

Non-targeted metabolomics identify polyamine metabolite acisoga as novel biomarker for reduced left ventricular function

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Abstract

Aims Chronic heart failure with reduced ejection fraction remains a major health issue. To date, no reliable biomarker is available to predict reduced left ventricular ejection fraction (LV-EF). We aimed to identify novel circulating biomarkers for reduced left ventricular function using untargeted serum metabolomics in two independent patient cohorts.

Methods and results Echocardiography and non-targeted serum metabolomics were conducted in two patient cohorts with varying left ventricular function: (1) 25 patients with type 2 diabetes with established cardiovascular disease or high cardiovascular risk (LV-EF range 20–66%) (discovery cohort) and (2) 37 patients hospitalized for myocardial infarction (LV-EF range 25–60%) (validation cohort). In the discovery cohort, untargeted metabolomics revealed seven metabolites performing better than N-terminal pro-B-type natriuretic peptide in the prediction of impaired left ventricular function shown by LV-EF. For only one of the metabolites, acisoga, the predictive value for LV-EF could be confirmed in the validation cohort ($r = -0.37$, $P = 0.02$). In the discovery cohort, acisoga did not only correlate with LV-EF ($r = -0.60$, $P = 0.0016$), but also with global circumferential strain ($r = 0.67$, $P = 0.0003$) and global longitudinal strain ($r = 0.68$, $P = 0.0002$). Similar results could be detected in the discovery cohort in a 6 month follow-up proofing stability of these results over time. With an area under the curve of 0.86 in the receiver operating characteristic analysis, acisoga discriminated between patients with normal EF and LV-EF < 40%. Multivariate analysis exposed acisoga as independent marker for impairment of LV-EF (Beta = -0.71 , $P = 0.004$).

Conclusions We found the polyamine metabolite acisoga to be elevated in patients with impaired LV-EF in two independent cohorts. Our analyses suggest that acisoga may be a valuable biomarker to detect patients with heart failure with reduced ejection fraction.

Keywords Heart failure; HFrEF; Ejection fraction; Biomarker; Metabolomics; Polyamine metabolism

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Introduction

Chronic heart failure with reduced ejection fraction (HFrEF) remains one of the major health issues in the world.¹ Patients suffering from HFrEF are frequently hospitalized for acute heart failure decompensation and show a 5 year mortality

above 75%.² HFrEF is defined by heart failure symptoms and left ventricular ejection fraction (LV-EF) < 40%.¹ N-terminal pro brain natriuretic peptide (NT-proBNP) serves as a gold standard biomarker for acute decompensated HFrEF. However, it may also be elevated in heart failure patients with mid-range and preserved ejection fraction

(HFmrEF and HFpEF), tachycardia, and valvular heart disease.^{1,3} NT-proBNP exhibits a higher negative than a positive predictive values in patients with non-decompensated, stable disease. Thus, it reliably allows to rule out suspected heart failure but poorly predicts LV-EF.^{4,5} NT-proBNP therefore is not a valuable indicator for left ventricular function, especially in acute decompensated heart failure.^{4,5} To date, no laboratory biomarker for LV-EF prediction is available.⁶ Advances in high-throughput technologies, including untargeted metabolomics, have given us novel insights into biomarker discovery. Thus, circulating metabolites may be valuable for screening of impaired LV-EF as well as guidance of clinical decisions. Moreover, by uncovering modulated biomarkers, untargeted metabolomics might give new insights into underlying pathomechanisms of heart failure and therefore into new therapeutic strategies in the future.⁷

In this hypothesis-generating pilot study, we aimed to identify novel circulating biomarkers for LV-EF impairment using untargeted serum metabolomics in two independent patient cohorts.

Methods

Discovery cohort

Patients with diabetes mellitus type 2 as well as cardiovascular disease (reduced LV-EF by ischemic cardiomyopathy with no coronary event in the past three months) were recruited at RWTH Aachen University hospital between 12/2015 and 07/2016 as described before (NCT03131232, approved by

the ethics committee of the RWTH Aachen Medical Faculty; all patients gave informed consent).^{8,9} Serum collection, recording of clinical data, and echocardiography were performed at baseline. Fortytwo patients were included, 17 individuals had to be excluded from the metabolomics analysis due to lack of serum collection, lack of echocardiography, or withdrawal of consent. Following initial echocardiography and serum collection patients received 10 mg empagliflozin per day. After 6 months, follow-up echocardiography and serum collection were performed within 13 patients of the cohort. Twelve patients had to be excluded due to lack of serum collection, discontinuation of study-medication, lack of echocardiography, or withdrawal of consent. Due to the exploratory character of this study, the sample size was not determined by statistical methods. Baseline data are provided in *Table 1*. Strobe diagram is shown in *Figure S1*.

