Plasma protein biomarkers in relation to cardiac hemodynamics and across heart failure stages

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DOCTORAL DISSERTATION
Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on May 8th, 2026, at 9 am, Belfragesalen, BMC, Lund.

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Title: Plasma protein biomarkers in relation to cardiac hemodynamics and across heart failure stages

Abstract:

Background: Heart failure (HF) is a heterogeneous clinical syndrome characterised by progressive alterations in cardiac function, central hemodynamics, and systemic consequences. Disruptions in filling pressures, cardiac output, vascular function, and tissue perfusion are key determinants of symptoms and prognosis. Although circulating biomarkers, particularly N-terminal pro-B-type natriuretic peptide (NT-proBNP), are widely used in HF care, existing markers provide limited information across disease stages and hemodynamic phenotypes. Advances in affinity-based plasma proteomics has enabled large-scale characterisation of circulating proteins and offer novel opportunities to identify biomarkers reflecting disease progression, congestion, and central cardiovascular function.

Aims: The overall aim of this thesis was to comprehensively evaluate novel and existing biomarkers across heart failure stages and in relation to central hemodynamics to identify potential novel biomarkers that may be clinically useful. Specifically, the aims of the three studies were to: 1) Systematically assess molecular patterns of HF across key stages: decades before onset of symptoms, during manifest disease, advanced HF and after reversal of heart failure by heart transplantation. 2) Evaluate the diagnostic and prognostic performance of bioactive adrenomedullin (bio-ADM) in HF patients with congestion, in comparison to NT-proBNP and explore the potential of bio-ADM as a biomarker of systemic venous congestion and derive potential decision thresholds from invasive hemodynamic and population-based studies. 3) Identify a panel of circulating plasma proteins as a representation of central hemodynamics in HF, which may enable non-invasive hemodynamic monitoring and offer insights into the molecular mechanisms underlying HF progression.

Methods: Study I: Plasma proteomic profiling of >1300 proteins using an aptamer-based assay in study participants from three independent cohorts (population-based cohort, manifest HF cases, advanced HF cases before and after heart transplantation). Study II: Assessment of bio-ADM using a sandwich immunoassay in three independent cohorts (community-based cohort, HF patients undergoing central hemodynamic assessment by cardiac catheterization, acute dyspnea cohort from emergency room including HF cases). Study III: Aptamer-based proteomic profiles were obtained in advanced HF patients serially before and after heart transplantation and related to serial hemodynamic measures from right heart catheterization. Associations were assessed using multivariable mixed-effects models and machine learning approaches to identify informative biomarkers and evaluate discriminatory performance.

Results: In study I, we identified circulating proteins associated with HF incidence, implicating immunological, complement, coagulation, natriuretic, and matrix remodelling pathways. We observed further divergence of these proteins from the general population in advanced HF, and partial regression after heart transplantation, and provide evidence that several of the proteins are likely to be of cardiac origin. In study II, we found that bio-ADM correlated with invasive measures of systemic venous congestion in patients with decompensated heart failure, discriminates elevated right atrial pressure, and associates with heart failure diagnosis and increased mortality independently of NT-proBNP, supporting its use as a clinical biomarker and providing a proposed decision cut-off. In study III, we identified 201 circulating proteins that significantly associated with central hemodynamic parameters, including filling pressures, ventricular workload, vascular resistance, and systemic oxygen utilization. Most proteins associated with multiple hemodynamic domains. Importantly, the most informative proteomic signatures demonstrated good discrimination for clinically relevant hemodynamic thresholds.

Conclusions: By application of plasma proteomic profiling, our results provide a comprehensive perspective of circulating proteins linked to heart failure development and progression over time. Furthermore, we identify plasma proteins that inform on congestion and central hemodynamics. We suggest that such biomarkers, notably including bio-ADM, may serve as non-invasive tools for phenotyping, monitoring, and precision-based management of heart failure.

Key words: Heart failure, biomarkers, hemodynamics, congestion, bioactive adrenomedullin, prognosis, proteomics

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MADE IN SWEDEN

To my husband Jan and my children Philip, Julia and Carl

"Det mesta är ännu ogjort. Underbara framtid."

-Ingvar Kamprad

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List of papers

This thesis is based on the following papers, referred to in the text by their Roman numerals.

I. **Egerstedt A**, Berntsson J, Smith ML, Gidlöf O, Nilsson R, Benson M, Wells QS, Celik S, Lejonberg C, Farrell L, Sinha S, Shen D, Lundgren J, Rådegran G, Ngo D, Engström G, Yang Q, Wang TJ, Gerszten RE, Smith JG. Profiling of the plasma proteome across different stages of human heart failure. Nature Commun 2019; 10: 5830.

II. **Egerstedt A,** Czuba T, Bronton K, Lejonberg C, Ruge T, Wessman T, Rådegran G, Schulte J, Hartmann O, Melander O, Smith JG. Bioactive adrenomedullin for assessment of venous congestion in heart failure. ESC Heart Failure 2022; 9: 3543–3555.

III. **Egerstedt A**, Czuba T, Gidlöf O, Lejonberg C, Lundgren J, Rådegran G, Gerszten R, Smith JG. Plasma proteomic representation of central hemodynamics over time in heart failure. Unpublished manuscript.

Author's contribution to the papers

I. First author. Responsible for collection and characterisation of data and contributed to design and interpretation of data analyses, conducted together with experienced bioinformaticians and statisticians. The manuscript was drafted by the supervisor with critical input from the author and co-authors. Revised and submitted together with supervisor.

II. First author. Responsible for data collection and led the data analysis with support from biostatistician. Drafting of the manuscript and served as corresponding author and revised it for publication.

III. First author. Responsible for design, data collection and led the data analysis with support from biostatistician and supervisor. Drafting of the manuscript and serves as corresponding author.

Abbreviations

ACC	American College of Cardiology
ACE	Angiotensin converting enzyme
ACEi	Angiotensin converting enzyme inhibitor
ADM	Adrenomedullin
ADYS	Acute dyspnea cohort
AHA	American Heart Association
AHF	Acute heart failure
ARB	Angiotensin receptor blocker
ARNI	Angiotensin receptor neprylisin inhibitor
AUROC	Area Under the Receiver Operating Characteristic Curve
Bio-ADM	Bioactive adrenomedullin
BSA	Body surface area
CI	Cardiac index
CO	Cardiac output
COPD	Chronic obstructive pulmonary disease
CRT	Cardiac-resynchronization therapy
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
ESC	European Society of Cardiology
HDL	High density lipoprotein
HF	Heart failure
HFmrEF	Heart failure with mildly reduced ejection fraction
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HF-RHC	Heart failure–right heart catheterization cohort
HR	Heart rate
ICD	Cardioverter-defibrillators
IHD	Ischemic heart disease
LDL	Low density lipoprotein
LV	Left ventricular
LVEF	Left ventricular ejection fraction
MAP	Mean arterial pressure
mPAP	Mean pulmonary arterial pressure

MPP	Malmö Preventive Project
mRAP	Mean right arterial pressure
NT-proBNP	N-terminal pro-B-type naturetic peptide
NYHA	New York Heart Association
PAWP	Pulmonary artery wedge pressure
PRA	Plasma renin activity
PVRI	Pulmonary vascular resistance index
RAP	Right arterial pressure
RAAS	Renin-angiotensin-aldosterone system
ROC	Receiver-operating characteristics
RV	Right ventricle
RVPd	Right ventricle pressure diastolic
SaO_2	Arterial oxygen saturation
SGLT2	Sodium-glucose linked transporter 2
SV	Stroke volume
SVI	Stroke volume index
SvO_2	Systemic venous oxygen saturation
SVR	Systemic vascular resistance

Introduction

Heart failure (HF) is a syndrome, which may include several manifestations, that results from failure of the heart to function as a pump which supports all tissues in the body with oxygenated blood. Clinical signs and symptoms of heart failure include shortness of breath, swelling of the legs and excessive fatigue. Heart failure often leads to failure of other vital organs like liver and kidney and confers profound impairments in health.

The lifetime risk of HF has increased over the last 25 years from 1 in 5 individuals to 1 in 3, with an estimated prevalence of 64 million people globally. HF is a growing public health issue that will challenge healthcare systems in many countries as several parts of the world experience a demographic shift towards a larger proportion of elderly in populations, further fuelled by higher incidence of risk factors like hypertension and obesity at younger ages compared to previous generations.

Fortunately, the treatment and preventive strategies for HF have developed rapidly over the past 25 years with the introduction of new pharmacological therapies such as SGLT2 and neprilysin inhibitors which have become part of the first-line treatment. Second-line therapy has been reinforced with implantable cardioverter-defibrillators (ICDs) and cardiac resynchronisation therapy (CRT) devices, while mechanical heart pumps and heart transplantation are available as last option for selected patients.

Despite such therapeutic improvements, mortality in patients with HF remains high and treatment is typically provided as “one size fits all” with limited consideration of the aetiology of the disease and individual response to therapy. Therefore, new and more personalised treatment strategies are warranted, where a precision-based approach integrating genetic, environmental and lifestyle factors may guide both evaluation and therapy.

Circulating biomarkers play a central role in precision medicine for their ability to serve as easily obtained measurable indicators on the state of the disease, response to treatment and as an indicator of biological processes. The systematic characterisation of circulating proteins across different stages of HF could provide pathophysiological insights and help identify therapeutic targets. Through identification of sensitive biomarkers, clinicians could then guide and select suitable personalised therapies for patients with HF.

This thesis aims to provide a broad picture of relevant biomarkers in different stages of heart failure, assess their correlation with hemodynamic measures, and evaluate the diagnostic and prognostic performance of specific proteins in relation to established biomarkers and specific clinical contexts. The three included studies span the continuum from discovery proteomics, validation of candidate biomarkers to deeper mechanistic insights. Together, they seek to advance the understanding of biomarker biology and improve risk stratification and clinical management in heart failure

Background

Heart failure: a common clinical syndrome resulting from cardiac dysfunction

HF is defined, based on international consensus, as a clinical syndrome consisting of typical symptoms (including breathlessness [dyspnea], ankle swelling and fatigue) that may be accompanied by clinical signs (including elevated jugular venous pressure, pulmonary crackles and peripheral oedema). This syndrome results from structural or functional abnormalities of the heart, manifesting as elevated intracardiac pressures or inadequate cardiac output at rest or during exercise.[1-3]

Clinical presentation and diagnosis

The clinical presentation of patients with HF is highly variable and depends on disease severity and underlying aetiology. Common symptoms include dyspnea, which initially manifests during exertion but may progress to occur at rest as disease severity and particularly as pulmonary congestion progresses. Orthopnea, characterised by breathlessness in the supine position, is frequently reported and is mitigated by upright repositioning. Fatigue is another common symptom, reflecting decreased cardiac output and impaired tissue perfusion.

On physical examination, signs of volume overload such as peripheral oedema and elevated jugular venous pressure are often evident. Pulmonary auscultation may reveal crackles resulting from pulmonary oedema, as well as the presence of abnormal heart sounds, such as an S3 or S4 gallop, indicative of increased ventricular filling pressures.[4] In advanced HF with pulmonary congestion, frothy or blood-tinged sputum may be present, although rales can be absent despite significant fluid accumulation. Other symptoms may include peripheral oedema, particularly in the lower limbs, chest pain, and cachexia. Cachexia is often related to hepatic congestion and bowel oedema in combination with impaired splanchnic circulation. Due to ascites or hepatic congestion, patients may also experience abdominal discomfort.

Accurate diagnosis relies not only on these clinical findings but is also based on objectives signs including cardiac circulating biomarker assessment (natriuretic peptides), imaging modalities such as ultrasound imaging of the heart

(echocardiography), and electrocardiography (ECG) (Figure 1). Timely and precise diagnosis is crucial for initiating appropriate therapeutic interventions aimed at improving patient prognosis and quality of life.

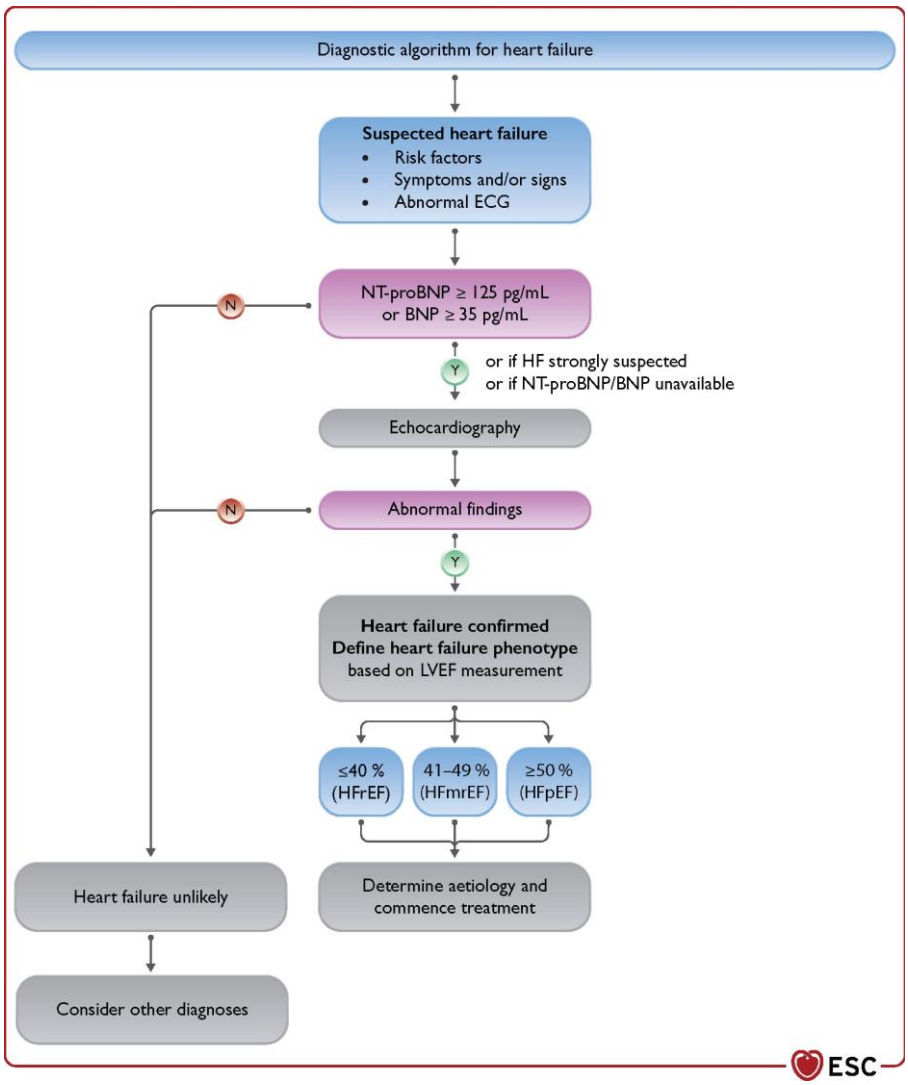

Figure 1. Diagnostic algorithm for heart failure. BNP, B-type natriuretic peptide; ECG, electrocardiogram; HFmrEF, heart failure with mildly reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B type natriuretic peptide. Reprinted with permission from Eur Heart J, Volume 42, Issue 36, 21, 2021.

