the CULPRIT-SHOCK trial, blood from patients with AMICS was drawn at hospital admission before angiography and PCI. EDTA-anticoagulated plasma was isolated and stored at −80 °C until further use. All patients with available biobanking from both randomization groups of the CULPRIT-SHOCK trial were included in the validation analyses of candidate biomarkers at pre-PCI plasma levels.

Time-Series Analysis

Candidate proteins were analyzed in a third cohort of patients with AMICS to allow a subsequent time-series analysis. A separate cohort of patients was recruited, similar to the exploration cohort, from the SYSTEMI study (NCT03539133) as described above. Shock criteria were identical to the ones defined in the exploration cohort. Inclusion in the time-series cohort were all patients with AMICS not part of the exploration cohort and with available clinical information and at least day 0 and day 1 plasma material (if alive). Blood was drawn following AMI diagnosis at hospital admission before starting coronary angiography and revascularization (day 0), after 1 day, after 5 days, and after 180 days. While blood was taken in the catheterization laboratory, on subsequent time points blood was collected together with routine sampling in the morning. EDTA-anticoagulated plasma was isolated and stored at −80 °C until further analysis.

Plasma Proteome Screening

The plasma proteome profile (2942 proteins) was analyzed in EDTA plasma using the PEA by Olink (Olink Explore 3072; Uppsala, Sweden)[18] to discover differences in the signature related to AMICS. Briefly, in PEA, 2 different antigen-specific antibodies bind to the target protein bringing them into spatial proximity. The antibodies are conjugated to DNA oligonucleotides that contain complementary sequence segments allowing hybridization and serving as templates for polymerase chain reaction-based amplification and subsequent readout by next-generation sequencing. Olink expression data are indicated as Normalized Protein Expression, an arbitrary unit that is in $\log_2$ scale. These data were subsequently analyzed in GraphPad Prism 9. The R library ComplexHeatmap was used to generate the heatmap. All biomarkers that demonstrated statistical significance in the comparison between the cohorts of AMI patients with and without AMICS were identified as candidate biomarkers and included in the secondary analysis. To account for multiple comparisons, the Benjamini-Hochberg correction method was applied to control the false discovery rate. Biomarkers were considered significant if they met both predefined criteria: an adjusted $P<0.05$ and an absolute $\log_2$-fold change >0.5. This threshold was chosen to ensure that only biologically meaningful changes in protein abundance were considered, avoiding subtle variations that may lack clinical relevance.[19]

VEGFR1 Analysis by ELISA

ELISA was used to evaluate VEGFR1 (vascular endothelial growth factor receptor 1) levels in plasma samples from the CULPRIT-SHOCK collective. Plasma samples stored at −80 °C were defrosted, and the subsequent ELISA procedures on 96-well plates were performed according to instructions supplied by the manufacturer. The VEGFR1 kit (abx352395, Abbexa, Cambridge, United Kingdom) was used to measure plasma VEGFR1 levels between 0.078 ng/mL and 5 ng/mL. Plasma samples were prediluted 1:4 and 1:2 for VEGFR1 assays. Protein concentrations were determined by comparing the absorbance at 650 nm wavelength in a microplate reader (BMG LABTECH GmbH, Offenburg, Germany) to the standard curve.

Statistical Analyses

Patient characteristics are displayed either as number (n)/percentage (%) for categorical variables, as mean±SD for

normally distributed variables, or as median and interquartile range for skewed variables. Statistical testing between the groups was performed by χ^2 test (sex, catecholamine use, comorbidities, risk factors), the Student *t* test (age, blood pressures, heart rate, serum lactate), or Mann-Whitney *U* test (body mass index). To analyze the validity of identified proteins, patients with AMICS from the CULPRIT-SHOCK trial were divided by the cutoff established by the median value and the calculated Youden index using ROC statistics. Survival between the stratified groups was analyzed by the Kaplan-Meier estimator using the log-rank (Mantel-Cox) test to assess 180-day mortality. The univariate and multivariable associations between initially identified biomarkers and mortality were assessed by fitting Cox regression models in an unadjusted model and a model adjusted for Simplified Acute Physiology Score (SAPS) II, which includes the following variables: age, heart rate, systolic blood pressure, body temperature, Glasgow Coma Scale, mechanical ventilation or continuous positive airway pressure, arterial partial oxygen pressure, fraction of inspired oxygen, urine output, blood urea nitrogen, sodium, potassium, bicarbonate, bilirubin, white blood cell count, chronic diseases, randomized treatment assignment, and type of admission. This score provides accurate information for assessing severity in patients with AMICS. For the time-series analysis, grouped bar plots comparing biomarker levels at various time points between patients who survived (alive) and those who did not (dead) were generated. Patients were categorized as survivors if they survived beyond 180 days post-incident, while nonsurvivors were those who died within 180 days of follow-up. Statistical comparisons between groups at each time point were conducted using a Student *t* test. Model performance was evaluated using the Akaike Information Criterion (AIC) to compare the predictive capabilities of SAPS II alone versus SAPS II combined with the respective biomarker. AIC was selected as it provides a measure of relative model quality while accounting for model complexity. Lower AIC values indicate superior model fit, with differences exceeding 2 points suggesting meaningful improvement in model performance. Models were constructed using Cox proportional hazards regression with all-cause mortality as the primary end point. Significance was assumed if $P<0.05$ (2-sided). The statistical analyses were performed in STATA, V.18.0 (StataCorp, College Station, TX).

