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Circulating metabolomic and lipidomic profiles distinguish patients with acute heart failure *de novo* vs. worsening of heart failure

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ABSTRACT

Introduction: We hypothesize that acute heart failure (AHF) de novo and worsening of pre-existing disease (WHF) reflect distinct underlying metabolic states.

Objectives: To compare circulating metabolomic and lipidomic profiles between AHF de novo and WHF.

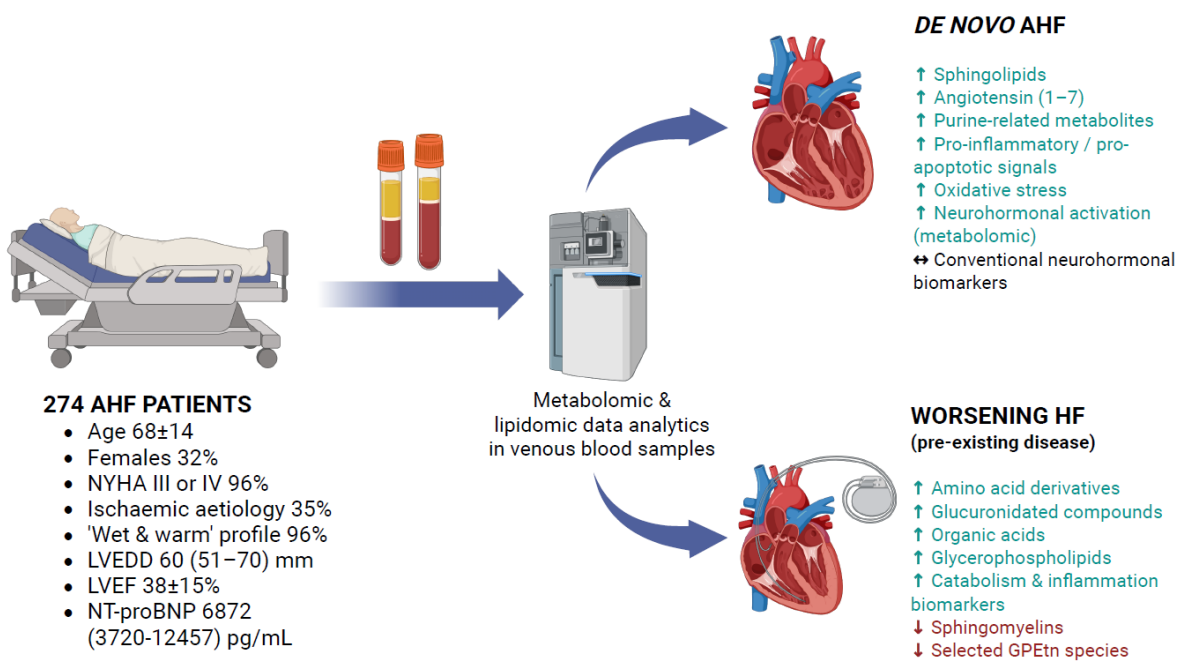
Patients and methods: We performed metabolomic and lipidomic analyses in venous blood collected from 274 patients admitted for AHF to a single tertiary referral cardiology center between 2021 and 2023.

Results: WHF patients (67%) were older, more often had coronary artery disease, and presented with slightly higher left ventricular ejection fraction. *De novo* AHF was characterized by higher levels of sphingolipids, angiotensin (1–7), and purine-related metabolites, indicating a pro-inflammatory and pro-apoptotic state and oxidative stress, with metabolomic signals of neurohormonal activation despite no differences in conventional neurohormonal biomarkers. WHF was associated with elevated levels of amino acid derivatives, glucuronidated compounds, and organic acids, which reflect altered energy metabolism and detoxification adaptations. Lipidomic analysis revealed higher levels of glycerophospholipids (GPEtn, GPGro, GPA, SQDG) and lower levels of sphingomyelins and selected GPEtn species in WHF, which may be related to impaired cellular membrane integrity or cellular injury. Biomarkers of catabolism and inflammation were also associated with pre-existing disease. In exploratory Cox modeling, several metabolites demonstrated reproducible associations with 12-month all-cause mortality independent of clinical covariates.

Conclusions: *De novo* AHF was characterized by a metabolomic profile indicative of a pro-inflammatory state and neurohormonal activation, while patients hospitalized for exacerbated pre-existing disease presented with metabo-lipidomic signatures of abnormal energy metabolism, catabolic state, and cellular injury.

KEY WORDS: acute heart failure, biomarkers, metabolomics, lipidomics

DE NOVO AHF WAS CHARACTERIZED BY A PRO-INFLAMMATORY METABOLOMIC PROFILE AND NEUROHORMONAL ACTIVATION, WHEREAS PATIENTS HOSPITALIZED FOR WORSENING PRE-EXISTING DISEASE EXHIBITED METABO-LIPIDOMIC SIGNATURES OF DISRUPTED ENERGY METABOLISM, CATABOLIC STATE, AND CELLULAR INJURY.



Abbreviations: AHF, acute heart failure; NYHA, New York Association; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; HF, heart failure.