Classification

Traditionally, HF classification has been based on the measurement of left ventricle ejection fraction (LVEF) as a measure of cardiac pumping efficiency. This classification originates from original treatment trials in HF that showed substantial improvements in outcomes for patients with LVEF ≤40%. According to recent ACC/AHA guidelines for HF from 2022 and ESC Guidelines from 2021, HF patients should be classified based on symptoms and LVEF into three subtypes (Table 1).[2,3]

Table 1. Definition of heart failure with reduced ejection fraction, mildly reduced ejection fraction and preserved ejection fraction

Type of HF	LVEF	Other parameter(s) needed for the definition
HFrEF	≤ 40%	Symptoms ± signs of HF[a]
HFmrHF	41% - 49%[b]	Symptoms ± signs of HF[a]
HFpEF	≥ 50%	Objective evidence of cardiac structural and/or functional abnormalities consistent with the presence of LV diastolic dysfunction/raised LV filling pressures, including raised natriuretic peptides[c]

a) Signs may not be present in the early stages of HF (especially in HFpEF) and in optimally treated patients. b) For the diagnosis of HFmrEF, the presence of other evidence of structural heart disease (e.g., increased left atrial size, LV hypertrophy or echocardiographic measures of impaired LV filling) makes the diagnosis more likely. c) For the diagnosis of HFpEF, the greater the number of abnormalities present, the higher the likelihood of HFpEF.

HF, heart failure; HFmrEF, heart failure with mildly reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LV, left ventricle; LVEF, left ventricular ejection fraction.

To describe the severity of HF, the New York Heart Association (NYHA) classification is used, based on functional capacity and severity of HF symptoms limiting physical activity (Table 2). A patient's NYHA class can change over time, representing a dynamic measure of functional status. However, even patients with mild symptoms may still be at high risk of death or hospitalisation, and more sensitive prognostic indicators are needed.[5]

Table 2. New York Heart Association functional classification based on severity of symptoms and physical activity

Class	Patient Symptoms
I	No limitation of physical activity. Ordinary physical activity does not cause undue breathlessness, fatigue or palpitations.
II	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in undue breathlessness, fatigue or palpitations.
III	Marked limitation of physical activity. Comfortable at rest but less than ordinary activity results undue breathlessness, fatigue or palpitations.
IV	Unable to carry on any physical activity without discomfort. Symptoms at rest can be present. If any physical activety is undertaken, discomfort is increased.

Incidence and prevalence

HF has an estimated prevalence of 64 million individuals worldwide according to the Global Burden of Disease study.[6] However, such estimates are limited by differences in availability of healthcare, detection and coding of the syndrome and nonadherence to the uniform staging and diagnosis of the disease. Although the age-adjusted incidence of HF has declined slightly over time, the prevalence is and will likely continue to increase due to changing demographics with an increasing proportion of elderly people in most populations, improved HF treatments and younger individuals presenting with HF risk factors at younger age than previous generations.[7,8]

The lifetime risk of being diagnosed with HF has risen from 1 in 5 to 1 in 3-4 in the last 25 years (Smith JG, manuscript under review).[9,10] In the United States: 1.9% to 2.6% of adults have HF and the prevalence of HF is expected to increase further, affecting >8 million people ≥18 years of age by 2030. This represents an increase in prevalence in the adult population of 2.4% in 2012 to 3.0% in 2030.[11]

In Europe, the incidence of HF in the population is about 3/1000 person-years and in the adult population, about 5/1000 person-years.[12,13] Prevalence differs among countries and has been estimated to 1-2% in the adult population (Figure 2).[14,15]

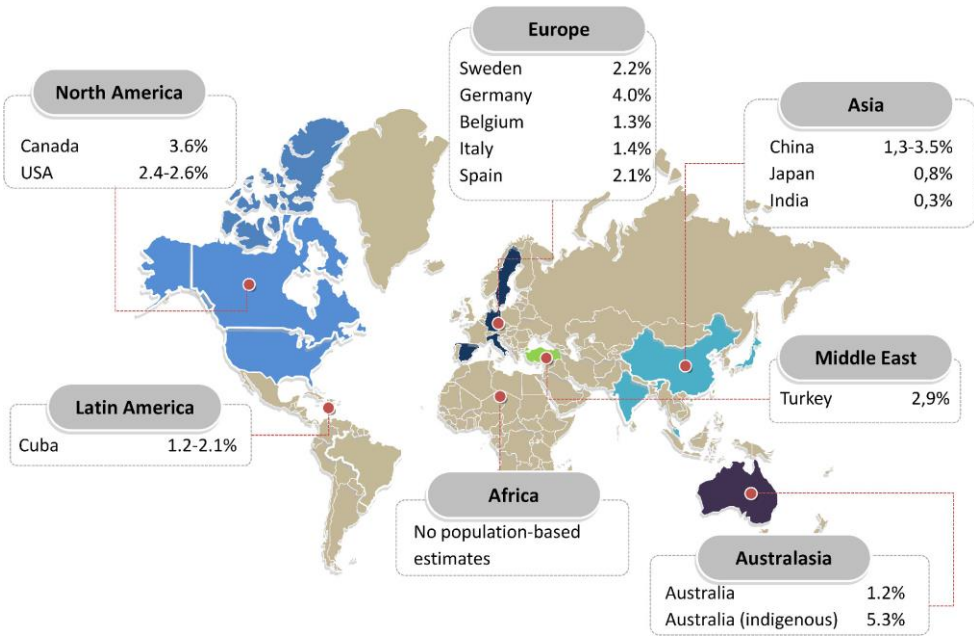

Figure 2. Prevalence of HF in population-based studies in percentage per region. Reprinted with permission from Eur J Heart Fail, 22: 1342-1356.

As most studies focus on clinically diagnosed HF patients, rather than screening of undiagnosed cases, the true prevalence is likely to be higher.[16] A meta-analysis based on echocardiographic screening studies in the general population found that the prevalence of 'all type' heart failure in developed countries is around 11.8% in those aged 65 years and over, including previously undiagnosed cases. This would translate to a calculated prevalence in the general population of 4.2% which is almost twice as high as reported prevalence based on registries containing only established cases.[17]

The difference between 4.2% and the around 2% reported in many studies, illustrates that even for such a severe syndrome as HF with substantial morbidity and mortality, may patients remain undiagnosed in over half of the cases. Especially heart failure with preserved ejection fraction (HFpEF) may be easily missed. Up to 76% of the unrecognised cases of heart failure represent HFpEF.[17] The causes for not recognising heart failure are multiple, including misclassification as chronic obstructive pulmonary disease, decreased physical fitness, ageing, or obesity due to a similarity in symptoms, and unavailability of echocardiography in primary care.[18]

Gender

HF is amongst the diseases with the most pronounced gender differences.[19,20] Differences between men and women are evident for HF aetiology, age at diagnosis, risk factors, biomarkers, pathophysiology, comorbidities, prevalence and clinical presentation.[20-25] Women below 75 years of age have a significantly lower incidence of heart failure compared to men.[26] In the Swedish HF registry, women accounted for only 29% of all HFrEF patients, but 55% of all HFpEF patients which is more commonly seen at higher age.[27] Several other community-based studies also report higher proportions of women in HFpEF cohorts and lower proportions of women in HFrEF cohorts.[20,28] In the Framingham Heart Study, female gender was associated with lower risk of HFrEF but not with increased risk of HFpEF.[29] The somewhat diverging results for HFpEF may result from differences in the population at risk including life expectancy.[30,31]Although efforts have been made to facilitate equal inclusion of men and women in HF clinical trials, women remain underrepresented.[32] This imbalance limits the generalisability of findings as sex specific differences in pathophysiology, clinical presentation and treatment response may be overlooked.

Risk factors and comorbidities

The development of HF is strongly linked to a range of well-established risk factors and comorbid conditions. Prominent among these are coronary heart disease, hypertension, diabetes mellitus, family history of cardiovascular disease, obesity,

chronic pulmonary diseases, and metabolic dysfunction. Additional risk factors include exposure to cardiotoxic agents such as cocaine or chemotherapeutic drugs with known cardiotoxic potential (e.g. anthracyclines including doxorubicin, and trastuzumab in breast cancer treatment),[33,34] as well as chronic inflammatory states, persistent infections and excessive alcohol intake.[35,36]

Early identification of individuals at elevated risk for incident HF represents a central strategy to reduce population-level incidence and a potentially cost-effective public health intervention. Particular emphasis should be placed on modifiable risk factors such as hypercholesterolemia, hypertension, tobacco use, obesity, excessive alcohol consumption, and diabetes mellitus.[37,38] A deeper understanding of the pathophysiological interplay among these factors may enable target preventive interventions, capable of modulating the complex biological pathways that precede the onset of HF (Figure 3).

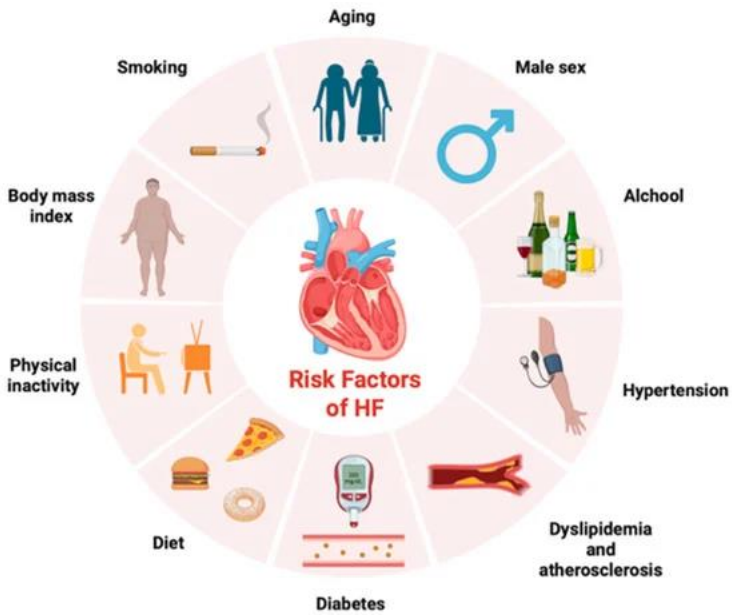

Figure 3. Risk factors for developing heart failure. Reprinted with permission from Int. J. Mol. Sci.2025, 26(16), 8046.

Among non-modifiable risk factors, advanced age is a particularly strong risk factor in HF, followed by sex and ethnicity. Obesity is an established independent risk factor for HF, with particularly strong associations with HFpEF.

Epidemiological evidence shows that male sex, older age, and elevated resting heart rate are associated with incident HF irrespective of prior myocardial infarction (MI).

In individuals with a history of MI, smoking, diabetes, and impaired renal function have been identified as predictors of new-onset HF, whereas these associations are not observed in patients without prior MI. Conversely, elevated body mass index, increased systolic blood pressure, and hyperglycaemia are significantly associated with HF onset in individuals without previous MI, but not in those with prior MI.[39] Systems-level analyses of protein networks in ischemic conditions further highlight activation of distinct pathophysiological pathways related to inflammation, endothelial dysfunction, coagulation and atherosclerosis.[40]

Aetiology

A wide range of aetiologies may result in HF, which represents the end stage of all heart disease. The epidemiology of HF has gained increased interest since 1997, when it was highlighted as a "new epidemic" by Dr Eugene Braunwald in his well-known editorial titled "Cardiovascular Medicine at the turn of the Millennium". He based his observation on increasing incidence leading to a high burden on healthcare systems and shift of focus from treatment of acute events of HF to more seriously addressing the chronic and growing challenge of HF management. Identification of the underlying aetiology of cardiac dysfunction is important in the diagnostic process of HF, as the specific pathology may guide therapeutic decisions.

Heart failure arises from a wide range of cardiac and systemic conditions, broadly categorised into primary myocardial disease and secondary causes. Aetiologies also vary considerably between high-income and developing countries.[41] The most common aetiology globally is ischemic heart disease (IHD), followed by hypertensive heart disease**,** valvular disease, and cardiomyopathies**.** Regional variations exist, where high-income countries report more ischemic HF and chronic obstructive pulmonary disease (COPD), while lower-income regions have more hypertension-related and rheumatic HF.[42]

Ischemic HF results from reduced myocardial perfusion, leading to systolic dysfunction. Valvular heart disease, often rheumatic in origin among youth and degenerative in the elderly can cause both pressure and volume overload. Hypertension**,** even without overt ischemia, contributes via increased afterload and ventricular remodelling. Aggressive treatment of hypertension has been shown to lower the incidence of HF.[43] These four aetiologies accounts for about two-thirds of HF cases.

Cardiomyopathies are currently classified as dilated, hypertrophic, restrictive, arrhythmogenic, and non-dilated. Genetic predisposition plays a significant role, together with environmental modifiers like toxins, pregnancy, or metabolic disease. Special subtypes include Takotsubo cardiomyopathy**,** triggered by stress and characterised by transient systolic dysfunction, and peripartum cardiomyopathy, linked to pregnancy-related hemodynamic stress and possible genetic factors.

Inflammatory cardiomyopathy typically stems from viral myocarditis, though bacterial, autoimmune, or parasitic causes (e.g., Chagas disease) are notable as well. Infiltrative conditions such as amyloidosis**,** sarcoidosis, and hemochromatosis induce restrictive physiology and often complicate standard HF therapy due to preload dependence.

Other contributors include tachyarrhythmias**,** thyrotoxicosis, and high-output failure, the latter seen in conditions like thiamine deficiency, liver disease, and AV shunting. These typically present with preserved EF but elevated filling pressures and elevated natriuretic peptides.

HF aetiology also varies between women and men and across age groups.[44] With age, the proportion of HF caused by IHD increases in both sexes, with a higher proportion of males having HF compared to females. Hypertension is the most common cause of HF in females from age 40 till 80, whereafter IHD is the domination cause. Alcohol cardiomyopathy is more prevalent in males compared to females in younger ages 20-49 years.[44] Women are more likely to experience mitral valve rheumatic heart disease or mitral valve prolapse, while men are more likely to suffer from aortic valve diseases such as regurgitation or stenosis.[45] Endocarditis is also more common in men.[46]

Prognosis

To translate the natural history of heart failure into a prognosis for the individual patient is very difficult. This is the main reason why physicians are reluctant to communicate quantitative information about prognosis to HF patients. Several prognostic markers and prognostic scores for hospitalisation and death have been described but the clinical value of such prognostic models is modest.

Escalating healthcare expenditure in HF has led to an intensified focus on reducing hospital readmissions.[47,48] Hospitalisation rates for acute HF are only available for Europe and the US and they both exceed 1 million annually and has tripled since the 1990s[7,49,50] and is the most frequent cause of unplanned hospitalisation in patients over 65 years of age.[51]

Hospitalisations for acute HF predominantly concern patients with a pre-existing HF diagnosis who present with acute-on-chronic deterioration, accounting for 70–80% of admissions; only 20–30% represent new-onset or advanced HF.[47,52] Despite advances in HF management, acute HF (AHF) hospitalisation remains associated with poor outcomes, with in-hospital mortality rates reported between 4–6%, and 10–30% for rehospitalisation, post-discharge and one-year mortality.[47,52-54]

In the ASCEND-HF registry, international variation in length of stay for AHF was examined, and each additional hospital day was independently associated with a 14% reduction in all-cause readmission and a 21% reduction in HF-specific

readmission.[55] These findings are consistent with earlier reports supporting the strategy of achieving adequate decongestion during the index hospitalisation to prevent recurrent admissions and reduce mortality.[56] Recurrent hospitalisation itself is a powerful prognostic marker: patients experiencing three or more hospitalisations have a one-year mortality of approximately 50%.[56,57] Although the risk of rehospitalisation and death is highest shortly after discharge, it remains substantially elevated for at least 18 months thereafter.[58]

Undertreatment at discharge is multifactorial. One contributing factor being physicians reluctance to initiate guideline-directed therapies due to their hemodynamic effects. Many recommended pharmacotherapies lower blood pressure or heart rate, and low blood pressure is independently associated with worse outcomes, which complicates titration in the acute setting.[59,60] Withholding beta-blockers at discharge following decompensated HF has been associated with increased post-discharge mortality.[61,62] The post-discharge period therefore constitutes a clinically vulnerable phase in which persistent clinical or subclinical congestion likely represents a modifiable driver of adverse outcomes warranting better prognostic tools for risk stratification.[63,64]

Symptom burden further correlates with prognosis. Across multiple studies employing the NYHA functional classification, patients in class IV exhibit an estimated 50% mortality within 1–2 years, compared with 20–30% among those in class III.[56] Despite substantial advances in guideline-directed therapy and an overall reduction in HF-related mortality over the past 25 years, recent epidemiological analyses indicate that age-adjusted HF mortality has been increasing in the United States since 2011 across several demographic groups, including adults aged 15–44 years and those ≥75 years.[65,66]

Pathophysiology of heart failure

As described, HF has a broad spectrum of aetiologies and phenotypes. This makes the identification of all the cellular and molecular mechanisms involved complex, and many remain poorly understood.[67] All types of cardiac insults can damage the myocardium and initiate compensatory mechanisms attempting to maintain cardiac output (CO) and meet systemic demands. This often result in maladaptation where prolonged stimulation of the sympathetic nervous system (SNS) leads to decreased responsiveness of beta-adrenergic receptors and depletion of adrenaline reserves. These changes negatively impact cardiac cell regeneration, promote myocardial hypertrophy, and enhance contractile function of the heart.[68] Additionally, heightened sympathetic activity triggers the renin-angiotensin-aldosterone system (RAAS), resulting in systemic vasoconstriction and increased sodium retention.[68,69] The activation of RAAS also promotes the release of angiotensin II, a key factor in

driving cardiac hypertrophy and interstitial fibrosis, both of which contribute to pathological remodelling of the myocardium.