RESULTS

Exploration Cohort: Plasma Proteome of AMICS Versus AMI Patients

The plasma proteome of patients with AMICS was compared with AMI patients without shock. The baseline characteristics of this patient cohort are presented in Table 1. Plasma proteome analysis used novel PEA technology and included 2942 proteins of which 2925 proteins were successfully quantified. Of those, 17 (0.6%) were below the detection threshold. In AMICS, expression of 23 proteins was found lower (<50%) while that of 857 proteins was higher (>200%) compared with AMI. One protein was significantly higher in patients with AMICS: VEGR1 (3.84 Normalized Protein Expression in AMICS versus 1.28 Normalized Protein Expression in patients with AMI, $P<0.0001$). This finding was validated using ELISA with a defined calibrated concentration curve (VEGFR1; 3.96±0.93 ng/L in AMICS versus 1.28±0.92 ng/L in patients with AMI, $P<0.001$; Figure S1) as the only protein meeting the predefined statistical significance threshold. Figure 1A illustrates a heatmap of all proteins analyzed and Figure 1B a volcano plot highlighting the regulated proteins between the 2 groups. Interestingly, while VEGFR1 reached statistical significance, traditional cardiac biomarkers such as cardiac troponin I and NT-proBNP were not significantly increased in AMICS in the PEA analysis.

Table 1. Baseline Characteristics of the AMI and AMICS Patients of the Discovery Cohort

Characteristics	AMI (n=9)	AMICS (n=8)	*P* value
Age, y; mean±SD	72±13	70±7	0.695
Body mass index, kg/m²; mean±SD	24.1±3.9	29.5±4.5	0.020
Sex (male), n (%)	5 (56)	6 (75)	0.620
Systolic arterial pressure, mm Hg; mean±SD	149±14	113±18	<0.001
Diastolic arterial pressure, mm Hg; mean±SD	85±11	66±10	0.002
Heart rate, bpm; mean±SD	76±15	100±20	0.016
Cardiogenic shock, n (%)	0 (0)	8 (100)	<0.001
Catecholamine use, n (%)	0 (0)	8 (100)	<0.001
Comorbidities, n (%)			
Previous myocardial infarction	2 (22)	3 (38)	0.620
Previous PCI	2 (22)	4 (50)	0.335
Previous CABG	0 (0)	0 (0)	>0.999
Atrial fibrillation	2 (22)	2 (25)	>0.999
Stroke	1 (11)	1 (13)	>0.999
Peripheral artery disease	2 (22)	2 (25)	>0.999
Cardiovascular risk factor, n (%)			
Current smoking	4 (44)	4 (50)	>0.999
Hypertension	6 (67)	7 (87)	0.576
Dyslipidemia	4 (44)	5 (63)	0.637
Diabetes	3 (33)	7 (87)	0.050
Serum lactate, mmol/L; mean±SD	1.3±0.4	3.8±2.1	0.012

AMI indicates acute myocardial infarction; AMICS, acute myocardial infarction with cardiogenic shock; CABG, coronary artery bypass graft; and PCI, percutaneous coronary intervention.

Validation Cohort: CULPRIT-SHOCK Patients

Verification and validation of the discovered proteins were performed in patients with AMICS of the CUPRIT-SHOCK trial. Biobanking of EDTA plasma before PCI was performed in 421 AMICS patients (Figure S2). Approximately half of these patients (n=211) survived more than 180 days. The complete baseline characteristics of