INTRODUCTION

Being an inflection point in the disease trajectory, an acute heart failure (AHF) episode represents a turning point in the patient's medical history [**1,2**]. It is not only an unfavorable medical event in terms of long-term prognosis, but it also necessitates a series of specific, evidence-based diagnostic and therapeutic decisions to be initiated in a timely manner [**3-6**]. In the broad population of patients hospitalized due to AHF, two major subgroups should be distinguished: patients with *de novo* AHF, in whom the index hospitalization is the first manifestation of the disease, and patients with previously diagnosed chronic heart failure (HF) experiencing a worsening of heart failure (WHF) episode [**7-9**].

Although symptomatology may be similar in both populations, the underlying pathophysiological milieu does not necessarily have to be identical [**10**]. A more precise biological characterization of these clinical scenarios may therefore carry relevant diagnostic

and therapeutic implications. For instance, in selected cases, suspicion of acute inflammatory cardiomyopathy may justify urgent endomyocardial biopsy, whereas decompensation of a known or suspected genetic cardiomyopathy would prompt a different diagnostic and management pathway [**11,12**]. Importantly, the distinction between *de novo* AHF and WHF is typically based on clinical history and available documentation, which may be incomplete or limited. Patient-reported history is not always precise, reliable, or verifiable. In some cases, long-standing comorbidities such as diabetes or chronic kidney disease suggest gradual disease evolution. However, objective circulating biomarkers that would support more informed diagnostic and therapeutic decision-making in this context are currently lacking. In this setting, identifying measurable biological differences between these scenarios may provide complementary information beyond conventional clinical assessment.

Accumulated evidence from experimental and clinical studies demonstrates that heart failure (HF) exerts multidimensional metabolic adaptations—for example, the modulation of utilization patterns of various biofuels at the tissue level [**13**]. Until recently, the diagnosis of often subtle metabolic alterations was laborious and challenging when using conventional methods (e.g., immunoenzymatic assays). However, the advancement of high-throughput omics technologies enables large-scale and detailed stratification of patient subgroups, while the analysis of multiple biomarkers simultaneously offers more profound insights into disease pathophysiology and holds significant potential for improving future diagnostic approaches [14-17].

The aim of this study was to verify the hypothesis of whether circulating metabolomic and lipidomic profiles differ between patients hospitalized with *de novo* AHF and those with a WHF (acute decompensation of pre-existing chronic disease).

PATIENTS AND METHODS

Patients and follow-up

Comprehensive clinical data were analyzed from patients enrolled in a prospective, observational, single-center AHF registry. Patients included in the present analyses were recruited between 2021 and 2023 among adults admitted on an unplanned basis to the Institute of Heart Diseases, Jan Mikulicz Radecki University Hospital in Wroclaw (USK), with a primary diagnosis of AHF. USK is a tertiary referral cardiology center with a heart transplantation program. Screening and enrollment were performed routinely by trained study personnel during the study period. Patients were enrolled in a non-consecutive manner because enrollment depended on the availability of study personnel, the possibility of obtaining written informed consent, and the feasibility of blood sampling according to the predefined study protocol. No preselection was made based on clinical phenotype, HF etiology, left ventricular ejection fraction, or expected metabolomic/lipidomic profile. Adult patients (>18 years) hospitalized on an unplanned basis for AHF with signs and symptoms of congestion were eligible if they were admitted within ≤48 hours, had N-terminal pro-B-type natriuretic peptide >2000 pg/mL on admission, and provided written informed consent. Key exclusion criteria comprised cardiogenic shock, acute coronary syndrome, acute pulmonary embolism, acute aortic syndrome, end-stage chronic kidney disease (estimated glomerular filtration rate <15 mL/min/1.73 m² prior to admission), advanced non-cardiac disease with life expectancy <6 months, and high risk of non-adherence to study procedures or follow-up. Patients were classified as *de novo* AHF or exacerbation of pre-existing HF (WHF) based on comprehensive clinical evaluation and mandatory verification of all available medical records by an experienced cardiologist.

Before laboratory assessment, a ≥6-hour washout period from the last loop diuretic dose was ensured. Diuretic therapy was administered according to standard clinical practice without

investigator interference. Blood samples were collected on the first morning after admission and again 24 hours later. For metabolomic and lipidomic analyses, serum samples obtained from the first collection were used and stored at −80°C in the institutional research laboratory until further processing. The registry protocol was approved by the local ethics committee (Bioethics Committee, Wroclaw Medical University), and all patients provided written informed consent. Patients were followed for 12 months, with structured telephone interviews conducted every 3 months. We analyzed the following outcomes: all-cause mortality, HF-related mortality, and rehospitalization due to HF.

Metabolomic and lipidomic profiling

Metabolomic and lipidomic profiling was performed at the Omics Research Center, Wroclaw Medical University, using an untargeted high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) workflow with in-house optimized sample preparation, acquisition, and data-processing procedures. Full details regarding chemicals, sample preparation, chromatographic separation, mass spectrometry acquisition, feature extraction, compound annotation, and pathway analysis are provided in the online Supplementary Methods.