This accelerates into a negative spiral where apoptosis leads to a decrease in cardiac output where elevated demands lead to increased neurohumoral stimulation and maladaptive hemodynamic and myocardial responses (Figure 4).[69] Apoptosis leads to impaired contractility and decrease in EF and incomplete LV emptying. Increased LV volume and pressure cause pulmonary congestion.[70]

Sodium and water retention is further potentiated through renal hypoperfusion that initiate release of antidiuretic hormone (ADH). An increase in central venous and intraabdominal pressure reduces renal blood flow and further decreasing glomerular filtration rate (GFR).[71] The cellular dysfunction is mirrored by elevated levels of norepinephrine and naturetic peptides like brain natriuretic peptide (BNP) and atrial natriuretic peptide (ANP), ineffective in counteracting the excess sodium and water retention.[71] These hormones are enzymatically broken down by neprilysin.

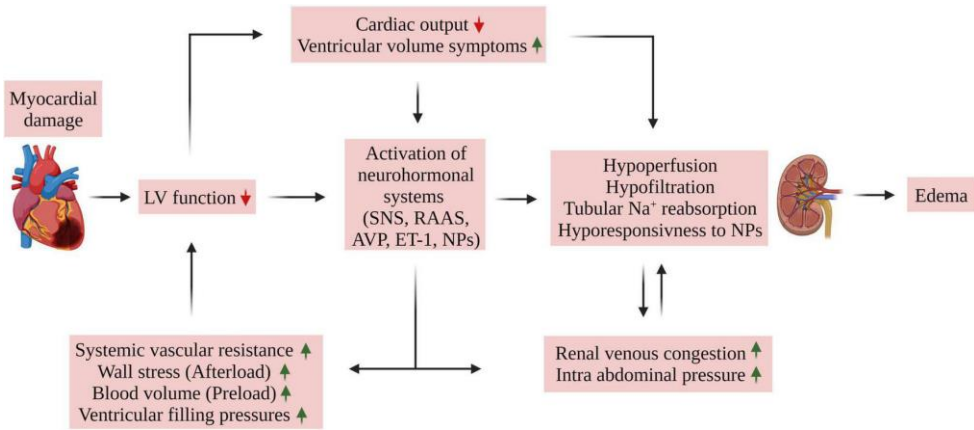

Figure 4. Mechanisms of oedema formation in HFrEF. ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; AVP, arginine vasopressin; ET-1, endothelin 1; LV, left ventricle; NPs, natriuretic peptides; RAAS, renin angiotensin aldosterone system; SNS, sympathetic nervous system. Reprinted with permission from Frontiers in Cardiovascular Medicine. 9. 933215.

The systematic mapping of the pathophysiology has led to effective treatment for HF where modulation of the activated systems is performed by beta-blockers, angiotensin converting enzyme inhibitors (ACEi) and angiotensin receptor neprilysin inhibition (ARNI) that has improved outcome and symptoms in heart failure patients with left ventricular dysfunction. The understanding of the underlying pathophysiology of heart failure is crucial to tailor and initiate the adequate therapeutic option for each patient.

Inflammation and oxidative stress

Recent evidence highlights that systemic inflammation and oxidative stress-related pathways play a crucial role in the pathophysiology of HF compared to the activation of the SNS and RAAS. Inflammation constitutes a pivotal mechanism in the pathophysiology of both principal phenotypes of heart failure.[72,73] In HFrEF, cardiomyocyte injury occurs as a consequence of an acute cardiac insult, typically myocardial ischemia or infection leading to an inflammatory response that promotes ventricular remodelling. Although the degree to which inflammation contributes to the persistence and progression of chronic heart failure remains uncertain. Numerous studies demonstrate elevated circulating concentrations of pro-inflammatory mediators, such as tumour necrosis factor-α (TNF-α), interleukin-1(IL-1), interleukin-6 (IL-6), and galectin-3 (Gal-3), suggest that inflammatory activity is sustained throughout disease progression.[74]

In contrast, HFpEF is under debate whether it should be conceptualised as a unified syndrome or as a spectrum of pathophysiological distinct disorders. A prevailing paradigm proposes that a chronic, systemic pro-inflammatory environment, caused by the comorbid conditions commonly observed in HFpEF, induces endothelial dysfunction and attenuates nitric oxide bioavailability leading to myocardial and vascular stiffening.[75]

Metabolic dysregulation and obesity further exacerbate these processes by facilitating expansion of epicardial adipose tissue and enhanced secretion of adipocytokines, thereby amplifying local inflammatory responses and promoting interstitial fibrosis within the myocardium. Proteomic profiling has underscored robust associations among inflammatory biomarkers, extracellular matrix remodelling, and the HFpEF phenotype.[76] These pathological alterations extend beyond the left ventricle to involve the left atrium, with atrial fibrillation frequently constituting an early clinical manifestation, particularly among individuals with obesity or diabetes mellitus.[77] However, the inflammatory hypothesis remains partially contested, as interventional studies targeting vascular inflammation have thus far failed to yield conclusive therapeutic benefit.[78]

Autonomic nervous system

Chronic heart failure is associated with complex disturbances of the autonomic nervous system (ANS), primarily characterized by sustained activation of the sympathetic nervous system and withdrawal of parasympathetic nervous system (PNS) activity.[79] These alterations initially arise as compensatory responses to impaired cardiac function and reduced systemic perfusion. With disease progression, however, persistent autonomic imbalance becomes maladaptive, contributing to adverse cardiac remodelling, progressive myocardial dysfunction, and worsening clinical outcomes.[79]

Longitudinal studies have demonstrated that the severity of autonomic imbalance in HF is strongly associated with increased mortality risk, although autonomic dysfunction is not uniformly present across all HF phenotypes.[80] Markers of autonomic dysfunction, such as elevated resting heart rate, reduced heart rate variability, and impaired baroreflex sensitivity, are independently associated with adverse prognosis.

Importantly, therapies that improve survival and reduce hospitalisation in HF also attenuate sympathetic activation and/or enhance parasympathetic modulation. These include angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, β-adrenergic blockers, mineralocorticoid receptor antagonists, digoxin, ivabradine, and cardiac resynchronization therapy (CRT). The favourable autonomic effects of these treatments underscore the central role of ANS dysregulation in HF pathophysiology.

Despite advances in guideline-directed therapy, morbidity and mortality in HF remain high.[3] The ANS therefore remains an important therapeutic target, as current pharmacological treatments do not fully reverse autonomic dysfunction, treatment optimisation is often limited by adverse effects and intolerance, and long-term adherence may be suboptimal in chronic disease.

Consequently, several novel therapeutic strategies aimed at modulating autonomic cardiovascular reflexes are under investigation. These include vagus nerve stimulation, renal sympathetic denervation, carotid body modulation, and baroreceptor activation therapy, as well as non-invasive approaches such as adaptive servo-ventilation in patients with sleep-disordered breathing. To date, clinical trial results have been heterogeneous, and the precise role of these interventions in HF management remains to be established and nuanced for a more phenotype-driven approach.

Hemodynamic alterations and congestion

Heart failure is characterised by the hearts inability to deliver required amounts of oxygenated blood, cardiac output, to the body in rest and during physical activity. Hemodynamics represent the dynamic interplay between cardiac function, vascular resistance, and blood volume distribution. A thorough understanding of hemodynamic principles is essential for interpreting cardiovascular physiology, diagnosing HF and guiding therapy to prevent end-organ damage and improve clinical outcome.

Fundamental hemodynamic principles

Hemodynamics is primarily determined by the interaction between cardiac output (CO), systemic vascular resistance (SVR), and blood volume. The volume of blood ejected by the heart per minute, is defined as the product of stroke volume (SV) and heart rate (HR). Stroke volume, in turn, depends on preload, afterload and myocardial contractility.[81]

$$CO = HR \times SV$$

CO=cardiac output; HR=heart rate; SV=stroke volume

Preload refers to the end-diastolic myocardial wall stress, commonly approximated by left ventricular end-diastolic volume (LVEDV). According to the Frank-Starling mechanism, stroke volume increase with rising preload within physiological limits. In HF, especially HFpEF, impaired compliance shifts the diastolic pressure-volume relationship upward, causing significant increase in filling pressures even at modest volumes. This explains why ventricular dilation in HF increases wall stress and oxygen demand.

Laplaces law (simplified modell of the LV):

$$Wall\ stress = (P \times r) / 2h$$

P=transmural pressure; r= ventricle radius; h=heart wall thickness

Afterload is the systolic wall stress the ventricle must overcome to eject blood. In clinical practice, systemic vascular resistance (SVR) and arterial elastance (Ea) serve as surrogate measures. Elevated afterload, resulting from hypertension or increased arterial stiffness, impairs stroke volume and promotes adverse remodelling, especially in HFrEF.

Contractility reflects the intrinsic ability of the myocardium to generate force independent of preload and afterload. In practice, contractility is inferred from indices such as ejection fraction (EF), and end-systolic elastance (Ees). In HFrEF, declining contractile reserve leads to reduced stroke volume and increased end-systolic volume. A lower end-systolic elastance indicates impaired contractility.

Cardiac output is the fundamental determinant of systemic oxygen deliver. In HF, CO may be reduced (HFrEF) or maintained at the cost of high filling pressures (HFpEF).

As described, physiological regulation of these parameters involves complex neurohormonal feedback mechanisms, including sympathetic activation and the RAAS, which modulate vascular tone, heart rate, and sodium-water balance. These systems maintain hemodynamic stability under varying physiological conditions but contributes to maladaptive responses in chronic cardiovascular disease. The intra cardiac pressures-volume relation is often depicted in a loop diagram where the x-axis represents volume and the y-axis represent the pressure in the left chamber. The loop is formed when pressure is measured continuously during a heart cycle depicted below in Figure 5.

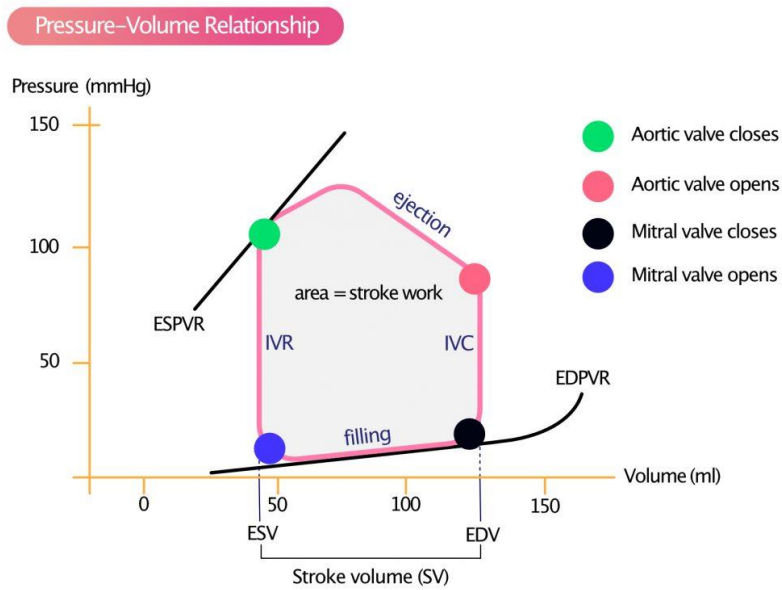

Figure 5. This diagram depicts the cardiac cycle, showing the relationship between pressure and volume in the ventricles during phases such as isovolumetric contraction, ventricular ejection, isovolumetric relaxation, and ventricular filling. It illustrates key events including stroke volume, end-diastolic and end-systolic volumes, and the pressure changes that occur during each phase of the heartbeat. Reprinted with permission.

Depending on type of heart failure the pressure-volume loop is displaced and tions explain clinical features such as pulmonary congestion, exercise intolerance, and susceptibility to decompensation:

- HFrEF: downward/rightward shift of the end-systolic pressure-volume relationship (ESPVR), increased volumes, reduced stroke work.
- HFpEF: upward/leftward shift of the diastolic pressure-volume relationship, reflecting reduced compliance and elevated filling pressures despite preserved EF.

Right heart catheterization

Direct hemodynamic assessment remains the gold standard for quantifying circulatory statues in patients with HF. Right heart catheterization (RHC) allows precise measurement of pressure and flows within the pulmonary and systemic circuits. The first reported right heart catheterization in human was performed by the surgical resident Werner Forssmann in Germany 1929, by inserting a 65 cm urethral catheter in his own left antecubital vein, advancing it to the right atrium to administer drugs directly into the right heart. Previously, equine venous cannulations had been made already in the 1700s. This combined with X-ray developed into the procedure right heart catheterization placing catheters in veins or arteries to access the heart to image, diagnose and treat conditions without having to open the thorax. In 1956, André F. Cournand, Dickinson W. Richards and Werner Forssmann were awarded the Nobel prize in Medicine for their contribution in developing central and peripheral catheterization procedures as we know them today.[82] The technique was used to study hemodynamics both in the heart and lungs in both patients with congenital heart disease, pulmonary disease and heart failure and the catheter was often referred to as the pulmonary artery catheter because mixed venous blood from the pulmonary artery often was collected to help measure the cardiac output.[82]

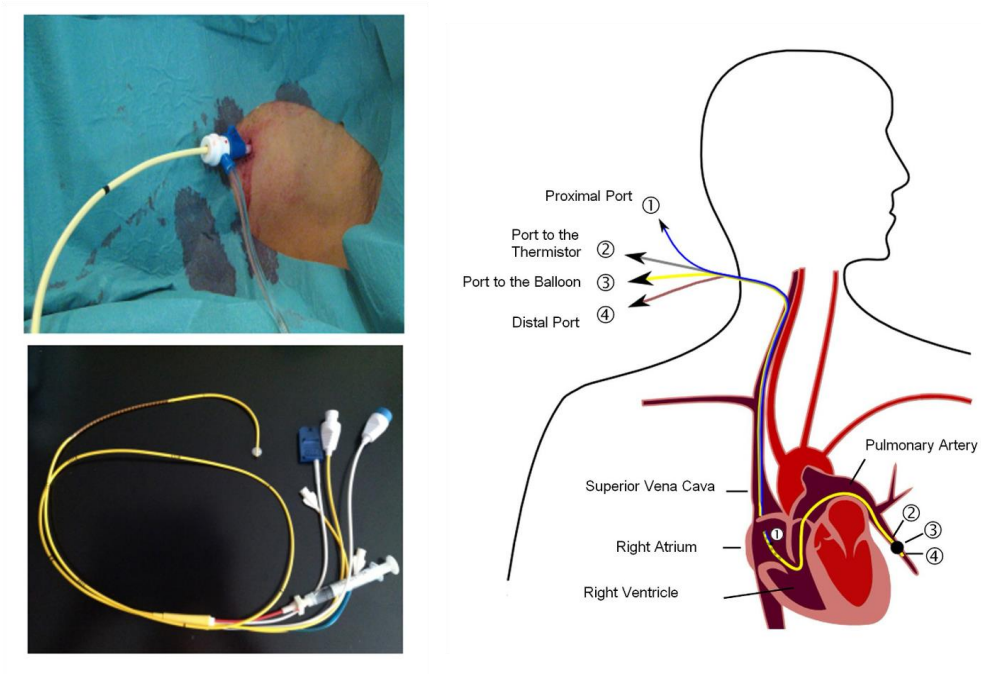

Figure 6. Swan-Ganz catheter with four lumen port. Number 1-4 indicates which port is used for measuring at different locations. Reprinted with permission.