Validation cohort

Patients hospitalized for ST-segment elevation myocardial infarction or non-ST-segment elevation myocardial infarction were included in this cohort between 02/2015 and 04/2016 at Tor Vergata University of Rome, Italy.⁹ All subjects gave written informed consent. Forty patients were enrolled in the study; 3 patients had to be excluded to various reasons. The study was approved by the local ethical committee ('AMIDIAB study 28.04/2008'). Serum, clinical data, and echocardiographic data were collected. Only male patients were included in this cohort. Baseline data are provided in *Table 2*. Strobe diagram is shown in *Figure S2*.

Table 1 Baseline characteristics of the discovery cohort

Parameter	LV-EF ≥ 50%	LV-EF 40–49%	LV-EF < 40%	P value
Number	14	6	5	
Age, years	64 ± 10.36	65 ± 10.12	64 ± 11.09	0.98
Female (%)	21	33	0	<0.0001
BMI, kg/m ²	31 ± 4.06	31.5 ± 4.18	33.8 ± 8.79	0.60
HbA1c, mmol/mol	67.99 ± 8.87	68.12 ± 11.69	73.12 ± 10.25	0.76
Creatinine, μmol/L	93.7 ± 20.33	86.63 ± 13.26	93.7 ± 29.17	0.84
eGFR, mL/min/1.73 m ²	79.69 ± 16.56	79.23 ± 11.5	77.48 ± 22.88	0.97
Serum Glucose, mmol/L	10.7 ± 2.6	11.1 ± 2.5	12.3 ± 2.8	0.50
NT-proBNP, pg/mL	466.1 ± 660.9	345.6 ± 157.4	2,348 ± 2,735	0.02
HDL cholesterol, mmol/L	1.02 ± 0.21	1.09 ± 0.33	0.84 ± 0.31	0.28
LDL cholesterol, mmol/L	2.75 ± 1.08	2.86 ± 1.63	2.15 ± 1.59	0.63
Sodium, mmol/L	136.8 ± 3.24	138.7 ± 1.97	135.2 ± 3.70	0.20
LV-EF, %	62.0 ± 3.67	45.3 ± 3.01	28.4 ± 8.08	<0.0001
GCS, %	−30.21 ± 1.37	−27 ± 2.53	−18.00 ± 3.87	<0.0001
GLS, %	−29.21 ± 2.23	−24.00 ± 2.83	−16.40 ± 6.12	<0.0001
LV-mass, g	117.6 ± 25.67	102.7 ± 11.06	109.4 ± 20.7	0.38
E/E'	7.79 ± 3.29	14.00 ± 4.56	7.40 ± 7.89	0.03
TAPSE, mm	20.7 ± 2.7	20 ± 0	16.0 ± 8.9	0.13
RVSP, mmHg	25.86 ± 4.85	26.83 ± 6.77	30.80 ± 3.53	0.30

Baseline characteristics of the patients with type 2 diabetes mellitus included in the discovery cohort. Analyses were performed by one-way ANOVA or χ^2 test. Data are shown as mean ± standard deviation. Significant values ($P < 0.05$) are bold.

BMI, body mass index; eGFR, estimated glomerular filtration rate; GCS, global circumferential strain; GLS, global longitudinal strain; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LV-EF, left ventricular ejection fraction; RVSP, right ventricular systolic pressure; TAPSE, tricuspid annular plane systolic excursion.

Table 2 Baseline characteristics of the validation cohort

Parameter	LV-EF \geq 50%	LV-EF 40–49%	LV-EF < 40%	P value
Number	21	10	6	
Age, years	60.71 \pm 11.82	56.8 \pm 9.60	62.17 \pm 12.06	0.58
Female, %	0	0	0	
Creatinine, μ mol/L	94.16 \pm 22	82.72 \pm 15.84	98.56 \pm 42.24	0.40
eGFR, mL/min/1.73 m ²	80.73 \pm 23.74	91.70 \pm 18.64	84.47 \pm 38.88	0.54
HDL, mmol/L	1.07 \pm 0.26	1.05 \pm 0.18	1.1 \pm 0.37	0.92
LDL, mmol/L	2.95 \pm 0.8	3.37 \pm 0.77	2.6 \pm 1	0.19
LV-EF, %	53.10 \pm 3.70	44.00 \pm 2.11	31.67 \pm 4.08	<0.0001
Diabetes, %	14.29	20	33.33	0.004
STEMI, %	38.1	80	50	<0.0001
NSTEMI, %	61.9	20	50	<0.0001

Baseline characteristics of patients included in the validation cohort. Analyses were performed by one-way ANOVA or χ^2 test. Data are shown as mean \pm standard deviation. Significant values ($P < 0.05$) are bold.

eGFR, estimated glomerular filtration rate; HDL, high-density lipoproteins; LDL, low-density lipoproteins; LV-EF, left ventricular ejection fraction; NSTEMI, non ST-segment elevation myocardial infarction; STEMI, ST-segment elevation myocardial infarction.