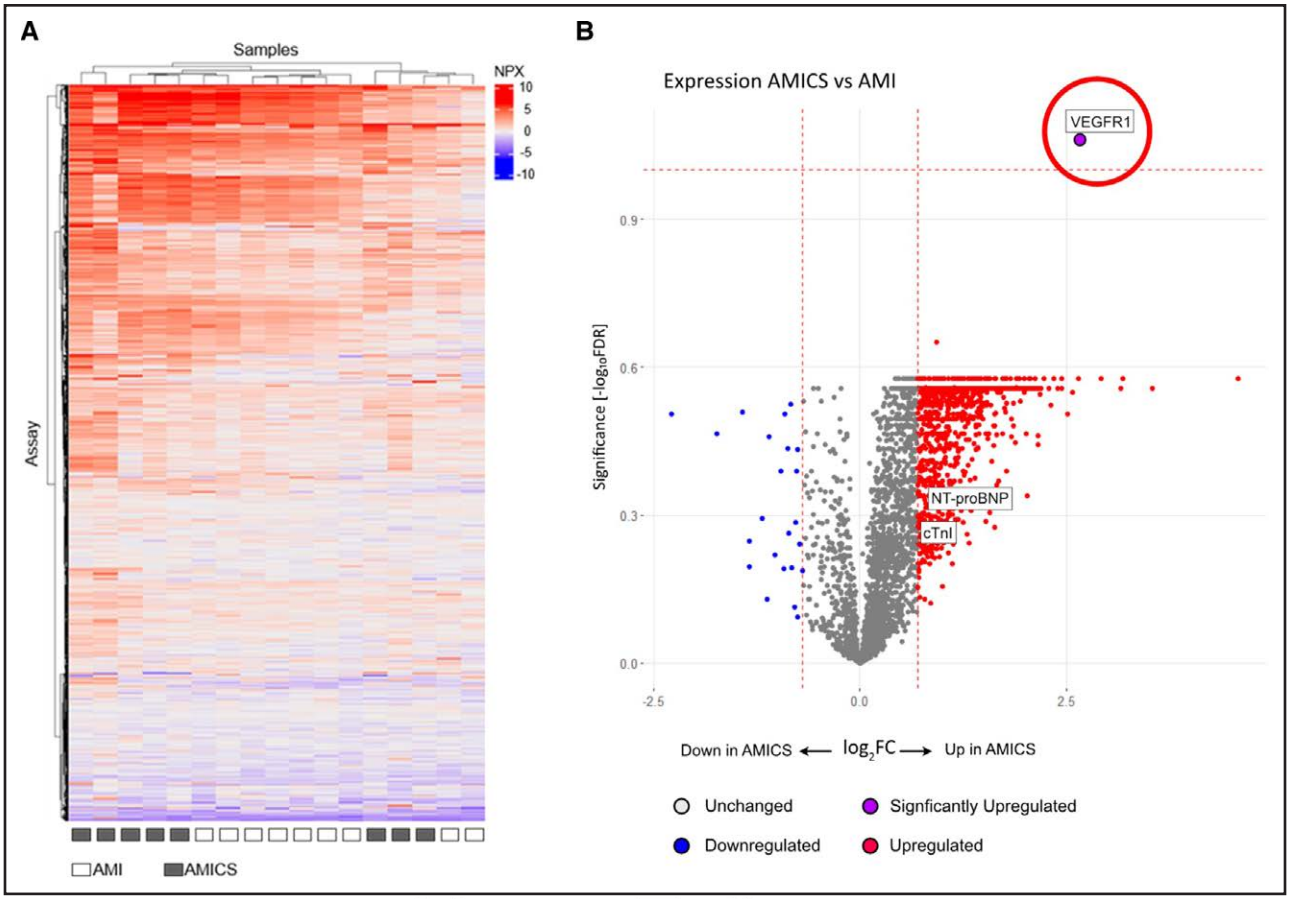

Figure 1. Proximity extension assay-based proteomics identifies VEGFR1 (vascular endothelial growth factor receptor 1) as candidate biomarkers in acute myocardial infarction cardiogenic shock (AMICS).
A, Heatmap representation of protein expression in the discovery cohort from AMI vs AMICS patients. The heatmap is constructed using a data frame of expression values (NPX, $\log_2$ scale) derived from the Olink assay. Each cell in the heatmap corresponds to an individual NPX value, with patients (Sample IDs) represented in columns and assays depicted in rows. The color scale in the heatmap represents the magnitude of NPX values, with red indicating higher expressions and blue indicating lower values. The assay results are grouped by patient groups, allowing for easy comparison between AMI and AMICS groups. The heatmap provides an overview of the distribution and variation of expression across different proteins assayed in each patient group, facilitating a visual exploration of the potential differences in protein expression levels between AMI and AMICS. All analyzed proteins are shown. **B**, Volcano plot with up- and downregulated proteins in AMICS vs non-CS AMI patients. VEGFR1 is significantly upregulated.

patients with AMICS stratified by 180-day survival are shown in Table 2.