Today the most used pulmonary artery catheter is the "Swan-Ganz" catheter.[83] Distinctive for this catheter is a balloon at the tip of the catheter which allows the catheter to be places via flotation bedside. It also provides the measurement of the right atrium and the pulmonary arteries continuously. Through a thermistor at the tip of the catheter, cardiac output can be measured directly (Figure 6).

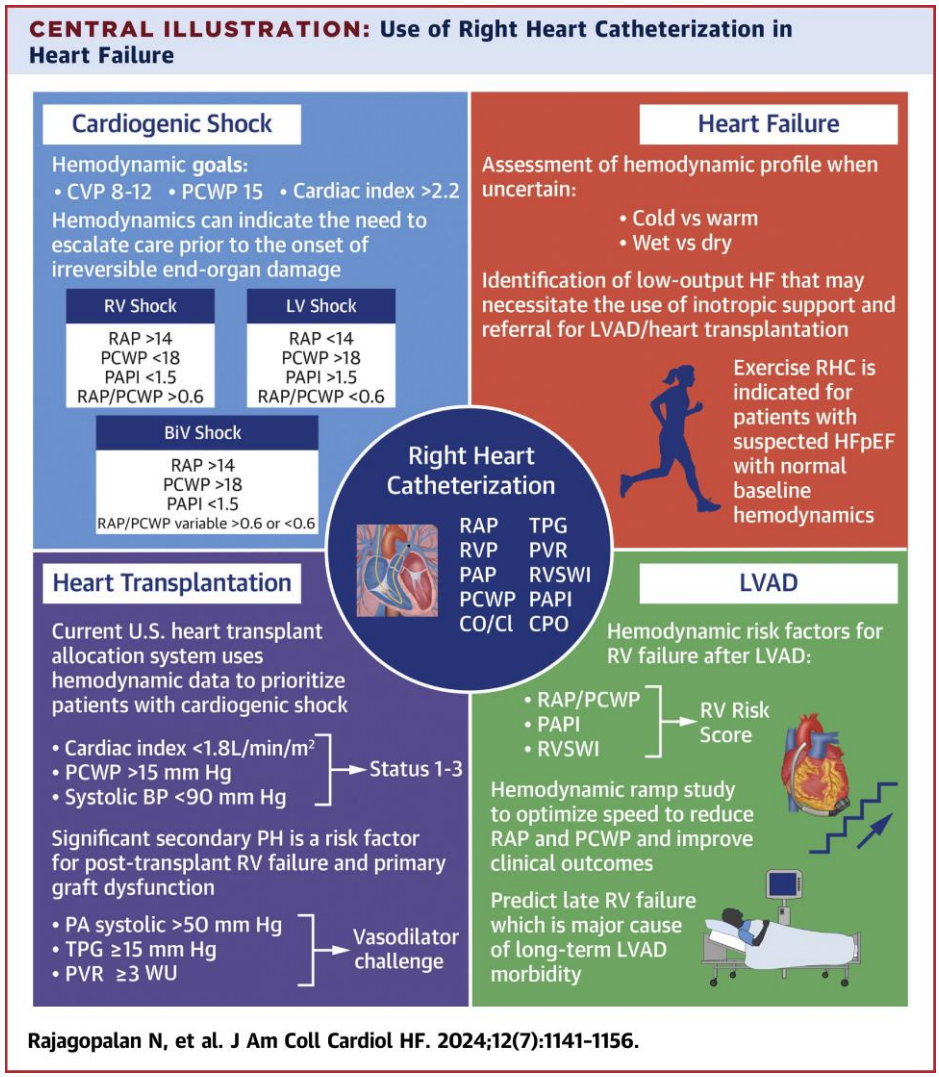

Figure 7. RHC provides important diagnostic and prognostic information for the full spectrum of heart failure, including cardiogenic shock, prioritization of candidates for heart transplantation, and treatment of LVAD patients. BiV, biventricular; CO, cardiac output; CVP, central venous pressure; HfpEF, heart failure with preserved ejection fraction; LV, left ventricle; LVAD, left ventricular assist device; PH, pulmonary hypertension; PVR, pulmonary vascular resistance; RHC, right heart catheterization; RV, right ventricle; RVSWI, right ventricular stroke work index; TPG, transpulmonary gradient. Reprinted with permission from J Am Coll Cardiol HF. 2024;12(7):1141-1156.

Right heart catheterization has a solid position in evaluation for cardiac transplantation, surveillance status post-cardiac transplant and for assessment pre-implantation off left ventricle assist devices and as well as for monitoring and optimizing the device. Finally, it's used for evaluation of patients with dyspnoea to diagnose or exclude heart failure with preserved ejection fraction, restrictive cardiomyopathy, constructive pericardial disease, pulmonary hypertension or as an assessment of patients with valve disease (Figure 7). Preforming the catheterization, the pulmonary catheter advanced into the vein approximately 15 cm after which the balloon is inflated to facilitate the access to the right atrium at about 20 cm where a pulsatile waveform is observed.

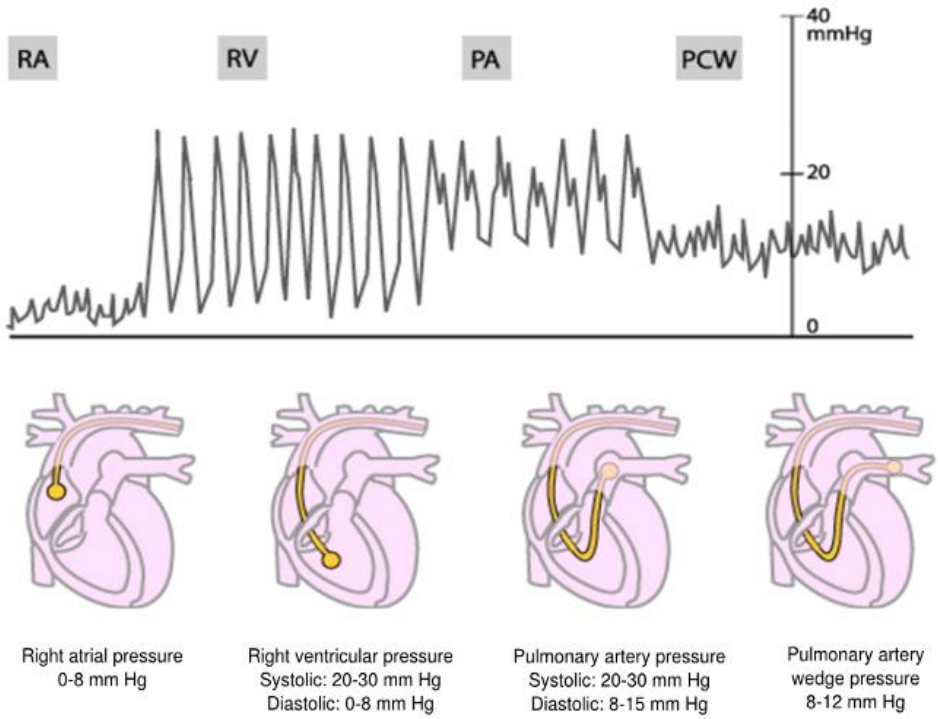

Figure 8. Swan-Ganz catheter in yellow advancing through the right heart. RA, right atrium; RV, right ventricle; PA, pulmonary artery; PCW, pulmonary wedge position.

Before recording, the system is equilibrated with atmospheric pressure. After recording the pressure in the right atrium(mRAP) the catheter is manipulated to turn towards the right ventricle where the right ventricle pressure (mRVP) is obtained. Finally, the catheter is advanced to the wedge position to measure the pulmonary capillary wedge pressure (PAWP) after which the balloon is deflated and brought back a few centimetres into the pulmonary artery where the

pulmonary artery pressure (PAP) (Figure 8). At this point a blood sample is withdrawn from the yellow port to get the mixed venous saturation. Arterial blood is also obtained separately and analysed to calculate the cardiac output using the Fick method.[84] The Fick principle is based on the idea that the oxygen consumption rate by peripheral tissues is directly proportional to the product of the amount of oxygen delivered to the tissues and the arterial-venous oxygen concentration difference, expressed in this equation:

$$CO = VO_2 / (C_aO_2 - C_vO_2)$$

- Cardiac output (CO) is the blood volume pumped by the heart per minute (measured in L/min).
- Tissue oxygen consumption (VO_2) is the rate at which oxygen is consumed by the body tissues per minute (measured in mL O_2/min).
- Arterial oxygen content (C_aO_2) is the oxygen content in arterial blood (measured in mL O_2/L).
- Mixed venous oxygen content (C_vO_2) is the oxygen content in mixed venous blood, typically sampled from the pulmonary artery (measured in O_2 mL/L).[85,86]

Calculating cardiac output and cardiac index can also be done by using thermodilution, where cold saline is injected into the blue port (right atrium) and mixed with blood. The difference in temperature is then measured by a thermistor and the procedure must be repeated at least three times to obtain an average cardiac output and cardiac index.[84] CO and CI can also be calculated through this formula using body surface area (BSA)m^2:

$$CI = CO / BSA$$

Echocardiography

Echocardiography is one of the cornerstones of non-invasive hemodynamic evaluations. Doppler-derived indices such as E/e´ ratio, vena cava diameter, and stroke volume estimation allow approximation of filling pressures and cardiac output. The stroke volume has several clinical significances in hemodynamics, most notably in Point-Of-Care ultrasound (POCUS) to assess fluid responsiveness in

critically ill patients who are in active shock and to determine the aetiology of the shock, intraoperative cardiac output monitoring, or as CO calculation method.

Lung ultrasound

Lung ultrasound (LUS) as part of POCUS has emerged as a complement in identifying pulmonary congestion in patients with undifferentiated dyspnoea and several observational and randomized clinical trials have demonstrated higher accuracy than clinical examination and chest X-ray.[87,88] Findings on LUS suggestive of congestion, B-lines, has been shown in studies, can identify individuals at high risk for decompensation or death, both in ambulatory HF cohorts and in hospitalised patients with persistent B-lines at discharge.[89-91]

The question not yet answered is if LUS-detected lung congestion might lead to improved congestion management and result in improved outcome and it is important to compare it to alternative strategies used to achieve the same goal bearing in mind reproducibility, cost and level of expertise needed.

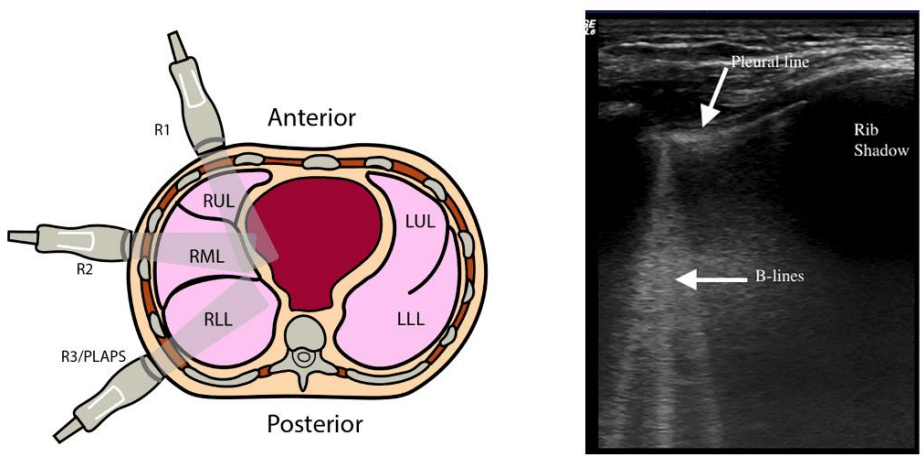

Figure 9. Lung ultrasound. Probe positions showing different projections as a part of a structured protocol examining the lungs. The picture at the right showing the pleural line, rib shaddows and B-lines. Reprinted with permission.

The pathophysiology of congestion

Central and peripheral congestion

Congestion refers to the pathological accumulation of fluid in organs and tissues due to elevated cardiac filling pressures and is a central component in the definition of HF.[1,92] Impaired ventricular function, leads to increased venous pressure, causing fluid to leak from the vasculature into the lungs (pulmonary congestion) or the systemic circulation (peripheral congestion). It may arise from impaired forward

flow, increased venous return, or fluid retention mediated by neurohormonal activation. Most HF patients have a combination of both intravascular and tissue congestion. Each of these two forms of congestion has a different pathophysiology and requires a different diagnostic approach.

The deterioration in ventricular function results in both systolic and diastolic functional alterations, characterized by cardiac fibrosis, ventricular hypertrophy, and subsequent unfavourable remodelling. This can result in diastolic dysfunction and reduced cardiac compliance. Diastolic left ventricular dysfunction, as a result of increased upstream pressures, affects the left atrium, pulmonary circulation, and therefore the right ventricle. The resulting hemodynamic alterations are responsible for the onset of the various comorbidities, including atrial fibrillation and pulmonary hypertension, leading to a poorer prognosis.[93] Systolic dysfunction develop as a consequence of the ventriculus–arterial coupling[94] which can lead to delays in the ventricular conduction, seen as "widened QRS complex" (>100 ms) on the ECG, an independent predictor of adverse outcomes and a target for device treatment.[95]

At a molecular level, persistent activation of the sympathetic nervous system and RAAS promotes sodium retention, venoconstriction, and plasma volume expansion. Increased capillary hydrostatic pressure subsequently drives transudation of fluid into interstitial spaces, while impaired lymphatic drainage exacerbates tissue oedema. Importantly, congestion may occur even in the presence of preserved cardiac output, particularly in diastolic dysfunction, highlighting the dissociation between pressure overload and pump failure.

Clinical assessment

In clinical practice, the evaluation of congestion in heart failure patients traditionally depends on physical examination findings such as swelling, lung crackles, and elevated jugular venous pressure. Jugular venous distension (JVD) is a critical clinical finding and should be routinely assessed. A positive hepatojugular reflux defined as a sustained JVD increase of more than 4 cm during abdominal pressure at a 45° incline indicates elevated left-sided filling pressures. In cases of cardiogenic shock, signs of hypoperfusion may include hypotension, delayed capillary refill, cold extremities, altered mental status, and oliguria.