Non-targeted metabolomics

Metabolomics analysis of serum samples were performed as described previously.^{8–11} After collection, samples were stored at -80°C . Prior to analysis at the Helmholtz Zentrum München (Germany), samples were thawed on ice and randomized on the day of extraction.

A 100 μL of the serum were pipetted into a 2 mL 96-well plate together with 100 μL of human reference EDTA plasma (Seralab, West Sussex, UK) and 100 μL human reference Serum (Seralab, West Sussex, UK). These samples were used as technical replicates throughout the data set in order to assess process variability. As process blank, water samples were used and placed into six wells of each plate.

After protein precipitation, the metabolites were extracted with 475 μL methanol, containing four recovery standard compounds to monitor the extraction efficiency. The supernatant after centrifugation was split into four aliquots of 100 μL onto two 96-well microplates. Two aliquots were used for analysis by two separate reversed-phase/ultra-performance liquid chromatography–tandem mass spectrometry (RP/UPLC–MS/MS) methods with positive ion mode electrospray ionization (ESI), one for analysis by RP/UPLC–MS/MS with negative ion mode ESI and one for analysis by (HILIC)/UPLC–MS/MS with negative ion mode ESI. The samples were dried on a TurboVap 96 (Zymark, Sotax, Lörrach, Germany) and reconstituted with 50 μL formic acid (FA) 0.1% prior to UPLC–MS/MS in positive ion mode, and with 50 μL of 6.6 mM ammonium bicarbonate (pH 8.0) prior to analysis with negative ion mode. Both solvents contained internal standards that allowed monitoring of instrument performance and served as retention reference markers.

In order to minimize human error, all handling with liquids was performed on an automated MicroLab STAR[®] robot (Hamilton Bonaduz AG, Bonaduz, Switzerland). UPLC–MS/MS analysis was performed on a Q Exactive high-resolution/accurate mass spectrometer (Thermo Fisher Scientific

GmbH, Dreieich, Germany) combined with a Waters Acquity UPLC system (Waters GmbH, Eschborn, Germany)

Samples of the discovery cohort

Before the UPLC–MS/MS runs, the dried samples were reconstituted with 80 μL of solvents, which are compatible to each of the methods. Every solvent contained several standards at fixed concentrations to guarantee injection and chromatographic consistency. One aliquot chromatographically optimized for more hydrophilic compounds was analysed using acidic positive ion conditions. The extract was gradient eluted from a C18 column (Waters UPLC BEH C18-2.1 \times 100 mm, 1.7 μm) by using water and methanol containing 0.05% perfluoropentanoic acid (PFPA) and 0.1% FA. One aliquot chromatographically optimized for more hydrophobic compounds was analysed using acidic positive ion conditions. The extract was again eluted from a C18 column using water, methanol, acetonitrile, 0.05% PFPA, and 0.01% FA operating at an overall higher organic content. The third aliquot was analysed using a separate dedicated C18 column using basic negative ion optimized conditions. The basic extracts were gradient eluted from the column using methanol, water and 6.5 mM ammonium bicarbonate at pH 8. The UPLC gradient elution was developed linear from 0.5% to 70% methanol over 4 min, then to a gradient of 99% methanol in 0.5 min with a constant flow rate of 350 $\mu\text{L}/\text{min}$. The fourth aliquot was analysed using a gradient of water (15%), methanol (5%) and acetonitrile (80%) with 10 mM ammonium formate (pH 10.8) and a mixture of 50% water and 50% acetonitrile via negative ionization following elution from a HILIC column (Waters UPLC BEH Amide 2.1 \times 150 mm, 1.7 μm). UPLC separation was developed on a linear gradient from 5% to 50% of the water/acetonitrile-mixture in 3.5 min followed by a linear gradient from 50% to 95% of the mixture in 2 min at a constant flow rate of 500 $\mu\text{L}/\text{min}$. The MS analysis alternated between MS and

data-dependent MS scans using dynamic exclusion. They covered 70–1000 m/z but varied slightly between methods.

Samples of the validation cohort

Prior to UPLC-MS/MS in positive ion mode samples were reconstituted with 50 μL of 0.1% formic acid and those analysed in negative ion mode with 50 μL of 6.5 mM ammonium bicarbonate at pH 8.0. Further internal standards allowing monitoring of instrument performance and serving as retention reference markers were present in the reconstitution solvents for both ionization modes. Two separate columns (2.1 \times 100 mm Waters BEH C18 1.7 μm particle) were used for mobile phase conditions. Acidic conditions using 0.1% FA in water (Solvent A) and 0.1% FA in methanol (Solvent B) were used providing optimized conditions for positive ESI. Basic conditions using 6.5 mM ammonium bicarbonate at pH 8.0 (Solvent A) and 6.5 mM ammonium bicarbonate in 95% methanol (Solvent B) were used for negative ESI. Sample extracts were injected, and the columns were developed in a gradient of 99.5% Solvent A to 98% Solvent B at 350 $\mu\text{L}/\text{min}$ flow rate in a run time of 11 min. Eluent flow was directly connected to the ESI source of the mass spectrometer. Data dependent MS/MS scans with dynamic exclusion and full scan mass spectra (80–1000 m/z) were recorded in turns.