VEGFR1 Predicts Higher Mortality Risk in an Independent and Augmentative Manner

In patients from the CULPRIT-SHOCK trial, AMCIS non-survivors had higher VEGFR1 levels at initial hospital admission (2.27 [0.99–5.18] versus 4.62 [1.91–7.81] ng/L, $P<0.001$; Figure S3). In Cox regression, higher VEGFR1 levels were associated with higher mortality rates (unadjusted hazard ratio [HR] of 1.06 [95% CI, 1.04–1.08]; $P<0.001$ per ng/L). After adjustment for disease severity using SAPS II, VEGFR1 maintained its independent prognostic value with an adjusted HR of 1.06 (95% CI, 1.03–1.09, $P<0.001$) per ng/L. Model comparison using AIC showed superior fit for the combined model (VEGFR1 and SAPS II, AIC=2775) compared with SAPS2 alone (AIC=2795), supporting VEGFR1's incremental prognostic value beyond established severity assessment. Similarly, in the Kaplan-Meier estimator as secondary analysis stratifying the CULPRIT-SHOCK cohort by the median VEGFR1 concentration (3.15 ng/L), patients with AMICS above the threshold displayed higher mortality (Figure 2; $P<0.001$). The same results were observed when analyzing VEGFR1 at the optimal cutoff as determined by the Youden index (3.59 ng/L, $P<0.001$).

Since lactate is currently the most important biomarker in diagnosing and managing CS,[20–22] the augmentative role of VEGFR1 in predicting mortality risk was examined. In sensitivity analyses stratified by lactate Society for Cardiovascular Angiography and Interventions categories, we observed distinct patterns in the association between VEGFR1 levels and 30-day mortality. In patients with lactate <2 mmol/L, VEGFR1

Table 2. Baseline Characteristics of AMICS Survivors and Nonsurvivors From the CULPRIT-SHOCK Trial

Characteristics	Survivors (n=211)	Nonsurvivors (n=210)	*P* value
Age, y; mean±SD	66±11	71±11	<0.001
Body mass index, kg/m^2; mean±SD	27.3±4.0	27.7±4.2	0.34
Sex (male), n (%)	163 (77)	150 (71)	0.17
Systolic arterial pressure, mm Hg; mean±SD	109±30	105±33	0.19
Diastolic arterial pressure, mm Hg; mean±SD	68±19	65±23	0.11
Heart rate, bpm; mean±SD	88±31	92±29	0.23
Cardiogenic shock, n (%)	211 (100)	210 (100)	0.99
Comorbidities, n (%)			
Previous myocardial infarction	38 (18)	27 (13)	0.16
Previous PCI	50 (24)	32 (15)	0.034
Previous CABG	12 (6)	11 (5)	0.87
Atrial fibrillation	17 (8)	24 (11)	0.22
Stroke	12 (6)	14 (7)	0.65
Peripheral artery disease	19 (9)	26 (13)	0.24
Cardiovascular risk factor, n (%)			
Current smoking	72 (34)	37 (18)	<0.001
Hypertension	135 (64)	122 (59)	0.29
Dyslipidemia	81 (38)	53 (26)	0.006
Diabetes	63 (30)	79 (38)	0.067
Serum lactate, mmol/L; mean±SD	3.4±2.8	7.2±4.9	<0.001

AMI indicates acute myocardial infarction; AMICS, acute myocardial infarction with cardiogenic shock; CABG, coronary artery bypass graft; and PCI, percutaneous coronary intervention.

demonstrated a robust and statistically significant association with mortality (HR, 1.14 [95% CI, 1.09–1.20]; *P*<0.001). Among patients with intermediate lactate levels (2–4.9 mmol/L), the association was attenuated but approached statistical significance (HR, 1.05 [95% CI, 1.00–1.11]; *P*=0.056). However, in patients with severe lactate elevation (≥5 mmol/L), VEGFR1 showed no significant association with mortality (HR, 1.00 [95% CI, 0.98–1.03]; *P*=0.834). There was no difference in sensitivity analysis according to sex, use of mechanical circulatory support devices or 2-vessel against 3-vessel disease.

The Cox regression analysis investigating the relationship between biomarkers and 30-day mortality demonstrated that patients with both lactate and VEGFR1 levels above the median had a significantly increased risk of death compared with those with both biomarkers below the median (adjusted HR, 1.83 [95% CI, 1.48–2.25]; *P*<0.001), after adjustment for disease severity using SAPS II. Model comparison using AIC demonstrated superior model fit for the combined biomarker model (AIC=2715) compared with both SAPS II alone (AIC=2795) and SAPS II with single biomarkers (lactate: AIC=2724; VEGFR1: AIC=2776), supporting the incremental prognostic value of the combined biomarker approach. The addition of VEGFR1 to SAPS II and lactate models led to modest increases in AUC (SAPS II: 0.692→0.716; lactate: 0.745→0.753), though these changes did not reach statistical significance.