However, these signs are often overlooked or inconsistently assessed, as their detection is highly influenced by the examiner's subjective judgment and experience. Furthermore, such assessments tend to have low reproducibility between different clinicians and lack both high specificity and standardized scoring systems, making them less reliable indicators of congestion.[96-100] Given the substantial risk of rehospitalisation in this patient population, relying solely on clinical signs for congestion assessment is clearly insufficient.

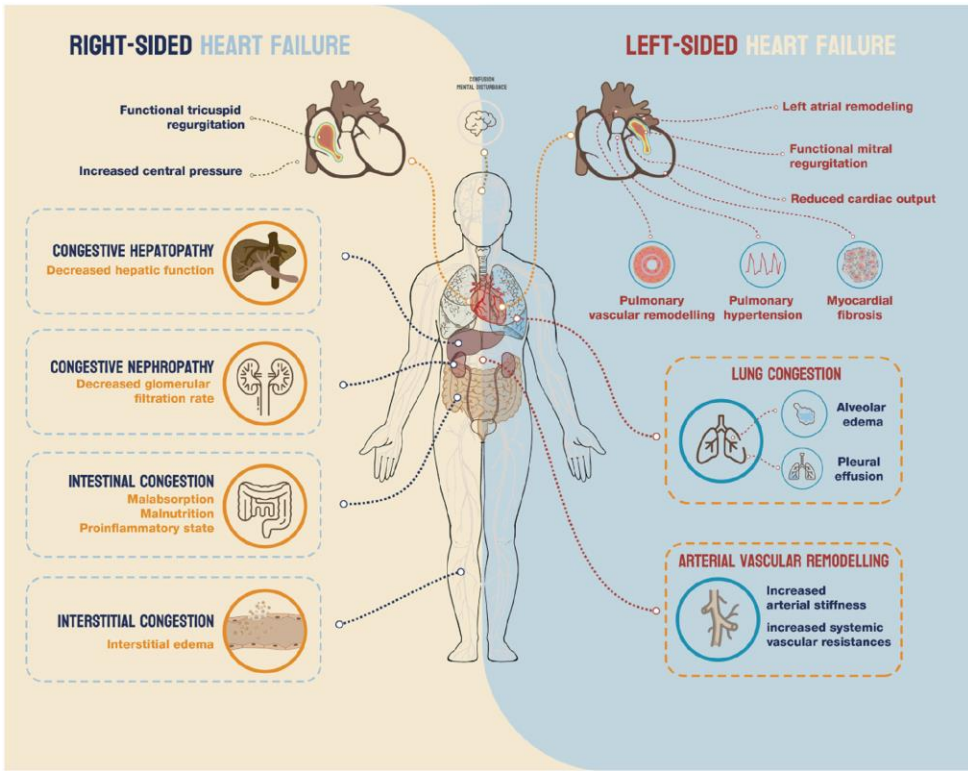

Figure 10. Clinical implications of organ congestion in heart failure. Congestion can lead to organ injury, dysfunction, and, ultimately, the failure of target organs. Reprinted with permission from Eur J Heart Fail, 24: 1751-1766.

Degree of congestion has traditionally been viewed upon as a surrogate for HF severity. This perspective has changed where fluid accumulation and redistribution are considered causally involved in HF and organ damage progression. Figure 10 depicts how congestion contributes to the HF-associated impairment of functional and structural changes in several organs and systems (heart, lungs, kidneys, liver, intestine, vessels, and brain).

Congestion of organs in the abdominal cavity is the net result of right-sided dysfunction and venous congestion. Passive congestive hepatopathy induced by increased central venous pressure may initially lead to cholestasis. However, chronic hepatic congestion may result in hepatic fibrosis and cirrhosis. Congestive nephropathy, caused by increased central venous pressure and extrarenal compression (i.e. intra-abdominal hypertension) may lead to a pressure-induced reduction in renal blood flow, renal hypoxia, increased interstitial pressure, and finally, interstitial fibrosis.[101,102] Intestinal congestion leads to increased gut permeability and translocation of endotoxins (pro-inflammatory state),

gastrointestinal hypoperfusion, protein-losing enteropathy, and cardiac cachexia. Furthermore, gut involvement complicates chronic heart failure treatment by decreasing intestinal absorption.[103] For instance, oedematous changes in the intestinal wall may alter the absorption rate of certain drugs such as furosemide. Additionally, the pro-inflammatory cytokine milieu resulting from bacterial and lipopolysaccharide translocation to the systemic circulation can also alter the expression of various drug-metabolizing enzymes and transporters.

Biomarkers in congestion

Biomarkers offer a promising solution due to their ease of measurement and consistent performance characteristics. Although natriuretic peptides are commonly regarded as the primary biochemical markers for congestion in heart failure, emerging research points to notable limitations.[104] These peptides primarily indicate cardiac wall stress and pressure, correlating mainly with intravascular volume excess, but they do not provide information about tissue and interstitial fluid accumulation. Therefore, developing and validating new objective biomarkers that specifically measure tissue congestion and reflect a patient's fluid status, remains a critical goal in advancing heart failure management.

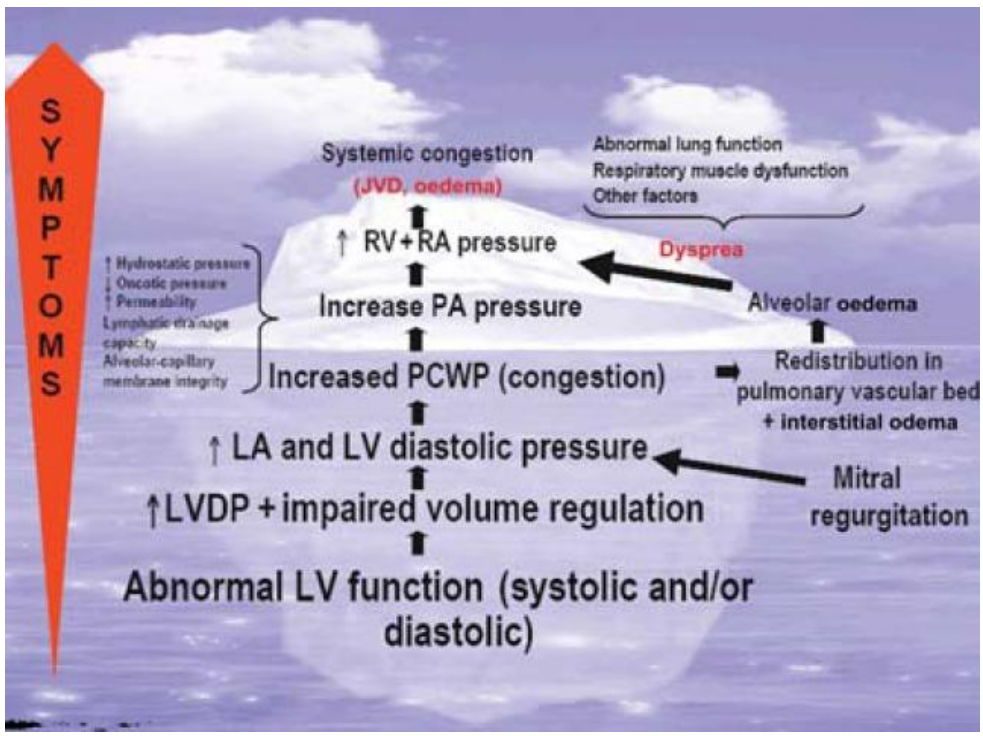

Figure 11. Assessing and grading congestion in acute heart failure. Reprinted with permission from European Journal of Heart Failure, 12: 423-433.

Quantitative grading

Invasive pressure thresholds like PCWP>18 mmHg and RAP>8 mmHg, traditionally define significant congestion and often result in overt clinical signs of HF. Elevation of left ventricular diastolic pressure (LVDP) in HF patients without overt clinical congestion has been termed 'hemodynamic congestion'.[98] Often, hemodynamic congestion precedes clinical congestion by days or even weeks.[105-108] Thus, clinical congestion may be the 'tip of the iceberg' of the hemodynamic derangements that precede symptoms (Figure 11). In fact, in chronic HF, even severe hemodynamic congestion rarely causes rales and/or radiographic pulmonary oedema.[98] This may be related to several adaptive pathophysiological changes such as increases in alveolar capillary membrane thickness, increased lymphatic drainage, and/or pulmonary hypertension.

Treatment of heart failure

The primary objective of the therapy for chronic HF is to relive symptoms, enhance quality of life, reduce hospitalisation admissions and ultimately lower cardiovascular mortality. Pharmacological treatment focuses on managing symptoms while initiating and optimizing medication that decrease morbidity and mortality.[2,109]

Pharmaceutical treatment

Guidelines now include four pillars for the therapy of HFrEF including sodium-glucose cotransporter-2 (SGLT2) inhibitor to add to angiotensin receptor-neprilysin inhibitor (ARNi) or, angiotensin-converting enzyme inhibitor(ACEi), angiotensin receptor blocker (ARB), beta-blockers, and mineralocorticoid receptor antagonists (MRA). Used together, these four pillars have great potential to improve outcome and it has been estimated a 73% relative risk reduction with a number needed to treat of four to prevent a death compared to no treatment.[2,109,110]

Other treatments like, Ivabradine can be useful in patients on optimal medical therapy with a heart rate of more than 70 bpm, providing mortality benefits and reducing HF hospitalisation.[3] Digoxin may be considered in symptomatic patients with sinus rhythm despite adequate goal-directed therapy to reduce the all-cause rate of hospitalisations, but its role is limited with conflicting results in studies.[3]

The titration of medications has gained more focus and an aggressive approach to achieve desired outcomes particularly after the STRONG-HF study[111] with the goal on being on all four drugs as soon as possible in tolerated doses.

Devices and mechanical support treatment

In heart failure, device therapy with cardiac resynchronization therapy (CRT) and implantable cardioverter-defibrillators (ICDs) is recommended in selected patients to improve symptoms and reduce mortality, in accordance with contemporary European guidelines.

CRT is indicated in patients with symptomatic heart failure (NYHA class II–IV), left ventricular ejection fraction (LVEF) ≤35%, and persistent symptoms despite optimal medical therapy:

- Class I: Patients in sinus rhythm with left bundle branch block (LBBB) morphology and QRS duration ≥150 ms
- Class IIa:
    - LBBB with QRS 130–149 ms
    - Non-LBBB morphology with QRS ≥150 ms
- Class IIb: Non-LBBB morphology with QRS 130–149 ms

CRT may also be considered in patients with atrial fibrillation if a high proportion of biventricular pacing can be ensured, often requiring AV node ablation (Class IIa).

ICD therapy is recommended for prevention of sudden cardiac death in patients with reduced LVEF (≤35%) despite ≥3 months of optimal medical therapy:

- Class I:
    - Primary prevention in patients with ischemic or non-ischemic cardiomyopathy, NYHA II–III, and expected survival >1 year
    - Secondary prevention in patients with prior sustained ventricular arrhythmias or cardiac arrest [2,3]

The CardioMEMS Heart Sensor, an implantable pulmonary artery pressure monitoring system, has shown variable results across studies. While the CHAMPION trial demonstrated a significant reduction in heart failure hospitalizations (approximately 28%), subsequent studies have yielded less consistent findings.

This approach represents an invasive and relatively costly strategy for hemodynamic monitoring. In the most recent European Society of Cardiology (ESC) guidelines, pulmonary artery pressure monitoring may be considered in selected patients with heart failure and a history of prior hospitalizations, corresponding to a Class IIb recommendation.[2,3,112,113]

Lifestyle intervention

Management of heart failure (HF) comprises both pharmacological and lifestyle-directed strategies, in which non-pharmacological modification constitutes an important component. Current ESC guidelines do not endorse a specific dietary pattern, whereas AHA/ACC recommend Mediterranean, plant-based, whole-grain and DASH diets(Dietary Approaches to Stop Hypertension), which have been associated with reduced HF incidence and possible protective effects.[2,3] The DASH diet, rich in potassium and antioxidants, may also lower HF-related hospitalisations, although findings remain inconsistent due to methodological limitations.

Weight reduction is recommended for HF prevention, yet its role in established disease is uncertain.[3] The so-called "obesity paradox," whereby higher BMI confers a survival advantage across HF phenotypes and comorbidity strata, complicates treatment decisions.

Sodium restriction remains controversial: ESC discourages intake <5 g/day and AHA/ACC recommends restriction in stage C HF,[3] but overly strict sodium limitation may provoke neurohormonal activation and has been associated with increased readmission and mortality, underscoring the need for individualised dietary guidance, particularly in nutritionally vulnerable patients.[114] Fluid restriction is commonly advised in severe HF with hyponatraemia, with ESC recommending 1.5–2 L/day and AHA/ACC noting uncertain benefit.[2] The FRESH-UP trial found no advantage of restriction versus liberal intake at 3 months and greater thirst burden in the restricted cohort, questioning routine implementation.[115]

Physical activity is broadly advocated, and both ESC and AHA/ACC support structured exercise, including supervised cardiac rehabilitation in advanced disease or frailty, to improve functional capacity, quality of life and reduce hospitalisations.[2,3]

Substance use is an under-recognised determinant of adverse HF trajectory. Alcohol, tobacco, cannabis and cocaine worsen outcomes, with heavy alcohol use linked to dilated cardiomyopathy and stimulants conferring particularly poor prognosis.[116] Tobacco increases HF risk via both coronary and non-coronary pathways and deteriorates outcomes in established HF. Cessation is strongly recommended, with evidence that even partial reduction confers benefit.[117] Further research is warranted to clarify the mechanistic and prognostic effects of substance use across the HF spectrum.

Heart transplant

For highly selected patients, cardiac transplant is indicated to improve survival and quality of life. The early experience with heart transplants was disappointing. In 1967, the first patient transplanted died of an infection after 17 days.[118] However,

with the introduction of immunosuppressive therapies and improved surgical techniques and a better understanding of human anatomy the numbers of patients undergoing heart transplant is steadily growing.[119] Outcomes and survival post-transplant have improved remarkably due to proper selection of the recipient, improved organ preservation techniques, timely referral for the eligible patients, careful surveillance and immunosuppression, and monitoring for any complication with prompt management.[119]

Unfortunately, many patients with advanced heart failure who meet the indications for a heart transplant are unfit due to contraindications like severe liver, kidney and lung failure, morbid obesity, uncontrolled diabetes, recent lung embolism and stroke, active infections and malignancy.[120,121]

Role of biomarkers in cardiovascular disease

Cardiovascular disease (CVD) remains the leading cause of morbidity and mortality worldwide, despite major advances in prevention and treatment. Early identification of individuals at risk and timely diagnosis of disease are critical to improving outcomes. In this context, biomarkers have emerged as an essential tool in modern cardiovascular medicine. The definition of a biomarker is:

"A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention"[122]

In CVD, biomarkers provide valuable information that complements clinical assessment and imaging findings, offering insights into disease onset, progression and prognosis. They play a crucial role across the continuum of cardiovascular care, from risk prediction and primary prevention, to diagnosis, prognosis, and monitoring of treatment response. Biomarkers can be classified as:[122]

1. A *diagnostic biomarker* that detects or confirms the presence of a disease or condition of interest or identifies an individual with a subtype of the disease.
2. A *monitoring biomarker*, when the biomarker can be measured serially to assess the status of a disease or medical condition for evidence of exposure to a medical product or environmental agent, or to detect an effect of a medical product or biological agent.

3. A *pharmacodynamic/response biomarker*, when the level of a biomarker changes in response to exposure to a medical product or an environmental agent.