For both cohorts, raw data were extracted using Metabolon's hardware and software (Metabolon, Inc., USA), Metabolites were annotated by curation of the LC-MS/MS data against proprietary Metabolon's chemical database library (Metabolon, Inc., USA). All mentioned metabolites were detected at the highest level of metabolite identification (Level 1, Tier 1) according to the Metabolon's proprietary standards.^{12,13}

Statistical analysis

Data were analysed and visualized using GraphPad Prism 9.0 and MetaboAnalyst 4.0.¹⁴ Statistical analyses were performed using correlation analysis, simple linear regression, receiver operating characteristic (ROC), two tailed t test, and ANOVA, when appropriate. Data are presented as means \pm standard deviation (SD). P values <0.05 were considered to be statistically significant. Metabolomics data were corrected for multiple testing by false discovery rate.

Results

We explored non-targeted serum metabolomics of 25 diabetic cardiovascular high-risk patients (discovery cohort) to identify novel biomarkers and pathways associated to reduced left ventricular ejection fraction (LV-EF). The cohort was divided into three groups according to the LV-EF ranges of normal LV-EF, HFmrEF, and HFrEF: LV-EF $\geq 50\%$, LV-EF

40–49%, and LV-EF $< 40\%$ (Table 1). The LV-EF means of each group were as followed: $62.0 \pm 3.7\%$, $45.3 \pm 3.0\%$, and $28.4 \pm 8.1\%$. As expected, global circumferential strain (GCS), global longitudinal strain (GLS) (both $P < 0.0001$), and NT-proBNP ($P = 0.02$) differed significantly among the groups. Patients were 64 ± 10 , 65 ± 10 , and 64 ± 11 years old ($P = 0.98$). Of all other baseline characteristics only gender was not homogeneously distributed ($P < 0.0001$). No significant difference could be observed for body mass index (BMI), HbA1c, estimated glomerular filtration rate (eGFR), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) (Table 1).

By non-targeted metabolomics, we measured 1269 serum metabolites (863 chemically identified and 406 not identified). NT-proBNP was included in the analysis as an established cardiac biomarker. Of 1269 measured, eight already identified metabolites significantly correlated with LV-EF ($P < 0.05$, false discovery rate <0.2) also revealing a strongly negative correlation to left ventricular strain (Figure 1A and 1B). Seven identified metabolites performed better than NT-proBNP (Figure 1A). Analysis of variance between patients with LV-EF $<40\%$ (HFrEF), LV-EF 40–50% (HFmrEF) and patients with LV-EF $>50\%$ revealed that ribitol and lactate were also increased in patients with LV-EF 40–49%, while all other biomarkers were only increased in patients with an LV-EF below 40% (Figure 1C). Although differences in single metabolites between the LV-EF groups were noticed, no separation of the groups was observed by principal component analysis of the metabolome (Figure S3).

To validate our findings in an independent cohort, we studied non-targeted serum metabolomics of 37 patients referred to the hospital for myocardial infarction (validation cohort). Patients were 61 ± 12 years (LV-EF $\geq 50\%$), 57 ± 10 years (LV-EF 40–49%), and 62 ± 12 years (LV-EF $< 40\%$) old. Mean LV-EF were as followed: $53.1 \pm 3.70\%$, $44.00 \pm 2.11\%$, and $31.67 \pm 4.08\%$. A significant difference in diabetes mellitus and myocardial infarction status (non ST-segment elevation myocardial infarction vs. ST-segment elevation myocardial infarction) was present between the LV-EF tertiles of the cohort. No difference was observed for age, gender, eGFR, HDL, and LDL (Table 2). In this cohort, only acisoga of the previously identified metabolites confirmed its significant correlation to LV-EF ($r = -0.38$, $P = 0.0207$) (Figure 2A). In patients with LV-EF $< 40\%$ levels of acisoga were significantly increased ($P = 0.04$, Figure 2B).