To further evaluate the clinical utility of this biomarker combination, patients were stratified into 3 distinct risk groups based on biomarker elevations: group I (no elevation of either biomarker; lactate <2.0 mmol/L and VEGFR1<3.59 ng/L), group II (elevation of either biomarker), and group III (elevation of both biomarkers). This stratification revealed a clear mortality gradient (Figure 3), with group I showing the lowest 180-day mortality (19%), group II displaying intermediate mortality (45%, HR, 2.35 [95% CI, 1.48–3.74]; *P*<0.001 versus group I), and group III exhibiting the highest mortality (59%, HR, 3.88 [95% CI, 2.43–6.19]; *P*<0.001 versus group I). Notably, these associations remained robust after adjustment for disease severity using SAPS II, with group II maintaining significantly higher mortality (adjusted HR, 1.99 [95% CI, 1.24–3.30]; *P*=0.005) and group III showing the strongest association (adjusted HR, 3.62 [95% CI, 2.23–5.88]; *P*<0.001) compared with group I. In addition, a multiplicative interaction testing was performed with lactate and VEGFR1 using both biomarkers as continuous variables in our logistic regression model. The analysis showed no significant interaction (OR, 0.9999936; *P*=0.206). Interestingly, while lactate remained strongly associated with mortality (OR, 1.328; *P*<0.001), VEGFR1 showed borderline significance in this interaction model (OR, 1.000072; *P*=0.05). The correlation analysis reveals a moderate positive correlation between lactate and VEGFR1 levels (r=0.4217; *P*<0.0001).

There was no interaction between VEGFR1 and the assignment to the treatment or control group. Of note, there were no differences in VEGFR1 levels regarding sex, use of temporary circulatory assist devices, and severity of coronary artery disease.

Time-Series Analysis

To better understand the time-dependent change of VEGFR1 in patients with AMICS, an additional analysis was conducted. The baseline characteristics of this cohort are given in Table 3. Of note, VEGFR1 was consistently elevated in nonsurvivors compared with survivors during the hospital stay (Figure 4). In the time-dependent analysis a significant difference between survivors and nonsurvivors in lactate measurements was only observed on day 0 (Figure S4A). The plasma levels of VEGFR1 and lactate on day 0 showed significant correlation (Figure S4B).

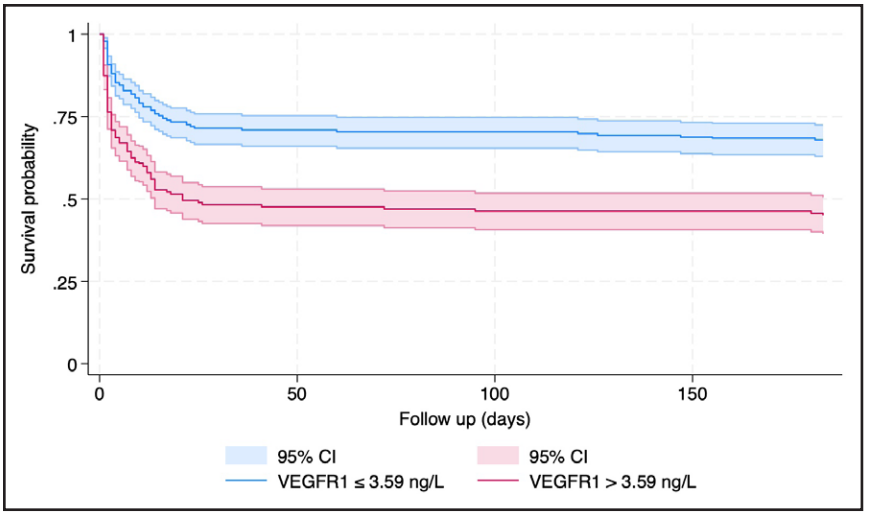

Figure 2. VEGFR1 (vascular endothelial growth factor receptor 1) level elevation (>3.59 ng/L) predicts higher mortality risk in patients with acute myocardial infarction cardiogenic shock (AMICS) from the CULPRIT-SHOCK study.
Kaplan-Meier curve representing 180-day survival of patients with AMICS divided by the threshold for VEGFR1 of 3.59 ng/L. Log-rank (Mantel-Cox) test *P*<0.001.

DISCUSSION

In summary, the current study (1) successfully performed a proteome screening with the analysis of nearly 3000 proteins in AMI patients with and without CS using PEA technology; (2) investigated the prognostic relevance of the significantly different candidate VEGFR1; and (3) confirmed VEGFR1 as a novel independent prognostic marker in AMICS.