The perfect biomarker in HF should be (1) measured non-invasively and at low cost, (2) highly sensitive to allow for the early detection of the disease, (3) unaffected or minimally affected by comorbid conditions, and (4) responsive to treatment effects.[123]

Traditional biomarkers such as low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL) remain central in assessing arteriosclerotic risk, while high-sensitivity C-reactive protein (hs-CRP) provide additional information on systemic inflammation and residual cardiovascular risk. In the acute setting, cardiac troponins (cTn) are the gold standard for detecting myocardial injury, and B-type natriuretic peptides (BNP, NT-proBNP) serve as key indicator of heart failure and ventricular stress.

The development of biomarker is a multidisciplinary process that integrate molecular biology, bioinformatics and clinical research. In the discovery phase, potential biomarkers are identified using high-throughput technologies such as genomics, proteomics, or metabolomics. Analytical validation ensures that the biomarker can be reliably and reproducibly measured, while clinical validation confirms its relevance for diagnosis, prognosis, or therapeutic response. The goal of biomarker development is to improve patient stratification, enable personalised medicine, and enhance the efficiency of drug development and disease management.

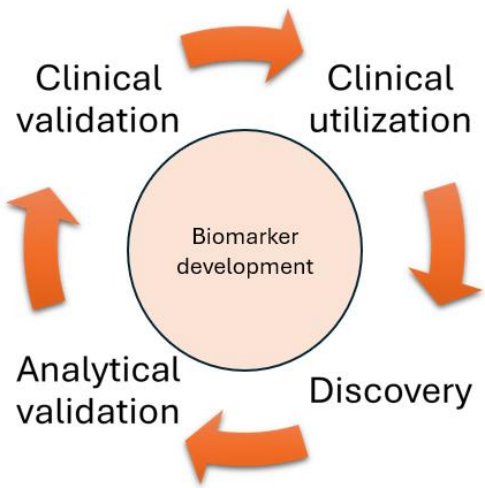

Figure 12. Evaluation of biomarkers.

Established biomarkers in heart failure

In accordance with recent guidelines for HF,[2,3] integrating clinical history with various clinical investigations, evaluating signs and symptoms to physical examination, imaging, and blood biomarkers, is recommended to facilitate the management of HF and to identify heart failure phenotypes (Figure 1).[124] Increased filling pressures, reduced cardiac output are hemodynamic alterations that impact cardiac compliance leading to increased vasoconstriction, vascular permeability, activation of the RAAS, dysregulation of the autonomic nervous system, and activation of inflammatory processes. All these systemic alterations determine the release of biomarkers and with the potential of insight in disease stage, prognosis and potential therapeutic targets. A handful of biomarkers have an established role in present cardiovascular medicine.

Natriuretic peptides

The discovery of the cardiac endocrine system 30 years ago, has led to the clinical use of peptides with natriuretic and vascular smooth muscle-relaxing activity functions. Atrial natriuretic peptides (ANP) and brain natriuretic peptide (BNP) and its inactive precursor NT-proBNP, were first isolated in porcine brain cells and later in high concentrations in cardiac cells and are secreted by cardiomyocytes in response to volume or pressure overload, induced stress on the myocardial wall.

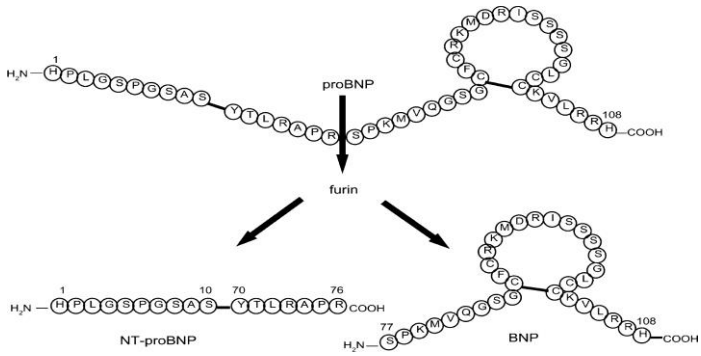

Figure 13. Biochemistry of NT-proBNP. Reprinted with permission from European Journal of Heart Failure, 6: 257-260.

Today they provide clinicians with robust diagnostic tools to differentiate HF from other causes of dyspnea in both acute and chronic stages, with its high negative predictive value.[125] NT-proBNP is invaluable for monitoring disease progression, adjusting treatment and predicting adverse outcome such as hospitalisation,

readmission and mortality in HF patients.[126] In clinical practice, natriuretic peptides are integrated into HF guidelines[2,3] and decision-making algorithms.

Several studies have demonstrated that natriuretic peptides are useful to stratify the risk of HF patients. In the Acute Decompensated Heart Failure National Registry (ADHERE), including 48 629 patients with acute HF, association between BNP entry levels and intra-hospital mortality was shown. This relationship was independent of other clinical and laboratory parameters and the HFrEF vs. HFpEF status.[127]

Further, several studies have shown that relative risk of all-cause death increases by 35% for each 100 ng/L increase in admission BNP can predict the short-and-long term prognosis in patients with acute HF.[128,129]

Despite their established role, natriuretic peptides have significant limitations. Even though there are established cut-offs for practical purpose, they should be interpreted to a great extent on their trend over time.[130] There is also a problem with specificity, where BNP and NT-proBNP levels may increase due to conditions not related to HF e.g. sepsis, renal dysfunction, pulmonary hypertension and older age.[131,132] In patients with comorbidities, the lacking specificity can complicate the diagnostic process. In obese patients, levels can be falsely low.[133]

The HF phenotype also impact the diagnostic utility of BNP and NT-proBNP. In HFpEF, which accounts for almost half of all HF cases, natriuretic peptides are rarely as elevated as in HFrEF. The lower myocardial wall stress in HFpEF results in BNP and NT-proBNP values that may not reach diagnostic thresholds, except in the presence of congestion. As a consequence, these limitations may lead to diagnostic inaccuracies and suboptimal care for HFpEF patients.[133] This discloses gaps in the use of established biomarkers in more challenging contexts highlighting the need for new biomarkers.

Tabel 3. The main advantages and limitations of clinical use of NT-proBNP.

Advantages	Limitation
Early diagnosis marker in patients with diabetes in the absence of a clear clinical expression of HF	The increase in BNP's and NT-proBNP may also depend on other comorbidities such as chronic renal failure or atrial fibrillation
In the absence of a defined cardiovascular pathology, the level of NT-proBNP values could predict the onset of heart failure, coronary artery disease and stroke	The value of NT-proBNP's should also be correlated with age, sex and BMI
NT-proBNP values are significantly associated with increased odds of advanced HF	There is a significant "grey area" in which the diagnosis is rather indeterminant
Correlation between NT-proBNP values and risk of adverse events in patients with HFpEF	

Cardiac troponins

Cardiac troponins (cTn), constitute a protein complex, consisting of three subunits: troponin T, troponin I, and troponin C. They are regulatory proteins in cardiomyocytes and contributes to the calcium-mediated interaction between actin and myosin.[134] Cardiac troponins are mostly known as typical biomarkers in the management of acute coronary syndrome. However, they are also recognised as useful prognostic biomarkers of HF. Elevated levels of troponins in patients with HF is linked to myocardial stress and death of cardiomyocytes related to subclinical, subendocardial ischemia.[135]

Elevated levels of cTn are reported to associate with advanced HF, poor prognosis and mortality risk. It is also associated with an increased risk of developing HF in patients with prior acute myocardial infarction, as higher cTn concentrations tend to correlate with a greater likelihood of HF onset.[135] In patients with acute HF, patients with elevated baseline cTn levels and AHF are hospitalised more frequently, have more intensive care unit admissions and higher in-hospital mortality.

Patients with chronic HFrEF and elevated cTn levels, have been reported to associate with greater long-term mortality and increased rehospitalisation rates due to HF.[136,137] Just like many other biomarkers, cTn values varies by gender, race, prevalence of cardiovascular disease, advanced age, hypertension, left ventricular hypertrophy, and chronic kidney disease. All this may lead to an increase in plasma cTn values. Similarly, clinical conditions not related to myocardial damage such as sepsis, acute respiratory distress syndrome, and stroke can increase cTn.[138]

Emerging and candidate plasma protein biomarkers

Biomarkers evaluated in heart failure has grown exponentially, but only a few of them have demonstrated sufficient evidence to justify their use in clinical practice. The majority of studies have focused on biomarkers for prognostic stratifications of HF patients and less on screening, diagnostic and treatment guidance tools for clinicians.

Metabolic and neurohormonal biomarkers

Key features of HF, such as fluid accumulation, vasoconstriction, and adverse cardiac remodelling arise primarily from neurohormonal imbalances, including the activation of both the RAAS and SNS. Selected molecules associated with these pathways are presented for their potential to serve as future biomarkers of HF.

Within the RAAS, many proteins have been studied in the search for potential prognostic biomarkers in HF, plasma renin activity (PRA) showing the most

significant findings.[139] In a study of 996 patients with chronic HF, PRA was found to be an independent predictor of cardiac death, providing additive value to NT-proBNP and LVEF.[140]

Adrenomedullin (ADM)

First characterized in 1993, ADM is a peptide hormone produced in vascular endothelial cells and smooth muscle and plays an important role in reducing systemic vascular resistance and increasing CO.[133,141,142] Due to its small size of 6 kDa it diffuses freely between blood and interstitium.[143] Originally identified in pheochromocytomas of the adrenal medulla, ADM receptors and binding sites are widespread in the body although cardiovascular and lung tissues have the highest density of binding sites.[144] ADMs main functions are vasodilatation, reducing systemic vascular resistance and blood pressure while augmenting tissue perfusion, and to maintain vascular integrity and decrease vascular leakage.

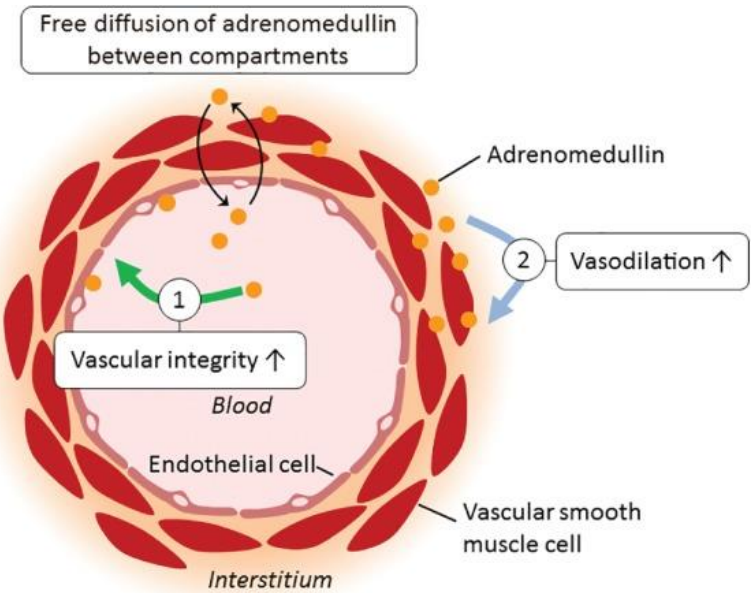

Figure 14. Adrenomedullin in heart failure. Simplistic representation of the mode of action of intravascular vs. interstitial adrenomedullin. (1) Adrenomedullin present within the blood vessels improved vascular integrity, thereby putatively reducing vascular permeability. (2) Adrenomedullin present in the interstitium acts on the vascular smooth muscle cells and causes dilatation of the vascular resistance and capacitance vessels. Reprinted with permission from Eur J Heart Fail, 21: 163-171.

Several studies have shown high levels in patient with sepsis and HF, diseases where vascular leakage and organ hypoperfusion is present. ADM expression can be induced by volume overload,[145] and loss of ADM function predisposes to increased

vascular permeability, oedema, and tissue congestion, as demonstrated in animal models lacking RAMP 2 or endothelial ADM expression.[146,147] ADM also modulates RAAS, where it suppresses aldosterone synthesis and counteracts angiotensin II-induced hypertrophy and renal injury, leading to cardioprotective effects.[148]

As shown in this thesis, there is an identified association between higher levels of bioactive ADM and progression of HF and adverse outcomes[133,149] and ADM is a strong predictor of residual congestion in AHF patients[150] and linked to increased risk of hospital readmission.[151]

Both ADM and NT-proBNP have shown prognostic value and can be used in HF patients to guide therapy. Studies suggest that integrating natriuretic peptides with ADM measurements could sharp diagnostics, but further validation in larger cohorts are needed.[152]

ADM is predominantly metabolized by neprilysin[153], a molecule included in sacubitril/valsartan, one of the most recent treatments for patients with heart failure.[3] Sacubitril is a neprilysin inhibitor, and thus is supposed to inhibit breakdown of ADM and several other peptide hormones. ADM is also cleared through binding with its receptors and subsequent internalisation and degradation.[154,155] Studies with exogenous ADM has been made primarily in patients with sepsis and could also be a therapeutic agent in HF.

Fibrosis and cardiac extracellular matrix remodelling biomarkers

Chronic stress on the heart of different origin can lead to fibrosis and cardiac remodelling causing impaired myocardial function and increased risk of arrhythmias. Candidate biomarkers involved in these pathophysiological mechanisms are:

Galectin-3

Galectin-3 (Gal-3) is a member of the lectin family, widely expressed in human tissues, acting as a galactoside-binding protein. Gal-3 is involved in many biological processes, such as controlling cell–cell and cell–matrix interactions, adhesions, proliferation, apoptosis, immunity, and inflammation.[158] In the pathology of HF, Gal-3 plays a biological role mainly through fibrosis and inflammation.[159] Gal-3 stimulates pathological remodelling and development of fibrosis, mainly by inducing fibroblast proliferation and collagen deposition, and is more of a "culprit" biomarker in HF compared to "bystanders" biomarkers, such as NT-pro BNP or C-reactive protein.[160]

The pathophysiological role of Gal-3, a β-galactoside-binding lectin, in HF is linked to its profibrotic and proinflammatory actions.[161] Gal-3 is secreted by activated

macrophages, promoting fibroblast proliferation and collagen deposition, leading to myocardial stiffness and ventricular dysfunction.[162] Preclinical studies have shown that inhibition of Gal-3 attenuates cardiac fibrosis and improves function in animal models, making it an attractive therapeutic target. Conflicting results has shown strong associations between Gal-3 and mortality, others point out that its prognostic significance diminishes when adjusted for confounding factors, such as renal function and comorbidities like diabetes.[163]

Soluble interleukin 1 receptor-like 1

Soluble interleukin 1 receptor-like 1(ST2) was first described in 1989 and is a member of the interleukin (IL)-1 receptor family. It exists both as soluble receptor named sST2 and as a transmembrane receptor, ST2L.[123,125,164,165] When the heart is subjected to stress, cardiac fibroblasts and cardiomyocytes secretes sST2 that compete with ST2L for the binding site of IL-33 and hence antagonize the cardioprotective effect of ST2L, leading to fibrosis and remodelling.[166,167]

Regarding ST2s prognostic information, in the Framingham Heart Study, which included 3428 participants, sST2 was associated with a higher risk of developing HF. [168] In acute HF, higher concentration of sST2 is associated with more severe pulmonary congestion[169] correlating with echocardiographic measures of right ventricular dysfunction and elevated central venous pressure.[170] The prognostic role of sST2 in acute HF was confirmed in a meta-analysis of 4835 patients with acute HF, which concluded that both admission and discharge sST2 were predictive of all-cause death and CV death, and that discharge sST2 predicted rehospitalisation for HF.[171]

Tissue Inhibitor of Metalloproteinases-1

Tissue Inhibitor of Metalloproteinases-1 (TIMP-1) regulates extra cellular matrix dynamics which is an important process in HF.[174] In patients with HFpEF, elevated levels reflect pathological fibrosis, leading to stiffness in the myocardium and diastolic dysfunction. In patients with HFrEF, TIMP-1 levels associate with negative ventricular remodelling and impaired cardiac function. These findings suggest a potential role as a prognostic biomarker and a therapeutic target in fibrosis modulation.[175]

Growth/differentiation factor 15

Growth/differentiation factor-15 (GDF-15), also referred to as macrophage inhibitory cytokine-1 (MIC-1) is produced trough stimulation by various cellular stressors, including inflammatory activation, myocardial ischemia, and malignancy. In individuals with HFpEF, combining measurements of GDF-15 with NT-proBNP has been shown to improve the discrimination between HF patients and healthy controls.[176,177] Elevated GDF-15 concentrations may also precede hospitalisation by up to three months and correlate positively with indices of left-ventricular

remodeling.[176,177] Experimental work suggests that GDF-15 can exert antihypertrophic actions, primarily through modulation of intracellular signalling cascade. However, the observation that higher levels of GDF-15 are linked to adverse clinical outcomes in HF raises questions about whether the molecule acts as a compensatory response or plays a role in disease progression. Although several studies support its protective and antihypertrophic functions, additional research is required to clarify these uncertainties and determine how GDF-15 might be integrated into clinical decision-making in HF.[176,177]

Inflammatory biomarkers

C-reactive protein

C-reactive protein (CRP) and its role in HF was first demonstrated back in 1953. Following, multiple studies have emphasized the prognostic value of inflammatory markers such as C-reactive protein (CRP), interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) in HF. These proteins all play a key role in the inflammatory pathways in HF. and have been associated with incident HF in elderly. However, their clinical utility remains limited due to their low specificity for HF and cardiovascular diseases. Also, no effective anti-inflammatory treatments specifically approved for HF exist.[178,179]

Carbohydrate antigen-125

Carbohydrate antigen-125 (CA125) is a protein involved in fluid and cell transport, inflammation, tissue repair, and the well-known tumour dissemination, known for its use in ovarian cancer. A recent published review, covering all studies of CA-125 in HF highlight an association of CA-125 with congestion in acute HF, high rates of mortality, rehospitalisation at 6 months and a role of CA-125 in guiding HF therapy.[180] Some studies report CA-125 being even better then NT-proBNP in different scenarios of acute HF. Several studies have reported increased levels of numerous tumour biomarkers, including CA 125 in HF patients before and after heart transplant.[181,182] Further studies are needed to implement CA-125 in clinical practice.