In the discovery cohort, acisoga showed a strong correlation to LV-EF ($r = -0.60$, $P = 0.0016$) with discrimination of patients with normal LV-EF, HFmrEF, and HFrEF ($P = 0.009$) (Figure 3A). Moreover, ROC analysis for the detection of LV-EF below 40% revealed an area under the curve of 0.86 (Figure 3A). Acisoga also strongly correlated with global longitudinal (GLS) ($r = 0.68$, $P = 0.0002$) and circumferential strain (GCS) ($r = 0.67$, $P = 0.0003$) as additional markers for left ventricular function (Figure 3B). Moreover, a significant correla-

Figure 1 Serum metabolomics reveal biomarkers for reduced left ventricular ejection fraction (LV-EF) in the discovery cohort. (A) Serum metabolomics performed in the discovery cohort ($n = 25$). Top-metabolites showing a significant Pearson correlation to LV-EF ($P < 0.05$) corrected for multiple testing by false discovery rate (FDR < 0.2). (B) Correlation heat-map showing the seven discovered metabolites and the coefficient of determination (r^2) to echocardiographic parameters (LV-EF, GLS, GCS, LV-mass, E/e', tricuspid annular plane systolic excursion, RVSP) and N-terminal pro brain natriuretic peptide (NT-pro-BNP). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. GCS, global circumferential strain; GLS, global longitudinal strain; LV-EF, left ventricular ejection fraction; LV-mass, left ventricular mass; RVSP, right ventricular systolic pressure; TAPSE, tricuspid annular plane systolic excursion. (C) Difference in serum metabolites according to LV-EF groups: LV-EF $\geq 50\%$, LV-EF 40–49%, and LV-EF $< 40\%$. The quantity of the metabolites is shown in fold change or pg/mL (NT-proBNP). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by one-way ANOVA with Tukey's post-test.

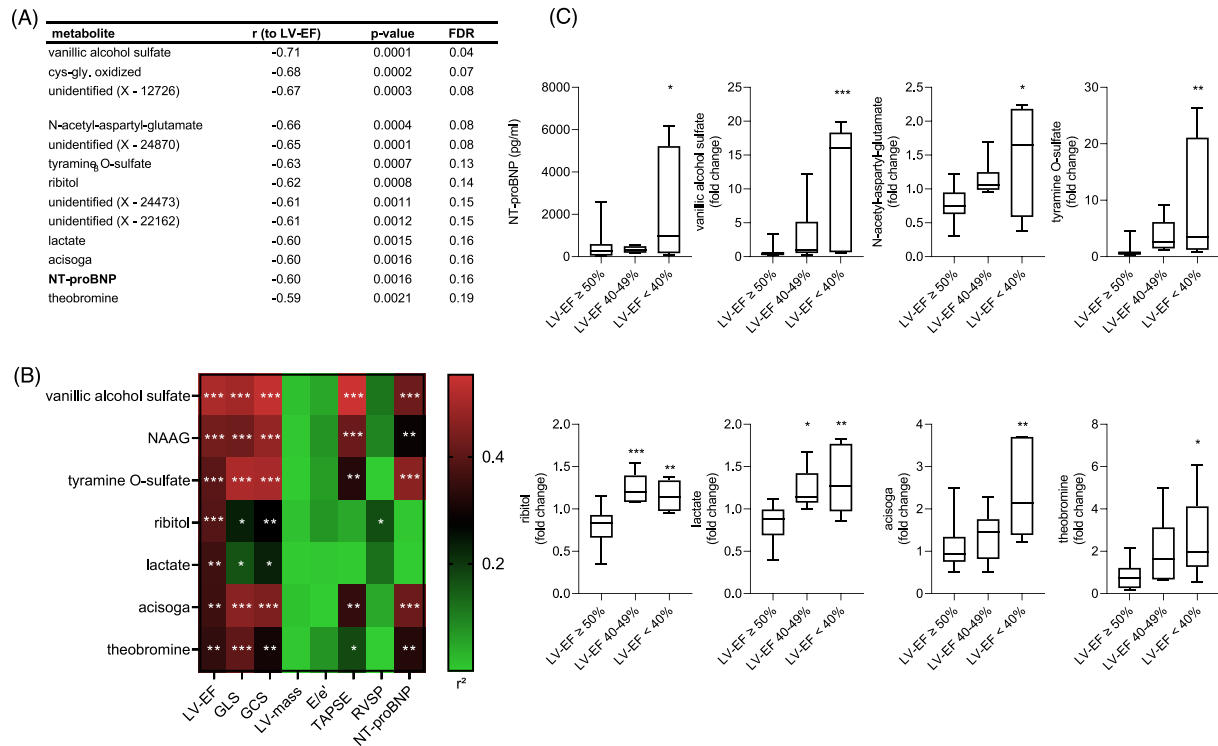


Figure 2 Confirmation of acisoga as biomarker for heart failure with reduced ejection fraction (HFrEF) in a validation cohort. (A) Correlation (Pearson) of acisoga and left ventricular ejection fraction (LV-EF) in the validation cohort, $n = 37$. (B) Acisoga is significantly enhanced in patients with HFrEF (LV-EF $< 40\%$) compared with patients with normal LV-EF. * $P < 0.05$ by unpaired two-sided t test.