The search for biomarkers in cardiogenic shock (CS) is not new, though many candidates have lacked independent prognostic value. Circulating lactate remains the clinical standard for detecting tissue hypoperfusion, with numerous studies defining its baseline levels and dynamics in AMICS. For example, a sub-analysis of the IABP-SHOCK II trial (Intraaortic Balloon Pump in Cardiogenic Shock II) showed that arterial lactate measured 8 hours after admission (>3.1 mmol/L) predicted outcomes better than baseline levels.[21] In addition, in a post hoc analysis of the DOREMI (Dobutamine Compared to Milrinone in the Treatment of Cardiogenic Shock) trial, lactate clearance at hospital admission and after 8 hours confirmed accurate prediction of mortality in patients with CS.[23] Several other biomarkers were tested, but they did not enter clinical use, mostly due to their missing prognostic relevance beyond serum lactate. Very recently, higher levels of circulating dipeptidyl peptidase-3, a protease implicated in Angiotensin II decomposition, forecasted a higher risk of CS and mortality (30 days and 1 year) in patients with AMI.[9] However, novel technologies such as Olink and comparable quantification methods and better search algorithms may help to establish additional and more accurate biomarkers. Accordingly, the current study investigated 2942 proteins in patients with AMICS and found the new potential candidate: VEGFR1.

The central finding was the identification of VEGFR1 as a biomarker that yields prognostic information. Our stratified analyses suggested variation in VEGFR1's prognostic utility over time across different shock severities, as defined by lactate categories. In patients presenting with mild shock (lactate <2 mmol/L), VEGFR1 demonstrated robust prognostic value, while this association was attenuated in moderate shock (lactate 2–4.9 mmol/L). Interestingly, in severe shock (lactate ≥5 mmol/L), VEGFR1's

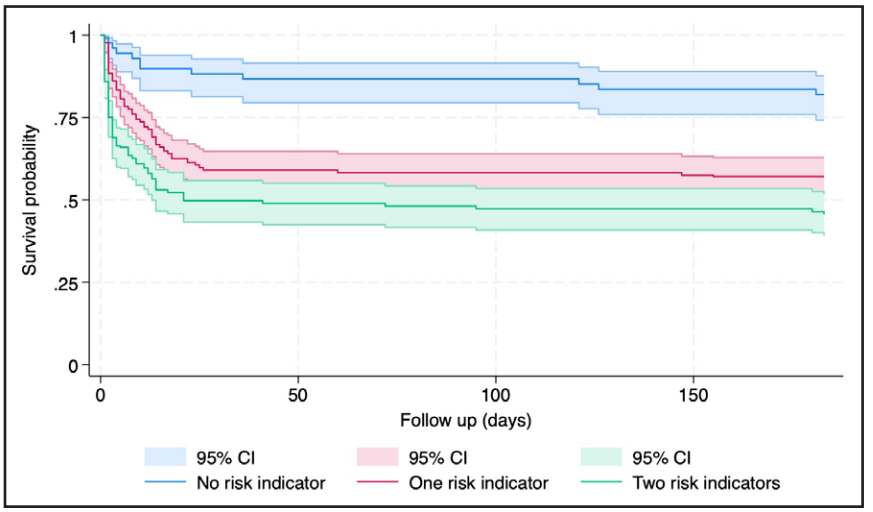

Figure 3. Incremental value of VEGFR1 (vascular endothelial growth factor receptor 1) and lactate together in predicting a higher mortality risk in patients with acute myocardial infarction cardiogenic shock (AMICS).
Kaplan-Meier curve representing 180-day survival of AMICS patients with no elevated biomarkers (blue, lactate <2.0 mmol/L, VEGFR1 <3.59 ng/L), either lactate or VEGFR1 elevations (red), or elevations of both biomarkers (green). Log-rank (Mantel-Cox) test *P*<0.001.

Table 3. Baseline Characteristics of AMICS Survivors and Nonsurvivors (Within 180 Days) From the Time-Series Analysis Cohort

Characteristics	Survivors (n=27)	Nonsurvivors (n=22)	*P* value
Age, y; mean±SD	61±10	68±15	0.059
Body mass index, kg/m²; mean±SD	26.0±2.8	26.9±4.3	0.417
Sex (male), n (%)	23 (85)	16 (73)	0.417
Systolic arterial pressure, mm Hg; mean±SD	97±20	88±17	0.08
Diastolic arterial pressure, mm Hg; mean±SD	63±15	58±13	0.25
Heart rate, bpm; mean±SD	101±23	101±19	0.925
Cardiogenic shock, n (%)	27 (100)	22(100)	>0.999
Catecholamine use, n (%)	21 (78)	22 (100)	0.055
Comorbidities, n (%)			
Previous myocardial infarction	2 (7)	1 (5)	>0.999
Previous PCI	8 (30)	10 (46)	0.398
Previous CABG	0 (0)	0 (0)	>0.999
Atrial fibrillation	4(15)	3 (14)	>0.999
Stroke	0 (0)	0 (0)	>0.999
Peripheral artery disease	5 (19)	3 (14)	0.943
Cardiovascular risk factor, n (%)			
Current smoking	15 (56)	4 (18)	0.018
Hypertension	15 (56)	9 (41)	0.464
Dyslipidemia	8 (30)	2 (9)	0.387
Diabetes	9 (33)	7 (32)	>0.999
Serum lactate, mmol/L; mean±SD	5.9±3.5	9.3±4.6	0.007