Proteomic technologies and analytical methods

By analysing proteins in tissue and body fluids, proteomics provides a broad understanding of the molecular mechanisms underlying HF and enables the identification of biomarkers that inform diagnosis, risk stratification and therapeutic strategies. Two main strategies are used, discovery proteomics and targeted

proteomics, each of which offers unique insights into protein function and disease mechanisms (Figure 15).

Discovery proteomics

Discovery proteomics employs an untargeted analytical strategy aimed at the simultaneous identification and characterisation of large numbers of proteins. The workflow typically comprises sample preparation, protein separation, molecular identification, and subsequent validation. Separation is commonly achieved using gel electrophoresis or high-resolution liquid chromatography (HPLC), which resolve proteins according to physicochemical properties such as molecular weight, charge, and affinity. Mass spectrometry (MS) is then applied to determine peptide mass-to-charge ratios (m/z), enabling protein identification and structural characterisation.[183,184] Top-down proteomics analyses intact proteins, whereas bottom-up proteomics relies on enzymatic digestion of proteins into peptides, yielding detailed sequence information. These approaches, combined with advanced MS modalities, have facilitated the discovery of numerous HF-related biomarkers.[185-187] In addition to mass spectrometry–based strategies, large-scale antibody-based screening platforms may also contribute to discovery analyses.

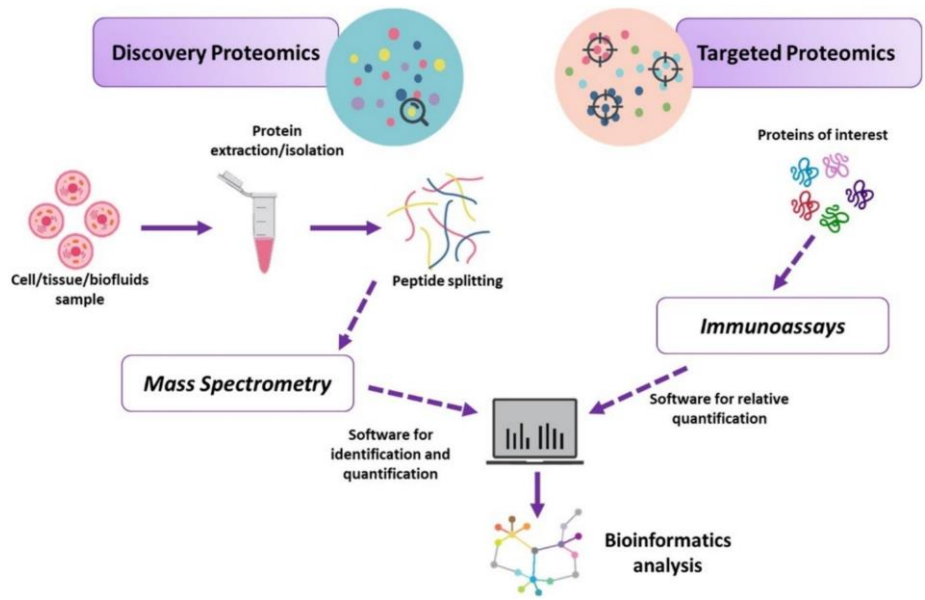

Figure 15. Schematic representation of the proteomic analysis approaches used in heart failure research. The figure illustrates the two main proteomics methods: discovery proteomics, which uses high-throughput techniques such as mass spectrometry (MS) to identify and characterize a large number of proteins without prior selection, and targeted proteomics, which focuses on the precise quantification of specific proteins. These approaches contribute to the identification of novel biomarkers and enable improved diagnosis, risk stratification and therapy monitoring in patients with heart failure. Reprinted with permission from Journal of Molecular and Cellular Cardiology Plus, Volume 11, 2025.

Targeted proteomics

Targeted proteomics enables precise quantification of selected proteins, bridging the gap between discovery and clinical application. Immuno-PCR–based methods, such as proximity extension assays (PEA), combine antibody specificity with DNA amplification, allowing sensitive, multiplexed detection from minimal sample volumes. While limited to available antibody targets and providing relative rather than absolute quantification, these platforms are well suited for large-scale clinical profiling. Robust biomarker validation remains essential to translate proteomic findings into clinically useful tools, for example in heart failure.[188]

Aptamer-based analysis

In 2010, an aptamer-based multiplex proteomic technology, the SOMAscan assay, was introduced, enabling the simultaneous measurement of approximately 1300–5000 human proteins (2026 the number is around 11 000 from 55 µL blood sample.[189,190] Aptamers are short DNA or RNA oligonucleotides that bind proteins with relatively low affinity and are intrinsically susceptible to nuclease degradation.[189] SOMAmers exhibit high epitope-level specificity for many human proteins.[191,192] In the SOMAscan workflow, SOMAmer–protein complexes are captured on biotin–streptavidin beads, nonspecific binders are removed with a polyanionic buffer, and specific SOMAmers are subsequently released by denaturation, hybridized to complementary microarray probes, and quantified by fluorescence.[189,191,192] This assay was used in paper I and III.

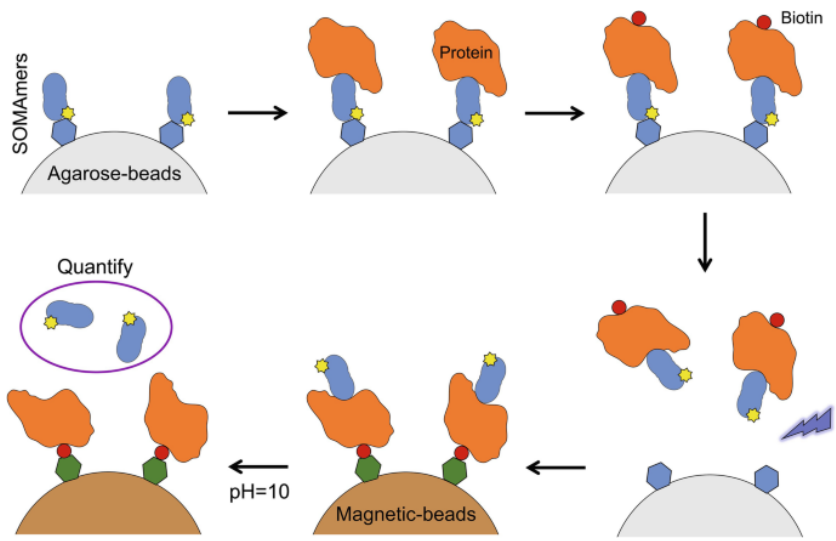

Figure 16. Aptamer-based proteomic assay (SOMAscanTM). Reprinted with permission from Methods in Molecular Biology, vol 2044.

Sandwich immunoassay analysis

In paper II, quantification of bio-ADM was performed using the Sphingotest® bio-ADM® immunoassay (SphingoTec GmbH, Hennigsdorf, Germany). This assay is an in-vitro diagnostic method designed for the quantitative determination of the biologically active form of adrenomedullin (ADM 1–52) in human EDTA plasma, based on a non-automated chemiluminescent sandwich immunoassay (immunoluminometric assay, ILMA) performed in a microtiter plate format.

Plasma samples are incubated with two specific monoclonal antibodies directed against distinct epitopes of the ADM 1–52 peptide. One antibody is immobilised on the solid phase, while the second is labelled with a chemiluminescent marker that enables quantitative detection of the antigen (antibody complex). The emitted light intensity, measured by a luminometer, is proportional to the concentration of bio-ADM in the sample.

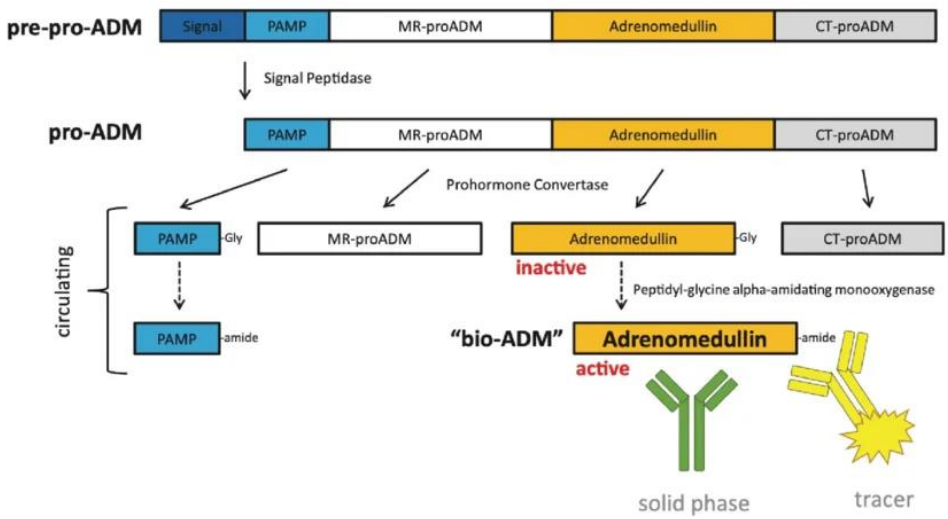

Figure 17. Scheme for biogenesis and measurement of bio-ADM. The various processing steps, which lead from the primary translational gene product pre-pro-ADM to biologically active ADM (bio-ADM). First in the signal sequence is clipped off. The resulting pro-ADM is then proteolytically cleaved in 4 fragments (PAMP, proadrenomedullin NH2-terminal 20 peptides; MR-proADM, midregional proadrenomedullin; Adrenomedullin-Gly, C-terminally glycine-extended adrenomedullin; CT-proADM, C-terminal proadrenomedullin). The resulting C-terminally glycinated ADM is biologically inactive and is converted into the biologically active C-terminally amidated ADM (bio-ADM). Using highly specific monoclonal antibodies directed against the middle portion of ADM and the amidated C-terminus, bio-ADM can be detected with an immunometric assay. Reprinted with permission from Eur J Emerg Med. 2022;21(2): 79-85.

Precision medicine

Modern medicine has the last decade shifted its focus from treating patients according to a one-size-fits-all paradigm toward a more individualised approach. This development reflects the long-standing medical ideal of treating the person rather than merely addressing the disease. At the forefront of this transformation is precision medicine, a clinical strategy that takes into account differences in people's genes, environmental exposure, and lifestyle with the aim to diagnose, and target the right treatment and prevention strategy to the right patient at the right time.

While precision medicine has already made substantial advances in oncology, its application in cardiovascular diseases, particularly heart failure, is gaining momentum. The complex and heterogeneous nature of heart failure with different aetiologies and diverse clinical trajectories, presents an ideal setting for the implementation of precision-based approaches. Patients with similar clinical presentations may exhibit markedly different molecular profiles, risk factors, and therapeutic responses, underscoring the need for tailored management.

The development of omics technologies, including genomics, transcriptomics, epigenomics, proteomics, metabolomics, and microbiomics, has significantly improved our understanding of the molecular processes involved in heart failure. Today, high-throughput platforms generate large-scale data, enabling a more nuanced understanding of disease biology. When combined with advanced computational tools and big data analytics, these technologies facilitate deep clinical and molecular phenotyping, paving the way for early detection, improved risk prediction, and more targeted therapeutic interventions with minimized adverse effects in patients with HF.

Aims of the thesis

The overall aim of this thesis was to expand understanding of proteins involved in the complex pathophysiology of heart failure, with a particular focus on their relationship to congestion and hemodynamic disturbances which may be of particular clinical importance. By evaluating selected proteins of interest, the thesis further sought to determine their potential value as clinically useful biomarkers capable of reflecting key physiological states relevant to patient assessment and management. In addition, the thesis aimed to evaluate the use of scalable and integrated multi-omics platforms for biomarker discovery. Given the multifactorial and heterogeneous nature of heart failure, it is unlikely that any single biomarker can provide sufficient information to meet all clinical needs regarding prognosis, risk stratification, or prediction of treatment response. Therefore, a broader systems-level approach may be necessary to capture the full biological complexity of the disease.

The specific aims of the included studies were:

- Paper I: To systematically assess molecular patterns of HF across key stages: decades before onset of symptoms, during manifest disease, advanced HF and after reversal of heart failure by heart transplantation.
- Paper II: To evaluate the diagnostic and prognostic performance of bio-ADM in HF patients with congestion, in comparison to NT-proBNP.
- Paper II: To explore the potential of bio-ADM as a biomarker of systemic venous congestion and derive potential decision thresholds from invasive hemodynamic and population-based studies.
- Paper III: To identify a panel of circulating plasma proteins as a representation of central hemodynamics in HF, which may enable non-invasive hemodynamic monitoring and offer insights into the molecular mechanisms underlying HF progression.

Methods and materials

Paper I

Study populations and design

The first study was based on three main cohorts of different design:

First, a random subset of 583 individuals was selected from the population-based Malmö Diet and Cancer-Cardiovascular Cohort (MDC-CC). The MDC-CC is a randomly selected subcohort of the Malmö Diet and Cancer Study (MDCS). MDCS was a prospective cohort study that enrolled 30 447 men (born 1923–1945) and women (born 1923–1950) residing in Malmö, southern Sweden. All participants in the MDCS underwent baseline examinations between 1991 and 1996, which included measurements of height, weight, and blood pressure, as well as completion of a comprehensive questionnaire.[193] From the larger cohort, 6103 individuals were randomly selected to participate in the MDC-CC, a study focusing on cardiovascular risk factors. In addition to peripheral venous blood sampling, data on smoking status, diabetes mellitus, and the use of antihypertensive and antidiabetic medications were obtained through a questionnaire. Diabetes mellitus was defined as fasting blood glucose > 6.0 mmol/L, self-reported physician diagnosis, or use of antidiabetic medication. To ensure comparability with the wider MDC-CC cohort, baseline characteristics were assessed and found to be consistent. Subjects who developed incident HF during follow-up (n = 185) were identified through nationwide Swedish registers, based on International Classification of Disease codes, for which high case validity have previously been observed.[194]

Second, a cohort of 84 patients with manifest HF were included from a nurse-led HF management program during 2013–2014. Peripheral venous blood samples were collected as part of an ongoing biobanking project at Skåne University Hospital (the Lund Cardiopulmonary Register).