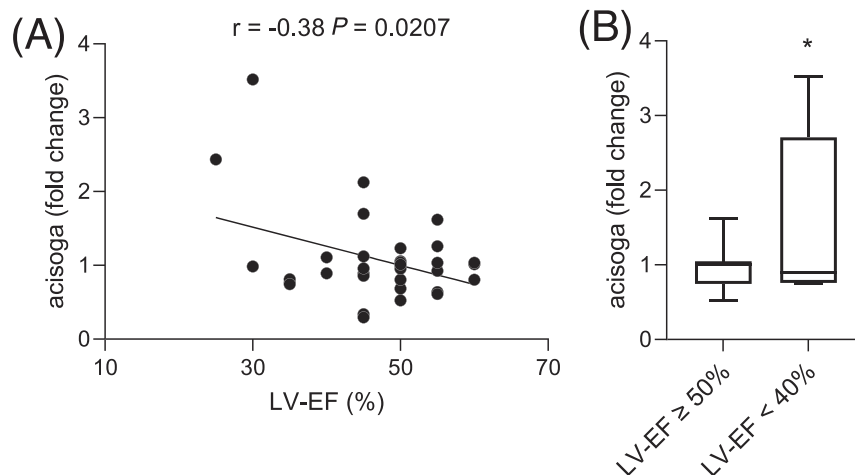
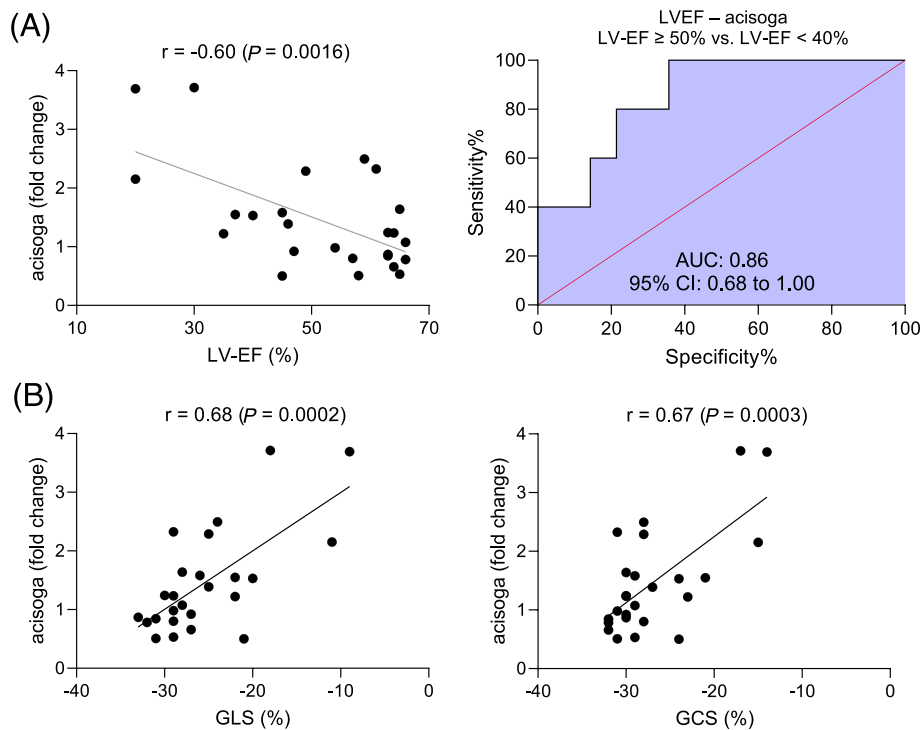


Figure 3 Acisoga correlates with echocardiographic parameters for left ventricular dysfunction. (A) Echocardiography and serum-metabolomics were performed in the discovery cohort ($n = 25$). Left: Correlation (Pearson) of left ventricular ejection fraction (LV-EF) (%) and acisoga (fold change). Right: Receiver operating characteristic-analysis of acisoga comparing patients with LV-EF ≥ 50 vs. patients with LV-EF $< 40\%$. AUC, area under the curve; CI, confidence interval. (B) Correlation (Pearson) of GLS (%) and GCS (%) to acisoga (fold change). GCS, global circumferential strain; GLS, global longitudinal strain.



tion between acisoga and tricuspid annular plane systolic excursion, as indicator for right ventricular function, could be observed. No correlation was noticed for right ventricular systolic pressure, E/e' , and LV-mass (Figure 1B).

After initial serum collection and echocardiography at baseline, patients of the discovery cohort received 10 mg empagliflozin per day. Echocardiography and blood sampling, including the same untargeted metabolomics approach, were repeated after 6 months. LV-EF, GCS, and GLS remained stable in this cohort. A trend towards an improvement of GCS after empagliflozin treatment ($P = 0.0532$) could be noticed. In accordance with this observation, levels of acisoga were relatively stable over time with a small, but significant, decrease after 6 months, which may reflect improvement of GCS. However, the correlation of acisoga to LV-EF, GLS, and GCS remained significant in this follow-up (Figure S4).