Statistical analysis

Clinical characteristics were summarized as mean (SD), median (IQR), or number (percentage), as appropriate. The normality of quantitative variables was assessed using the Kolmogorov–Smirnov test and visual inspection of distributions. Between-group comparisons between patients with *de novo* AHF and WHF were performed using the Student t test or the Mann–Whitney U test for continuous variables, as appropriate, and the Pearson chi-square test for categorical variables. For omics data, triplicate mass spectrometry measurements were averaged for each study participant, and variables with no measurable signal or those not suitable as biomarkers were excluded before modeling. Metabolomic and lipidomic between-

group comparisons were performed using the Mann–Whitney U test. Multivariable logistic regression models were used to identify metabolomic and lipidomic features associated with WHF, and results are reported as odds ratios (ORs) with 95% confidence intervals (CIs). Model assumptions, feature selection, multicollinearity assessment, PCA-based sensitivity analyses, and model performance assessment are described in detail in the Supplementary Methods. Associations between metabolites and 12-month outcomes were explored using Cox proportional hazards models with a complete-case approach; hazard ratios (HRs) are expressed per 1-IQR increase in metabolite concentration. Three exploratory outcome-modeling strategies were applied: clinical variables forced into the model with metabolites as candidates, metabolites only, and clinical variables and metabolites competing for inclusion. Bootstrap CIs were calculated for the final Cox models. Additional outcome-modeling results for HF-related mortality and HF rehospitalization are provided in the Supplementary Methods. Statistical inference was based on $\alpha = 0.05$. Analyses were performed using Statistica 13.3 and R software, with package details provided in the Supplementary Methods.

RESULTS

Baseline characteristics of examined HFH patients

Data from 274 patients with AHF were analyzed, of whom 90 (33%) were classified as having *de novo* AHF. Detailed clinical data were missing for one patient in each group (*de novo* and WHF). A ‘wet and warm’ hemodynamic profile predominated at presentation, occurring in 96% of patients. Patients with pre-existing HF (67%) were older, more frequently had coronary artery disease as the primary underlying etiology, and presented with slightly higher LVEF despite comparable left ventricular end-diastolic diameter. Regarding cardiac biomarkers, including those reflecting neurohormonal activation, both groups were comparable. Patients

with pre-existing HF had, however, a significantly higher burden of major comorbidities, including chronic kidney disease and diabetes (Table 3).

Metabolomic data

Univariable analyses according to pre-existing and *de novo* heart failure

In *de novo* AHF, higher serum levels of compounds associated with a pro-inflammatory and pro-apoptotic state, oxidative stress, and neurohormonal signalling (compared with WHF) were identified: SM(d18:0/24:1(15Z)(OH)) ($P < 0.001$), Cer(d18:1/22:0) ($P < 0.001$), Angiotensin(1-7) ($P < 0.001$), SM(d18:1/24:1(15Z)) ($P = 0.001$), N-Acetylcochinol-O-phosphate ($P = 0.02$), uric acid lactate ($P = 0.02$) and Cer(d18:1/25:0) ($P = 0.04$). In WHF higher concentrations of the following compounds were detected potentially linked to a catabolic state, altered energy metabolism, and detoxification: 1-methylhistidine ($P = 0.002$), indacatherol-8-O-glucuronide ($P = 0.004$), glucose lactate acetate ($P = 0.008$), methylphosphonfluoridate ($P = 0.01$), N-acetyl-S-allylcysteine ($P = 0.02$), and glucuronic acid ($P = 0.03$).

Three compounds: dimethylarsinic acid ($P = 0.02$), LysoPC(P-16:0/0:0) ($P = 0.02$), and inulin ($P = 0.03$), showed different detection patterns between the two subgroups, and were associated with many zero values (undetectable level) in one or both groups. For dimethylarsinic acid, at least 50% of the records associated with pre-existing HF were zeroes, unlike the other group, where the median value was 19.10. In case of LysoPC(P-16:0/0:0) both groups showed a median of 0, although in the pre-existing HF subgroup, the count of records with zero values was significantly higher, since the 3rd quartile in this group was also 0. While at least 50% of values associated with inulin were 0 in the pre-existing HF, the *de novo* group showed a median value of 75.24.

Key factors associated with the odds of pre-existing heart failure – insights from multivariable modeling

The multivariable model (**Table 1**) was based on the values of: Cer(d18: 1/22:0) (sphingolipid involved in cell signaling), arginine (precursor of nitric oxide contributing to vascular homeostatis), 1-methylhistidine (marker of catabolism), N-acetyl-S-allylcysteine (possible marker of detoxification processes), and allophanic acid (end-product of nitrogen metabolism). If the values of the values of the independent variables were hypothetically 0, the odds of pre-existing HF would be 2.29 (P = 0.002), accounting for the probability equal to 0.6960. These baseline odds would be decreased by 4.50% with every 1000-unit increase in Cer(d18: 1/22:0) (OR = 0.955, 95% CI: 0.932 – 0.980). Each 100-unit increase in arginine would lower the odds by 1.40% (OR = 0.986, 95% CI: 0.977 – 0.995). Additionally, the odds would be lowered by 1.30% for every 10% increase in allophanic acid (OR = 0.987, 95% CI: 0.976–0.998). The odds would increase alongside with 1-methylhistidine (by 7.80%, OR = 1.078, 95% CI: 1.037 – 1.121) and N-acetyl-S-allylcysteine (by 1.40%, OR = 1.014, 95% CI: 1.001 – 1.027).