AMICS indicates acute myocardial infarction with cardiogenic shock; CABG, coronary artery bypass graft; and PCI, percutaneous coronary intervention.

prognostic capability was diminished (HR, 1.00 [95% CI, 0.98–1.03]; *P*=0.834). However, formal testing for statistical interaction between VEGFR1 and lactate (as continuous variables) did not reach significance, indicating that the observed differences may reflect trends rather than true effect modification. These findings may still suggest that VEGFR1's prognostic value is most pronounced in early or moderate stages of shock, where it is less overshadowed by profound physiological derangement. VEGFR1, also known as Flt1 (fms related receptor tyrosine kinase 1), is 1 of 3 known VEGF receptors.[24] The VEGF family modulates angiogenesis and controls vascular permeability, traits acting as prerequisite for vascular development and maintained vasoactive regulation. Most of the beneficial effects of this protein family are considered to occur by signaling mediated upon binding of VEGF-A to VEGFR2.[25] Indeed, 1 remarkable function is the VEGFR2-mediated modulation of the endothelial nitric oxide synthase function,[26] a key regulator of blood pressure. However, VEGFR1 competes with VEGFR2 for binding VEGF-A as it shows high affinity for the ligand, yet binding results in low intracellular signaling activity. Thus, it acts as a scavenger and negative regulator of VEGF-A–VEGFR2 signaling.[27,28] Interestingly, VEGF-A levels associate with the degree of microvascular obstruction in ST-segment–elevation AMI.[29] Severe microvascular obstruction constitutes a risk factor that increases CS probability. In addition, a recent meta-analysis reported changes in VEGF-A levels in severe septic and critically ill patients and confirmed their predictive power.[30] Changes in VEGFR1 were similarly linked to states with impaired systemic circulation. For instance, a rise in soluble form of VEGFR1 in response to sepsis was observed in experimental studies on mice and later also confirmed in septic patients.[31,32] Similar to our results, soluble VEGFR1 isoform conferred a solid independent mortality prediction in patients with septic shock.[33]

In murine models of hypoperfusion, hindlimb ischemia led to a marked decrease in VEGFR2 and an increase in VEGFR1 on endothelial cells.[34] This suggests VEGFR1 upregulation may reflect vascular damage. However, in our cohorts, VEGFR1 levels were already elevated at admission, indicating an acute response to hemodynamic deterioration in AMICS. Supporting its relevance in critical illness, a common *FLT1* gene variant linked to higher VEGFR1 expression was associated with increased acute respiratory distress syndrome risk in sepsis.[35,36]

VEGF-B, another VEGFR1 ligand, plays a role in cardiac angiogenesis in ischemia with only little to no function in other organs.[37,38] It proved relevant for the myocardial cellular metabolism by supporting fatty acid uptake. Interestingly, changes in its expression levels and protein abundance were associated with dilative or ischemic heart diseases.[39] In patients, VEGF-B levels rise after AMI, but even small increases are linked to adverse 6-month remodeling. Overall, changes in VEGF family proteins clearly impact cardiac and circulatory function. While the underlying mechanisms need further study, our findings highlight VEGFR1 as a promising prognostic marker in CS and a potential indicator of disease severity. Point-of-care testing for VEGFR1 could help to identify high-risk patients with tissue hypoxia. Future studies should explore whether targeting VEGFR1 can alter disease progression. Importantly, VEGFR1 levels remained elevated in nonsurvivors at later time points (day 1 and day 5), supporting its stability as a prognostic marker to identify patients at risk. However, prospective studies are necessary to confirm these findings and investigate the role of VEGFR1 as tool to support decision making.

This study's main limitation is that VEGFR1 was identified by comparing the plasma proteome of AMICS versus AMI patients without CS (n=9 versus n=8) in a small cohort. To reduce false positives, we applied multiple comparison correction, which may have masked other relevant protein changes. Without this correction, additional proteins might have appeared significantly regulated. We chose to prioritize specificity but