In a multivariate regression analysis correcting for age, eGFR, BMI, HbA1c, and gender, acisoga (Beta = -0.71 , $P = 0.004$) remained an independent predictor for LV-EF in the discovery cohort at baseline (Table S1). Similar was observed in the validation cohort, where acisoga remained an independent LV-EF predictor (Beta = -0.38 , $P = 0.04$) in a

multivariate regression analysis corrected for age, BMI, eGFR, diabetes mellitus, and myocardial infarction status (Table S2).

Discussion

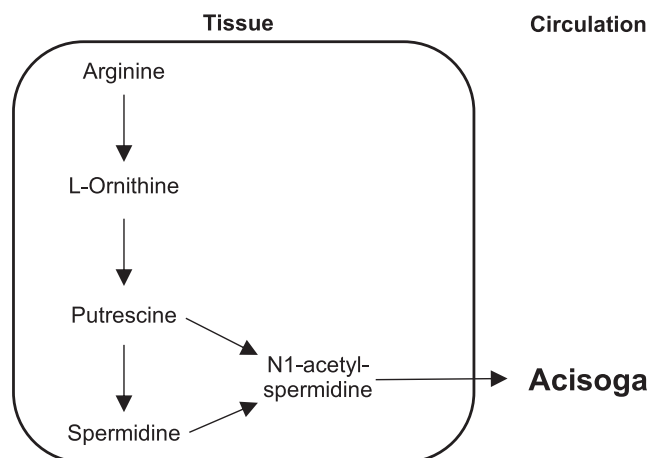
Acute and chronic heart failure remain severe, potentially life-threatening diseases with an enormous impact on both mortality and morbidity of patients. Unfortunately, to date no reliable laboratory test predicting the left ventricular function has been discovered. Established biomarkers, such as NT-proBNP, may also be elevated in response to other decompensated heart diseases independently of LV-EF. Untargeted metabolomics are an emerging and innovative tool to detect new biomarkers and to uncover disease pathways in cardiovascular research.¹⁵ Differences in the metabolism between non-heart failure, HFpEF, and HFrEF patients and therefore varying LV-EF have been shown via targeted metabolomics.^{16,17} These findings emphasize metabolomics as a promising tool to detect appropriate biomarkers for LV-EF deterioration. In this study, we ex-

explored metabolomics of two different cohorts to unmask novel biomarkers for impaired left ventricular function. Although detecting several candidates by an untargeted metabolomics approach in a discovery cohort, only one of the metabolites predicting LV-EF could be confirmed in the validation cohort. We found acisoga, a metabolite of the polyamine metabolism, to be enhanced in patients with LV-EF < 40% correlating significantly not only with LV-EF but also with several other echocardiographic parameters of ventricular function, like GLS or GCS.^{18,19} Even though it was one of few metabolites discriminating successfully between heart failure groups, it was the only metabolite showing reliability in two different cohorts and also a stability over time. Notably, acisoga's correlation to LVEF was independent of renal function, diabetes mellitus, age, and gender. Acisoga [IUPAC name: *N*-(3-(2-oxopyrrolidin-1-yl)propyl)acetamide; HMDB ID: HMDB0061384; CAS Registry Number 106692-36-8] is an organic compound belonging to the chemical group of *n*-alkylpyrrolidines.²⁰ Acisoga is part of the polyamine metabolism and the catabolic product of spermidine via N1-acetylspermidine.^{16,21} It originates from arginine, which is transformed to L-ornithin by arginase and further decarboxylated to spermidine precursor putrescine (Figure 4).²¹ Although the biological function of this metabolite remains to be elucidated, it is known to be increased in obesity and to some extent predict the occurrence of atrial fibrillation.^{22,23} In mouse models of heart failure by left anterior descending ligation and transverse aortic constriction, Sansbury *et al.* observed an elevation of the acisoga precursors spermidine and putrescine in an untargeted metabolomics analysis of cardiac tissue. Both metabolites significantly correlated with echocardiographic LV-EF in this model.²⁴ Moreover, Meana

et al. found a significant correlation between N1-acetylspermidine, another precursor of acisoga, and LV-EF in human heart tissue of patients undergoing cardiac surgery involving extracorporeal circulation. Even though the sample size in this study was small, it still indicates an alteration of polyamine metabolism in heart failure and fits our findings.²⁵ The fact that spermidine, putrescine, and N1-acetylspermidine were increased in failing cardiac tissue indicates enhanced polyamine metabolism in heart failure increases serum levels of acisoga rather than impaired degradation.^{24,25} In another untargeted metabolomics approach, acisoga was found to be an independent biomarker to discriminate pulmonary arterial hypertension patients from healthy individuals.²⁶ Patients with pulmonary arterial hypertension mostly have normal left ventricular function, but frequently exhibit reduced right ventricular function. In our cohort, acisoga correlated also with tricuspid annular plane systolic excursion. These findings suggest that acisoga might be elevated also due to right ventricular dysfunction. Echocardiographic imaging however is limited in exact prediction of RV-function. Other imaging techniques, for example MRI, enable a more precise characterization of the right ventricular function.²⁷ Moreover, heart failure patients frequently have combined LV and RV dysfunction. We can only speculate, but our findings may indicate that acisoga rather predicts global ventricular function rather than LV-EF alone.