Lipidomic data in heart failure hospitalization patients with *de novo* vs. pre-existing heart failure

Univariable differences in values of all measured metabolites

Patients with pre-existing HF had higher serum levels of: GPEtn(17:2/18:1) (P = 0.01), GPGro(23:0/20:5) (P = 0.03), GPA(11:0/20:2) (P = 0.03), and SQDG(18:1/20:0) (P = 0.04). Lower serum levels in pre-existing HF were observed for: GPSer(17:1/26:2) (P = 0.007), GPEtn(17:1/26:2) (P = 0.03), and SM(d15:0/24:4) (P = 0.03). In case of GPIns(18:4/16:0), although most individuals showed a lack of this metabolite, very high values were more frequently observed in individuals with pre-existing HF (P = 0.003).

Multivariable association between the metabolites and the odds of pre-existing HF

Multivariable analyses confirmed selected univariable findings, including associations for lipids of (sub)cellular membranes such as GPGro(23:0/20:5) and GPEtn(17:1/26:2). They also revealed independent predictive signals from lipids with less well-defined roles in human (patho)physiology—DGDG(11:0/26:2), MG(18:1/0:0/0:0), MGDG(14:0/24:0)—as well as from the GPEtn principal component, which reflects phospholipids involved in inflammation and oxidative stress balance.

In Model 1 (Table 2), a 1 SD increase in DGDG(11:0/26:2) was associated with a 52% increase in the odds of pre-existing HF ($P = 0.007$). In contrast, a 1 SD increase in MG(18:1/0:0/0:0) and MGDG(14:0/24:0) was associated with a 39.7% and 54.4% decrease in the odds, respectively (both $P < 0.001$). The GPEtn principal component was associated with a 28.2% decrease in odds per unit increase. In Model 2 (Table 2), a 1 SD increase in GPGro(23:0/20:5) was associated with a 58% increase in the odds of pre-existing HF ($P = 0.006$), whereas MG(18:1/0:0/0:0) and GPEtn(17:1/26:2) were associated with a 42.2% and 36.5% decrease in odds, respectively (both $P < 0.001$). Both models demonstrated comparable classification performance. Additional information on feature scaling is provided in Table 2.

Metabolomics and clinical outcomes

During 12-month follow-up period 53 patients died (19%), of whom 48 due to HF (18%). Rehospitalization for HF occurred in 30% of subjects, but this outcome was available only for 253 subjects (93% of the whole cohort). All-cause mortality was comparable between *de novo* AHF and WHF ($P = 0.10$ for the log-rank test), as well as HF-related mortality ($P = 0.23$) and rehospitalization for HF ($P = 0.14$).

Results of the exploratory metabolomics analyses for all-cause mortality are summarized in Table 4, presenting hazard ratios (HRs) per 1 interquartile range (IQR) increase in metabolite concentration, with both model-based (Wald) confidence intervals and bootstrap confidence

intervals. Across the three modeling strategies, several metabolites showed consistent associations with all-cause mortality. In Strategy 1 (clinical variables forced-in, metabolites as candidates), higher levels of glucose–lactate–acetate (HR per IQR 1.04, 95% CI 1.02–1.06; bootstrap CI 0.76–1.19) and nitrosyl acetate (HR 1.04, 95% CI 1.01–1.06; bootstrap CI 0.92–1.08) were associated with increased risk. Both metabolites remained selected in Strategy 3, where clinical variables and metabolites competed for inclusion, with similar effect sizes (HR 1.03–1.04 per IQR), suggesting that these associations were not solely driven by adjustment for clinical covariates. A robust inverse association was observed for 1,2-ethanedisulfonic acid, which was selected in both Strategy 1 (HR 0.20, 95% CI 0.07–0.59; bootstrap CI 0.02–0.51) and Strategy 3 (HR 0.26, 95% CI 0.11–0.65; bootstrap CI 0.07–0.68). This metabolite demonstrated one of the largest effect sizes across strategies, indicating a strong signal in this exploratory setting. Phosphothiophosphoric acid adenylate ester showed a consistent positive association with mortality in Strategy 1 and Strategy 3 (HR ≈1.10 per IQR), while allophanic acid was selected only in Strategy 1 (HR 1.45 per IQR), suggesting sensitivity of this signal to model specification.

In Strategy 2 (metabolites only), additional metabolites emerged, including raffinose (HR 1.60 per IQR), 3-oxalomalic acid (HR 1.16 per IQR), and xanthine (HR 0.68 per IQR). These associations were not retained when clinical variables were allowed to enter the model, indicating potential confounding or shared prognostic information with clinical covariates.

Finally, in Strategy 3, several metabolites (N-acetylcochinol-O-phosphate, α-hydroxysalmeterol, and 2-phosphophloretin) were selected only when competing directly with clinical variables, highlighting metabolites whose prognostic signal was sufficiently strong to be retained alongside established clinical predictors. Across metabolites with small IQRs in raw units (e.g., glutathione–bicarbonate), effect sizes were numerically close to unity (HR ≈1.001

per IQR) but consistent across strategies, underscoring the importance of interpreting HRs in the context of the reported IQR scaling rather than absolute HR magnitude.

Analyses regarding HF-related mortality and rehospitalization for HF are presented in the Supplementary Methods.