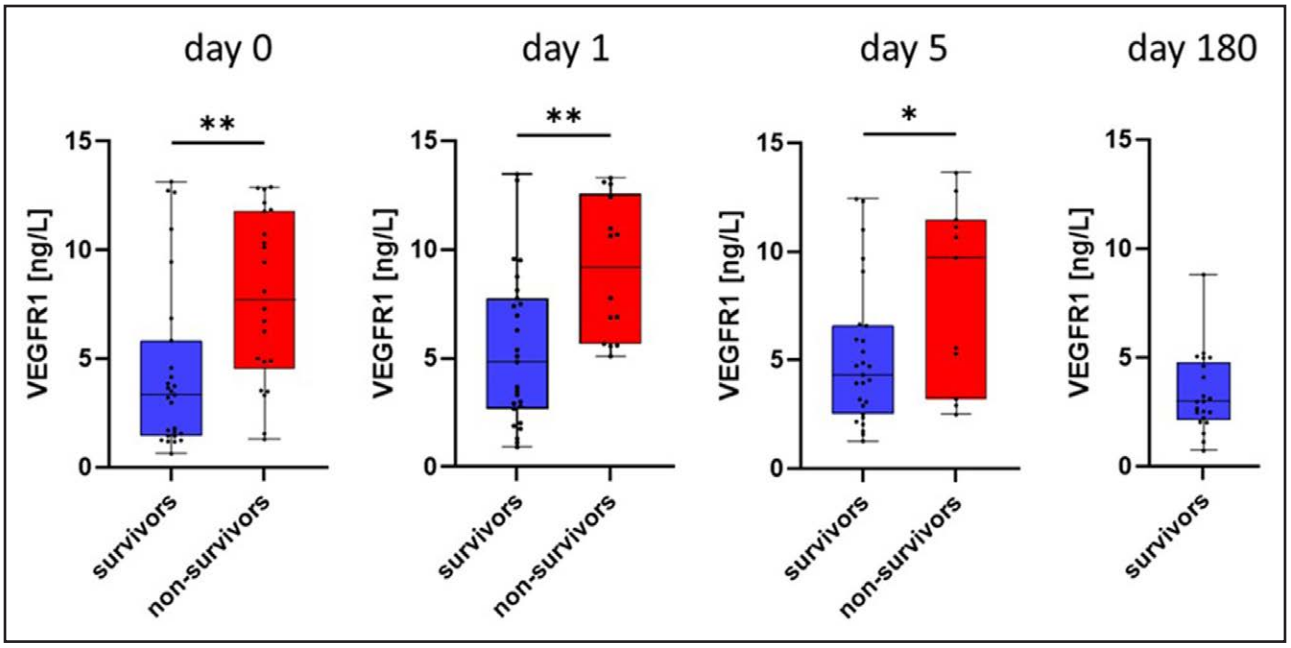

Figure 4. Time-dependent analysis of VEGFR1 (vascular endothelial growth factor receptor 1) in patients with acute myocardial infarction cardiogenic shock (AMICS).
Plasma concentration of VEGFR1 was measured by ELISA on day 0 (n=27 vs n=22), day 1 (n=27 vs n=14), day 5 (n=27 vs n=11), and day 180 (n=21) post-AMI and groups dichotomized in those surviving and nonsurviving until day 180. Comparisons by *t* test. Data are presented as mean±SEM. The number of replicates is indicated in each column. Survival was confirmed for 27 patients while only 21 of those were available for plasma sampling at day 180. The *P* values are shown for the groups compared as indicated by the respective lines. **P*<0.05; ***P*<0.01.

acknowledge this may have limited biomarker discovery. It is important to acknowledge that the discovery phase was hypothesis-generating with limited statistical power. While it identified VEGFR1 as potential biomarker it may have missed others, including those already described. The full data set is available to support further analysis using alternative thresholds. Notably, VEGFR1's stability in this clinical setting may contribute to its strong prognostic value in cardiovascular disease and mortality. The verification and validation of these proteins was performed in an AMICS-only collective, albeit in the largest prospective AMICS biobanking cohort to date. However, the CULPRIT-SHOCK trial initially investigated 706 patients. All patients with available biobanking were analyzed (n=421). Not all sites of this multicenter trial collected biomaterial, thus introducing a potential selection bias. Moreover, we acknowledge the limitation of using a Youden index cutoff for survival analysis which was derived from and used in the same cohort.

CONCLUSIONS

In summary, our findings indicate that elevated plasma protein levels of VEGFR1 in patients with AMICS predict an increased mortality risk, in addition to the recognized elevations in lactate. VEGFR1 has the potential to precisely identify high-risk patients with AMICS, allowing for individualized interventions and more accurate risk assessment, especially given the unacceptably high and stagnant mortality rates in AMICS.[40] Furthermore, emerging biomarkers could aid clinical research by pinpointing patients suitable for novel treatments. As mechanical circulatory support devices have produced beneficial outcomes in CS,[4,41,42] early detection of shock stages and prompt initiation of treatment remain critical for patient survival.

Our novel approach used multiparametric and highly sensitive proximity extension assay technology, leading to the identification of VEGFR1 as an early biomarker in patients with AMICS. Despite the complexity of our methodology, we propose a relatively straightforward method for risk stratification through a binary biomarker approach. In a cohort of well-defined patients with AMICS, VEGFR1 provided independent prognostic insights, adding to the established prognostic value of serum lactate. Given the challenges in improving patient outcomes in CS with devices or other innovative treatment strategies, improving CS characterization with new biomarkers such as VEGFR1 could be instrumental in refining patient selection for future prospective trials.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Figures S1–S5

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