Interestingly, acisoga has previously been detected in urine.^{28,29} We found that levels of acisoga predicted LV-EF independently of renal function. Further studies must evaluate whether urinary acisoga reflects serum levels and whether it has also predictive value for left ventricular function.

Figure 4 Synthetic pathway of acisoga. Arginine is transformed to L-ornithin by arginase and further decarboxylated via ornithin decarboxylase to putrescine. Putrescine is then transformed to spermidine via spermidine synthetase. Both, spermidine and putrescine can be transformed to N1-acetylspermidine via sperminidine-spermine-N1-acetyl transferase or polyamine oxidase. N1-acetylspermidine is then oxidated to acisoga via amine oxidase.



Our study has limitations. Due to the exploratory character of this study, two cohorts with dissimilar patient characteristics were compared. Nonetheless, both independent cohorts revealed a similar link between acisoga and left ventricular function. Our data provide novel evidence for altered polyamine metabolism in heart failure, which is in consensus with previous investigations. We only included a limited number of patients. Thus, this explorative study needs to be confirmed with a targeted analysis of acisoga in a larger cohort containing patients with normal as well as reduced EF, (potentially also reduced right ventricular function) as well as different diabetes status. Multivariate analyses in both of our cohorts, however, imply an independent up-regulation of acisoga in heart failure patients. To develop potential therapeutic targets, it is crucial to uncover the exact molecular pathways of polyamine metabolism in heart failure by basic research.

In conclusion, acisoga, a metabolite of the polyamine metabolism, may represent a new potential biomarker to rapidly detect impaired left ventricular function and serve as a follow-up marker to predict LV-EF deterioration or improvement in heart failure patients.

Conflict of interest

K.S. served as a consultant to Amgen, Boehringer Ingelheim, AstraZeneca, and Lilly; received grant support from Boehringer Ingelheim; and served as a speaker for Amgen, AstraZeneca, Boehringer Ingelheim, Lilly, MSD, Novo Nordisk, Novartis, and OmniaMed.

N.M. has received support for clinical trial leadership from Boehringer Ingelheim, Novo Nordisk, served as a consultant to Boehringer Ingelheim, Merck, Novo Nordisk, AstraZeneca, and BMS; received grant support from Boehringer Ingelheim, Merck, and Novo Nordisk; and served as a speaker for Boehringer Ingelheim, Merck, Novo Nordisk, Lilly, BMS, and AstraZeneca. N.M. declines all personal compensation from pharma or device companies.

M.F. served as a consultant for Boehringer Ingelheim, Merck Sharpe&Dohme, Lilly & Co, Janssen, Sanofi, and Amgen.

M.L. received grants and personal fees from Boehringer Ingelheim, MSD, and Novo Nordisk, personal fees from Amgen, Sanofi, AstraZeneca, Bayer, and Lilly.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. STROBE diagram of the discovery cohort.

Figure S2. STROBE diagram of the validation cohort.

Figure S3. Acisoga is a stable biomarker for left ventricular function over time. A) LV-EF (%), GLS (%), GCS (%) and acisoga (fold-changes) between patients at baseline and 6 months follow-up after empagliflozin treatment (10 mg o.d.) in the discovery cohort (n = 13). *: $P < 0.05$ by Paired Student's t-tests. LV-EF = left ventricular ejection fraction; GCS = global circumferential strain; GLS = global longitudinal strain B) Correlation (Pearson) analysis between left ventricular function (LV-EF, GLS and GCS) and acisoga.

Figure S4. Principal component analysis of metabolomics. Principal component analysis plot of the metabolome data set of the discovery cohort at baseline. Red dots = LV-EF $\geq 50\%$, green dots = LV-EF 40–49%, blue dots = LV-EF $< 40\%$.

Table S1. Multivariate analysis shows correlation of Acisoga to LV-EF independently of baseline characteristics in patients of the discovery cohort. n = 25, Significant p -values are bold. LV-EF = left ventricular ejection fraction, BMI = Body mass index, GFR = glomerular filtration rate, HbA1c = haemoglobin A1c.

Table S2. Multivariate analysis shows correlation of Acisoga to LV-EF independently of baseline characteristics in patients of the validation cohort. n = 37, Significant p -values are bold. LV-EF = left ventricular ejection fraction, BMI = Body mass index, STEMI = ST-segment elevation myocardial infarction, NSTEMI = non-ST-segment elevation myocardial infarction.

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