DISCUSSION

Current research demonstrates that circulating metabolomic and lipidomic profiles differ significantly between patients hospitalized for HF who are clinically classified as *de novo* versus pre-existing disease (WHF). Using omics-based methodology, we identified that these two subgroups exhibit distinct biomarker signatures across a broad spectrum of metabolites—including the metabolomic domain (sphingolipids, RAAS peptides, purine metabolites, amino acid derivatives, glucuronidated compounds, organic acids, and exogenous or environmental compounds). Compounds comprising the lipidomic domain (phospholipids, glycolipids, lysophospholipids, phosphatidylinositols, and monoacylglycerols) that are associated with membrane structure, cellular signaling, mitochondrial function, and inflammatory modulation, were also different between the study groups. Summarizing specific results, *de novo* AHF was characterized by a metabolomic profile indicative of a pro-inflammatory state and neurohormonal activation, whereas patients hospitalized for exacerbated pre-existing disease presented with combined metabolomic and lipidomic signatures of abnormal energy metabolism, a catabolic state, and cellular injury. Based on the obtained results, it can be cautiously hypothesized that *de novo* HF represents a pronounced inflammatory and neurohormonal “shock” to the organism. As the disease progresses, there appears to be an intensification of catabolic processes—potentially involving cellular degradation—along with disordered energy metabolism.

Notably, the energy-related signal derived from peripheral blood may be of particular relevance, as previous studies have largely lacked data on systemic energetic alterations beyond specific tissues such as the heart or skeletal muscle. It has been well established for decades through experimental and *in vitro* studies that HF is characterized by disturbed cardiac energetics and specific metabolic reprogramming at the myocardial tissue level [18-21]. However, the clinical translation of these findings remains limited due to the difficulties in assessing the metabolism of myocardial and other organs *in vivo* in patients. Omics-based studies now suggest that for HF research relevant information on disease mechanisms, metabolism (energetics, oxidative stress) and prognosis can, to some extent, be obtained from multi-compound analyses of peripheral blood samples. However, it is worth noting that the clinical interpretation of the nature, magnitude, and direction of detected changes remains uncertain, particularly in relation to cellular processes in the heart [21-25]. Preliminary metabolomic and lipidomic profiling of peripheral blood in patients with HF may offer a less invasive method (compared to e.g., tissue specimens analysis following an endomyocardial biopsy) into systemic metabolic (mal?)adaptations, complementing more routinely available data on neurohormonal activation and cardiorenal signaling [26,27]. It is, in fact, neurohormonal activation and disturbances along the heart–kidney axis—not energy metabolism per se—that constitute the cornerstone of contemporary HF therapy. Except for intravenous iron therapy [28], the issue of energy deficiency—despite the heart often being described as “an engine out of fuel” [29]—has not been adequately addressed in clinical trials in HF [30,31]. As our study indicates, a more extended disease history may translate into detectable circulating signatures of metabolic and lipidomic alterations, potentially enabling novel diagnostic approaches to estimate disease duration (and potentially progression), an aspect of substantial clinical relevance. Moreover, improved characterization of energy-related pathophysiology at both systemic and organ-

specific levels could enhance prognostication not only in overt AHF but also in earlier stages of the disease.

Omics-based approaches represent a relatively recent but rapidly expanding domain of translational research in HF, with growing evidence that comprehensive profiling of circulating metabolites can support clinically relevant decision-making processes [32-35]. Simultaneous interrogation of large panels of metabolites offers a cost-effective strategy for identifying novel biomarkers with potential diagnostic, prognostic, and even therapeutic implications. Beyond risk stratification for incident disease (e.g. in patients at risk of HF, i.e. corresponding with the universal definition of HF stage A or B) or adverse outcomes in established HF (stages C-D), pathway-level insights may uncover mechanistic targets amenable to therapeutic intervention [23, 32, 36-38]. Particularly compelling are emerging concepts of drug discovery and repurposing guided by omics datasets integrated with advanced computational and artificial intelligence–based analytical frameworks, an area that is likely to yield further breakthroughs in the near future [39]. At present, however, much of the field remains focused on refining prognostic assessment by integrating omics-derived signatures with established clinical and classic biomarker-based risk predictors. Indeed, already several years ago, distinct metabolite profiles in patients with HF and reduced LVEF were shown to translate into markedly worse long-term outcomes [40].

In this context, our analyses identify a set of metabolites showing reproducible associations with all-cause mortality across complementary exploratory modeling strategies. Given the post-selection nature of inference and the exploratory intent of the present study, these findings should be interpreted as hypothesis-generating and warrant confirmation in independent, externally validated cohorts. Nevertheless, certain biologically meaningful comparisons can be made. Several pathway-level signals identified in our study align with previously reported metabolomic determinants of prognosis in HF. Alterations in amino acid turnover and energy-

related metabolites—features that in our cohort differentiated *de novo* AHF and WHF patients—have been linked to adverse outcomes in HF [41, 42]. In this framework, our mortality analyses reinforce the concept that systemic metabolic stress constitutes a clinically meaningful axis in the pathophysiology of progressive HF. Recent systematic analyses have demonstrated that recurrent classes of metabolites—particularly amino acids and their derivatives—are consistently associated with adverse outcomes in HF across independent cohorts [43]. The convergence between these aggregated findings and our own observations at the pathway level enhances the biological plausibility and clinical interpretability of the present results, while simultaneously underscoring the need for the next step of incorporation into practical risk stratification tools. In addition to the metabolomic findings discussed above, our study also revealed distinct lipidomic patterns—particularly involving glycerophospholipids and sphingolipids—that clearly differentiated HF phenotypes. Although we did not specifically assess lipidomic markers in relation to mortality outcomes, emerging data from observational AHF cohorts [44] suggest that circulating lipidomic profiles, including lysophospholipids, may carry prognostic relevance. Taken together, these observations indicate that lipidomic remodeling constitutes a promising diagnostic area for future studies.

It should be emphasized that prior guideline-directed pharmacological treatment in patients with pre-existing HF may have influenced selected metabolic pathways and could have contributed to inter-group differences observed in our study. In particular, long-term exposure to agents modulating the renin–angiotensin–aldosterone system and sympathetic signaling, as well as sodium-glucose cotransporter type 2 inhibitors, may affect circulating biomarkers and certain metabolic signals [45,46]. However, the metabolomic and lipidomic alterations identified in our analysis extend beyond the primary pharmacological targets of these therapies and involve a broad range of pathways, including sphingolipid metabolism, amino acid derivatives, catabolic markers, and membrane-associated lipid species. While neurohormonal

and metabolic therapies can exert indirect systemic effects, they would not be expected to uniformly account for the overall pattern of differences observed across multiple, biologically distinct pathways [47]. Importantly, major biomarkers assessed at admission (NT-proBNP, C-reactive protein) were comparable between groups, arguing against a simple or exclusively treatment-driven explanation of the findings. Therefore, although prior therapy may have shaped the overall metabolic milieu to some extent, it is unlikely to fully explain the distinct omics profiles observed between *de novo* and worsening HF.

STUDY LIMITATIONS

This study has certain limitations that are specific to the research topic. First, we did not include all consecutive patients, which may affect the generalizability of the presented analyses. The wide range of left ventricular ejection fraction values [38 (15) %], indicating substantial variability across the EF spectrum, together with the fact that only approximately one-third of patients had ischemic HF etiology, support the view that the study population was phenotypically and aetiologically heterogeneous and therefore warrant cautious interpretation of the results. Certain metabolites present at low concentrations may have limited stability and durability under conditions of blood sample freezing at –80°C. Finally, results obtained from peripheral blood samples may not fully reflect organ-specific processes or provide insight into the metabolic and (patho)physiological roles of lipid-related compounds in individual organs, such as the heart, kidneys, or liver.

CONCLUSIONS

Our study demonstrates that *de novo* AHF and WHF are characterized by divergent metabo-lipidomic signatures, reflecting differences in inflammatory activation, neurohormonal signaling, energy metabolism, and cellular stress pathways. These results provide pathophysiological insight into the heterogeneity of AHF and suggest that integrated omics profiling may help characterize the biological stage of the disease at hospital presentation - an

often unresolved question for clinicians that may directly influence early diagnostic and therapeutic decisions. Future studies are warranted to determine whether these omics-derived signatures can be translated into clinically actionable diagnostic and risk stratification tools.

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CONTRIBUTION STATEMENT

Study design - MF, PP, JB

Data acquisition - MG, GI, RZ, PG, PF, GM, LL, MF

Statistical analysis - MT, PF, GM, LL, MF

Data interpretation - MT, MF, JB

Manuscript drafting - MT

Critical revision of the manuscript - MG, GI, RZ, PG, PF, GM, LL, MF, PP, JB

Supervision - MF, PP, JB

Revisions - MT, MF, JB, LL

All authors contributed to the article and approved the final version of the manuscript.

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Table 1. Multivariable classification model (outcome: pre-existing HF) based on metabolomic data

Feature	Value increment	βi	βi SE	Wald statistic	βi - 95% CI	βi 95% CI	*P*	OR	OR - 95% CI	OR 95% CI
Intercept	-	0.829	0.266	9.719	0.308	1.350	0.002	2.290	1.360	3.856
Cer(d18:1/22:0)	1000 units	-0.046	0.013	12.348	-0.071	-0.020	<0.001	0.955	0.932	0.980
Arginine	100 units	-0.014	0.005	8.840	-0.023	-0.005	0.003	0.986	0.977	0.995
1_methylhistidine	100 units	0.075	0.020	14.209	0.036	0.114	<0.001	1.078	1.037	1.121
N-Acetyl-S-allylcysteine	100 units	0.014	0.007	4.382	0.001	0.027	0.04	1.014	1.001	1.027
Allophanic_acid_log1.1(v+1)	10%	-0.013	0.006	5.493	-0.025	-0.002	0.02	0.987	0.976	0.998
H-L test *P* = 0.2070, AICc = 288.68, D = 276.36, Pseudo-R2 = 0.2964, learning AUC: 0.764 (0.030), testing AUC: 0.739 (0.032)										

The 'OR' column features odds ratios with an exception for the ‘Intercept’ row which reflects the baseline odds: baseline odds (in case of the intercept), odds ratios (in case of effects)

Table 2. Multivariable classification model (outcome: pre-existing HF) based on lipidomic data

Model 1: GLM (distribution = binomial, link = logit), stepwise exclusion/re-inclusion									
10-fold CV learning AUC: 0.695 (0.034), testing AUC: 0.657 (0.036)									
AIC = 322.68									
	βi	βi SE	Wald statistic	βi - 95% CI	βi 95% CI	p	OR	OR - 95% CI	OR 95% CI
Intercept	0.775	0.139	30.898	0.502	1.048	<0.001	2.170	1.651	2.852
MG(18:1/0:0/0:0)	-0.506	0.156	10.496	-0.813	-0.200	0.001	0.603	0.444	0.819
GPEtn_PC	-0.331	0.137	5.870	-0.598	-0.063	0.02	0.718	0.550	0.939
MGDG(14:0/24:0)	-0.786	0.204	14.865	-1.185	-0.386	<0.001	0.456	0.306	0.680
DGDG(11:0/26:2)	0.419	0.156	7.253	0.114	0.724	0.007	1.520	1.121	2.062
Additional information									
Feature	Scale/other information								
Intercept	For the following values of independent variables: 920188 MG(18:1/0:0/0:0), 22319450 GPEtn(20:4/18:0), 15089420								

	GPEtn(20:3/18:1), 7142291 GPEtn(22:6/18:1), 1272287 MGDG(14:0/24:0), 94180 DGDG(11:0/26:2)								
MG(18:1/0:0/0:0)	1 unit = 1 SD ≈ 1234725 raw units								
GPEtn_PC	Value ≈ -14498855*GPEtn(20:4/18:0) - 7936758*GPEtn(20:3/18:1) - 4284724GPEtn(22:6/18:1) (in raw units)								
MGDG(14:0/24:0)	1 unit = 1 SD ≈ 820425 raw units								
DGDG(11:0/26:2)	1 unit = 1 SD ≈ 51231 raw units								
Model 2: GLM (distribution = binomial, link = logit), stepwise inclusion/exclusion									
10-fold CV learning AUC: 0.694 (0.034), testing AUC: 0.659 (0.036)									
AIC = 322.54									
	βi	βi SE	Wald statistic	βi - 95% CI	βi 95% CI	p	OR	OR - 95% CI	OR 95% CI
Intercept	0.775	0.140	30.883	0.502	1.049	<0.001	2.172	1.652	2.855
MG(18:1/0:0/0:0)	-0.548	0.149	13.515	-0.840	-0.256	<0.001	0.578	0.432	0.774
GPEtn(17:1/26:2)	-0.454	0.138	10.831	-0.724	-0.183	0.001	0.635	0.485	0.832
GPGro(23:0/20:5)	0.456	0.164	7.703	0.134	0.778	0.006	1.578	1.143	2.177
Additional information									
Feature	Scale/other information								

Intercept	For the following values of independent variables: 920189 MG(18:1/0:0/0:0), 319861 GPEtn(17:1/26:2), 191428 GPGro(23:0/20:5)
MG(18:1/0:0/0:0)	1 unit = 1 SD ≈ 1234725 raw units
GPEtn(17:1/26:2)	1 unit = 1 SD ≈ 224045
GPGro(23:0/20:5)	1 unit = 1 SD ≈ 106804

Table 3. Clinical characteristics of patients hospitalized for heart failure according to acute heart failure status - *de novo* vs. worsening heart failure.

Clinical parameter	All patients with AHF (n=272[a])	*De novo* AHF (n=89)	WHF (n=183)	*P*-value
Age, years	68 (14)	64 (17)	70 (13)	0.003
Female sex, %	86 (32%)	27 (30%)	59 (32%)	0.75
New York Heart Association class on admission II/III/IV, n (%)	3/88/168 (1/33/63%)	2/35/48 (2/41/56%)	1/53/120 (1/30/69%)	0.09
Primary ischaemic aetiology, n (%)	96 (35%)	18 (20%)	78 (43%)	<0.001
Haemodynamic profile on admission - wet and warm, n (%)	260 (96%)	86 (97%)	174 (95%)	0.87
Echocardiography				
Left ventricular end-diastolic diameter, mm	60 (51-70)	61 (51-67)	59 (51-67)	0.72
Left ventricular ejection fraction, %	38 (15)	37 (16)	39 (15)	0.04
TAPSE, mm	16 (14-20)	18 (15-20)	16 (13-19)	0.007

Pharmacotherapy prior to admission				
Beta-blocker, n (%)	195 (72%)	35 (40%)	160 (87%)	<0.001
ARNI, n (%)	24 (9%)	0 (0%)	24 (13%)	<0.001
ACE-I/ARB, n (%)	139 (51%)	36 (41%)	103 (56%)	0.02
MRA, n (%)	116 (43%)	12 (14%)	104 (57%)	<0.001
SGLT2 inhibitor, n (%)	62 (23%)	2 (2%)	60 (33%)	<0.001
Loop diuretic, n (%)	158 (58%)	19 (22%)	139 (76%)	<0.001
Oral anticoagulant, n (%)	129 (48%)	16 (18%)	113 (62%)	<0.001
Laboratory tests				
Plasma NT-proBNP, ng/L	6872 (3720-12457)	6293 (3535-10554)	8024 (3826-14756)	0.06
High-sensitive cardiac troponin type I, pg/mL	27 (15-64)	27 (16-65)	28 (14-63)	0.84
eGFR, mL/min/1.73 m^2	62 (27)	75 (25)	57 (26)	<0.001
High-sensitive CRP, mg/L	10.2 (4.2-23.0)	8.7 (4.6-21.9)	10.5 (3.9-23.6)	0.94
AST, U/L	28 (22-39)	31 (23-42)	28 (22-38)	0.27
ALT, U/L	25 (16-39)	31 (18-46)	23 (15-37)	0.01
GGTP, U/L	77 (39-152)	72 (37-134)	82 (45-155)	0.25
Sodium, mmol/L	140 (8)	141 (4)	139 (9)	0.17
Potassium, mmol/L	4.3 (1.6)	4.1 (0.6)	4.3 (1.9)	0.31
Haemoglobin (g/dL)	12.6 (2.2)	13.1 (2.1)	12.4 (2.1)	0.02
Hospitalization details				
In-hospital death, n (%)	17 (7%)	2 (2%)	15 (9%)	0.06
Intensive cardiac care admission, n (%)	38 (15%)	10 (12%)	28 (16%)	0.42

Diuretic i.v., n (%)	268 (99%)	86 (97%)	182 (99%)	0.07
Vasodilator i.v., n (%)	104 (39%)	34 (38%)	70 (39%)	0.89
Inotropes, n (%)	47 (17)	6 (7%)	41 (22%)	0.001
Major comorbidities				
Ischaemic heart disease, n (%)	120 (44%)	17 (19%)	103 (57%)	<0.001
Arterial hypertension, n (%)	209 (77%)	57 (64%)	152 (83%)	<0.001
Atrial fibrillation, n (%)	176 (65%)	42 (47%)	134 (73%)	<0.001
Diabetes mellitus, n (%)	126 (46%)	24 (27%)	102 (56%)	<0.001
Chronic obstructive pulmonary disease/asthma, n (%)	46 (17%)	11 (12%)	35 (19%)	0.16
Smoking, n (%)	94 (35%)	38 (43%)	56 (31%)	0.049

Data are presented as a mean (standard deviation), a median (with lower and upper quartiles), or a number (with percentage), as appropriate.

Abbreviations: ACE-I, angiotensin-converting enzyme inhibitor; AHF, acute heart failure; ALT, alanine aminotransferase; ARNI, angiotensin receptor–neprilysin inhibitor; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; LDL, low-density lipoprotein; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SGLT2, sodium–glucose cotransporter type 2; WHF, worsening heart failure.

SI conversion factors: to convert C-reactive protein to nmol/l, multiply by 9.524; NT-proBNP to ng/l, multiply by 1; hemoglobin to g/l, multiply by 10.

[a] - in the full cohort (n=274), detailed clinical data were unavailable for one *de novo* AHF patient and one WHF patient.

Table 4. Results from exploratory modelling in context of all-cause death

Metabolite	IQR (raw)	STRATEGY 1 (HR [CI] {boot CI})	STRATEGY 2 (HR [CI] {boot CI})	STRATEGY 3 (HR [CI] {boot CI})
Glucose lactate acetate	981.6	1.04 [1.02–1.06] {0.76–1.19}	—	1.03 [1.01–1.04] {0.88–1.13}
Nitrosyl acetate	850.5	1.04 [1.01–1.06] {0.92–1.08}	—	1.03 [1.01–1.06] {0.83–1.06}
Glutathione bicarbonate	4.42	1.001 [1.000–1.002] {1.000–1.004}	1.001 [1.000–1.002] {1.000–1.002}	—
Phosphothiophosphoric acid adenylate ester	476.1	1.10 [1.04–1.17] {1.00–1.23}	—	1.10 [1.04–1.16] {1.02–1.17}
1,2-Ethanedisulfonic acid	2068	0.20 [0.07–0.59] {0.02–0.51}	—	0.26 [0.11–0.65] {0.07–0.68}
Allophanic acid	415.4	1.45 [1.12–1.87] {0.89–2.23}	—	—
Raffinose	10.78	—	1.60 [1.31–1.95] {0.97–2.08}	—
3-Oxalomalic acid	146.3	—	1.16 [1.07–1.25] {1.09–1.38}	—
Xanthine	5.39	—	0.68 [0.50–0.92] {0.46–0.87}	—
N-Acetylcochinol-O-phosphate	7027	—	—	0.45 [0.24–0.84] {0.21–0.67}

α-Hydroxysalmeterol	32853	—	—	0.69 [0.51–0.95] {0.41–0.83}
2-Phosphophloretin	29213	—	—	1.56 [1.13–2.15] {0.75–2.34}

Hazard ratios (HRs) are expressed per 1 interquartile range (IQR) increase in metabolite concentration, where the IQR is calculated in raw units and reported in the table. Wald 95% confidence intervals (CI) are derived from the final Cox model after bidirectional stepwise selection. Bootstrap confidence intervals (boot CI) are based on resampling with replacement using the fixed final model formula. Dashes (—) indicate that the metabolite was not selected in the final model under the given strategy. Results are exploratory and subject to post-selection inference. For details, see the 'Methods' section.

SHORT TITLE: Metabolomic and lipidomic profiles in AHF