Third, a cohort of 30 patients with advanced HF was included, with blood sampling at the time of RHC between 2012 and 2016. RHC was performed as part of the routine pre-transplant assessment at Skåne University Hospital, Lund, and stored in the Lund Cardiopulmonary Register until used. Follow-up samples from the same individuals were collected at the 6-month post-transplant evaluation which also includes RHC.

We confirmed that there was no overlap of participants across the three cohorts. Figure 18 below provides a schematic overview of HF progression and illustrates how the included cohorts represent different stages of the disease.

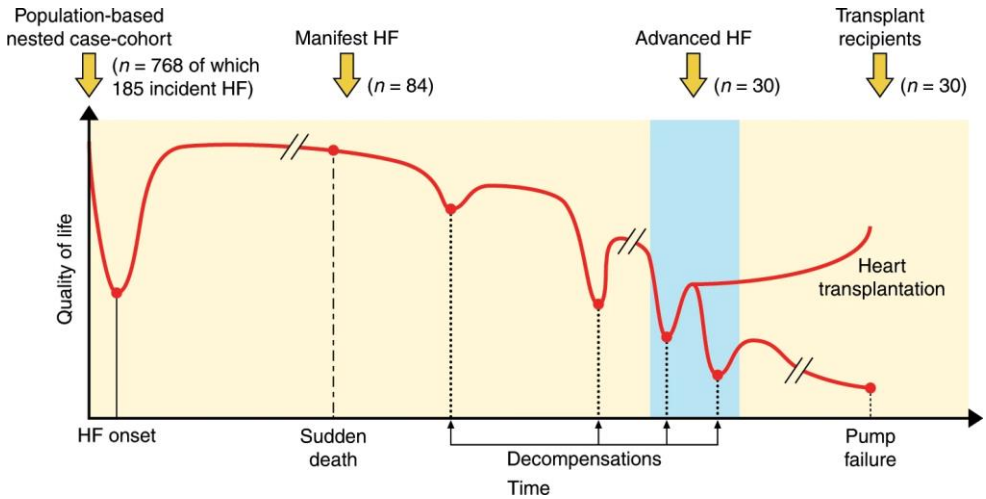

Figure 18. Sampling of study cohorts from different stages of heart failure. Schematic representation of the natural history of heart failure (HF) progression, from the initial timepoint of disease onset through a gradual decline with increasing episodes of worsening typically necessitating in-hospital care (decompensations) towards terminal pump failure. Sudden cardiac death may occur at any time. Cohorts for the current study were obtained from four stages: before disease onset (population-based cohort), during manifest HF, and serially in advanced HF before and after heart transplantation. Proteomic profiles in subjects with incident HF from the population-based cohort were compared to subjects without incident HF. Manifest HF patients were compared to the population-based cohort. Samples from heart transplant recipients were compared to the corresponding samples from the same patient before transplantation. Reprinted with permission.

To validate associations identified in relation to manifest HF, residual blood samples obtained during routine clinical care, samples that would otherwise have been discarded, which were collected between September 2014 and September 2015 within BioVU, the Vanderbilt University Medical Centre biorepository. This validation cohort comprised 48 HF cases and 47 control individuals without HF or other cardiovascular disease.

To further investigate the cardiac origin of selected proteins, paired blood samples were obtained simultaneously from the coronary sinus and the femoral vein in six patients with hypertrophic obstructive cardiomyopathy immediately prior to alcohol septal ablation at Massachusetts General Hospital in Boston, MA, USA.[195]

Proteomic analyses

Plasma samples from the five cohorts were randomly allocated across 96-well plates and analysed using a multiplex proteomic platform based on modified DNA

aptamers (slow-off rate modified aptamers, SOMAmers). Protein abundance was quantified as relative fluorescence units (RFU) using oligonucleotide microarrays (Agilent Technologies, Santa Clara, CA) (Figure 16).[192,196] The SOMAscan platform (SomaLogic Inc., Boulder, CO) enabled measurement of 1305 proteins at that time point, including secreted proteins (47%), extracellular domains (28%), and intracellular proteins (25%), thereby offering broad coverage across diverse biological pathways. A detailed description of the assay has been provided in the background section of this thesis.

Samples from the alcohol septal ablation cohort and the BioVU validation cohort were assayed using an earlier version of the SOMAscan assay containing aptamers targeting 1129 proteins, of which 1061 overlapped with the more recent version.[197] Each sample was diluted to three concentrations (40%, 1%, 0.05%) to enable accurate quantification of proteins across a dynamic range spanning eight orders of magnitude. Normalisation procedures included: (i) hybridization correction using 12 control sequences on each microarray, and (ii) median signal normalisation for each dilution level to adjust for inter-plate variability. Samples were accepted if their median signal fell within 0.4–2.5 of the plate median, consistent with historical performance.

Each SOMAmer was calibrated using five pooled human plasma samples on each 96-well plate. Plate-level calibration required that 95% of SOMAmers deviated by less than 0.4 from the plate median. High specificity and reproducibility of SOMAmer-based measurements have been previously demonstrated.[195,198]

Spatial transcriptomic analysis of human hearts

Spatial transcriptomic profiling was performed on tissue sections from two explanted hearts obtained from patients with ischemic cardiomyopathy and hypertrophic cardiomyopathy undergoing cardiac transplantation. Samples were frozen immediately after explantation, cryosectioned, and placed on Spatial Transcriptomics Library Preparation slides (Spatial Transcriptomics, Stockholm, Sweden) containing positional barcoded reverse-transcription primers, as described previously.[199]

Sections were fixed, stained, and imaged prior to tissue permeabilization, RNA hybridization, and cDNA synthesis. cDNA libraries were sequenced on an Illumina NextSeq 500 platform, generating 50 million paired-end reads (125 bp). Reads were aligned to the human reference genome using the ST pipeline (v1.6.2). Spatial alignment of histological images with array features was performed using ST Spot Detector Software. Variability across spatial features and between patients was explored for proteins with evidence of cardiac origin, and colocalization analysis was performed with transcripts specific to defined cardiac cell types.

Genome-wide association studies

Genotypes were available in 1421 subjects from the MDCS with SOMAscan, based on genome-wide genotyping. These subjects represented the 583 random cohort subset and 185 heart failure cases described here, and additional case subsets with cardiometabolic disease not contributing to the current study. Genotypes were called using Illumina GenomeStudio, and imputation was performed to the 1000 Genomes phase 1 version 3 (August 2012) reference panel using IMPUTE v2 for SNPs passing the following criteria: call rate ≥ 95%, pHWE ≥ 1×10^{-6}, and minor allele frequency ≥0.01.

Statistical methods

Of the 1305 quantified proteins, 912 (70%) exhibited a skewness coefficient >2, and visual inspection of histograms confirmed substantial right-skewness. Consequently, all protein concentrations were natural logarithm transformed to ensure distributional consistency. Protein levels were additionally standardised to a mean of 0 and a standard deviation of 1 to facilitate interpretation of effect sizes.

Associations between individual proteins and incident HF were evaluated using Cox proportional hazards regression models including 583 population-representative controls and 185 HF cases. Models were adjusted for age and sex and incorporated Prentice weighting to account for the case–cohort sampling design, with robust variance estimators applied.[200,201] Statistical significance was defined using a Bonferroni-adjusted threshold ($p < 3.8 \times 10^{-5}$), reflecting correction for 1305 independent tests. Independence from established cardiovascular risk factors, including body mass index, blood pressure (systolic, diastolic, and antihypertensive treatment), lipid concentrations (HDL and LDL), smoking, diabetes mellitus, and coronary heart disease, was examined through sequential multivariable adjustment, as described previously.[194]

A sensitivity analysis restricted to nonischaemic HF was conducted by censoring follow-up at first myocardial infarction, yielding 142 HF cases. Additional analyses confirmed independence of associations from renal function, storage time, and sample plate. Assumptions of normality for log-transformed proteins were evaluated by histogram inspection, and the proportional hazards assumption was assessed using Schoenfeld's global test. Pairwise correlations between proteins were estimated using Pearson correlation coefficients.

Associations with manifest HF were assessed using unconditional logistic regression comparing 84 HF cases with 583 population-based controls. Variance estimates were derived using bootstrap resampling, with models adjusted for age and sex. A secondary multivariable model additionally accounted for body mass index, estimated glomerular filtration rate, diabetes, and smoking status. External validity was evaluated by comparing odds ratios per standard deviation of log-

transformed RFU values, and their directional consistency, with results obtained from the BioVU cohort (48 HF cases, 47 controls).

Serial measurements in 30 heart transplant recipients were analyzed using the Wilcoxon signed-rank test. For proteins associated with incident HF, cardiac enrichment was explored descriptively using paired coronary sinus and peripheral venous plasma samples from six patients. Analyses were performed using STATA version 12 (StataCorp, College Station, Texas, USA).

Pathway-level insights were obtained using gene-set enrichment analysis (GSEA)[202] to test whether proteins associated with manifest HF were enriched for hallmark gene sets curated in the Molecular Signatures Database. Significance was evaluated using hypergeometric tests, and false discovery rate (FDR) q values were calculated using the Benjamini–Hochberg procedure.[203] Hallmark gene sets represent a refined, less redundant subset of functional annotations facilitating reproducibility.[204]

Genome-wide association analyses for protein quantitative trait loci (pQTLs) were conducted using general linear models assuming an additive genetic model, implemented in the R package GWAF v2.2.58. Genome-wide significance was defined as $p < 3 \times 10^{-9}$, corresponding to Bonferroni correction for one million independent variants across 16 proteins. A second-stage meta-analysis was performed with data from 759 participants in the Framingham Heart Study[26] for the eight proteins without genome-wide significant cis-pQTLs in the MDC cohort. For each protein, SNPs within ±1 Mb of the transcription start site with MAF > 0.05 and imputation quality >0.3 were included (2,894 SNPs in total). Bonferroni-corrected significance for this stage was set at $p < 2 \times 10^{-5}$.

Paper II

Study design and Cohorts

The second study was also based on three independent cohorts:

First, to establish normal reference values for bio-ADM, individuals from the Malmö Preventive Project (MPP) were used. MPP is a community-based lifestyle intervention program initiated in the early 1970s, in which 18 240 participants were re-examined between 2002 and 2006. During this phase, cardiovascular risk factors were assessed, and fasting plasma samples were obtained. From this population, a random sample of 5060 individuals was selected for the present analyses. Estimated glomerular filtration rate (eGFR) was calculated from serum creatinine using the CKD-EPI formula. A healthy sub-cohort was also derived from the MPP cohort, in order to explore how reference intervals vary with disease burden and risk factors. After exclusion of participants with a history of heart failure, atrial fibrillation, stroke, ischemic heart disease, diabetes, smoking, BMI >30 kg/m², hypertension, or

eGFR <60 mL/min/1.73 m², a total of 1128 subjects remained for reference intervals.

Second, to evaluate bio-ADM levels in relation to invasive hemodynamic measures, a cohort of 346 patients with HF undergoing right heart catheterization (RHC) at Skåne University Hospital in Lund between August 2011 and January 2019 was included. RHC was performed using a Swan-Ganz catheter (Baxter Healthcare Corp., Santa Ana, CA, USA) as part of transplant evaluations or diagnostic workup and was integrated into an ongoing biobanking project, Lund Cardiopulmonary Register (LCPR). A subset of 47 of these patients later underwent heart transplantation. In these individuals, a pre-transplant plasma sample was obtained during RHC at a median of 142 days before transplantation (range 4–1218 days), and a follow-up sample was collected using the same procedures during the routine 6-month endomyocardial biopsy visit (median 167 days; range 26–409 days).

Third, to assess the diagnostic and prognostic performance of bio-ADM in the context of a clinical dyspnea presentation, patients were extracted from the previously described[205] acute dyspnea (ADYS) cohort consisting of 1710 individuals aged ≥18 years presenting with dyspnea at the emergency department of Skåne University Hospital in Malmö on weekdays between December 2013 and July 2018. After written informed consent, a research nurse collected clinical data through structured interviews and medical record review, and blood samples were obtained through peripheral venepuncture. Complete data on both bio-ADM and clinical characteristics were available in 1539 patients.

Proteomic analyses

Bio-ADM was measured using a sandwich immunoassay from SphingoTec GmbH (Hennigsdorf, Germany), by an external laboratory blinded to clinical characteristics.[206] This biomarker is unique in measuring bio-ADM, and was used in several previous studies of this biomarker.[150,151,207-212] The lower limit of detection (LOD) is 3 pg/mL and the limit of quantification (LOQ) is 8 pg/mL, with interassay and intraassay coefficients of variation of 5–10% and 4–8%, respectively.[206]

N-terminal pro-B-type natriuretic peptide (NT-proBNP) was measured by a common clinical electrochemiluminescence sandwich immunoassay, in HF-RHC as part of routine clinical care through the department of clinical chemistry on the fully automated Elecsys E170 analyser, and in ADYS on a Cobas e411 analyser (both from Roche Diagnostics, Indianapolis, IN). Low intra-assay and individual variability have been described for both assays.[213]

Collection of clinical and hemodynamic data

RHC was performed in the supine position under local anaesthesia, with the catheter introduced through the right internal jugular vein using the Seldinger technique. The catheter was advanced to the pulmonary artery guided by ultrasound and

fluoroscopy, and the catheter position was verified by identifying the signature pressure curves. Pressure measurements were obtained in the pulmonary arterial wedge position (pulmonary arterial wedge pressure, PAWP), pulmonary artery (mean pulmonary arterial pressure, mPAP), right ventricle (diastolic right ventricular pressure, RVPd), and atrium (mean right atrial pressure, mRAP). Cardiac output was measured by thermodilution as the average of three measurements and indexed to body surface area as cardiac index.

Stroke volume index (SVI) was computed by dividing cardiac output by the heart rate. The mixed venous oxygen saturation (SvO2) was measured in blood drawn from the pulmonary artery on an ABL 90 Flex (Radiometer Medical ApS, Copenhagen, Denmark). Elevated filling pressures were defined based on published studies as mRAP >10 mmHg and PAWP >15 mmHg, while pulmonary hypertension was defined as mPAP >25 mmHg in accordance with guidelines from the European Society of Cardiology.[214] A cut-off of PAWP >18 was also explored, as many patients in a chronic context have higher values.

Statistical methods

First, the population distribution of bio-ADM in the MPP cohort was examined using histograms, which revealed a truncated pattern (Figure 19A), with a substantial proportion of samples clustered near the lower limit of quantification (LLOQ) and approximately 10% falling below the assay's limit of detection. Because this degree of left-censoring may bias conventional linear models, associations between clinical covariates and log-transformed bio-ADM were estimated using Type 1 Tobit regression, which treats values below the LLOQ as censored rather than missing and thereby yields unbiased parameter estimates. Reference intervals were subsequently derived using percentile-based methods with bootstrap resampling in both the full cohort and the healthy subcohort.

Second, the distribution of bio-ADM in the HF-RHC cohort was assessed and demonstrated less truncation but considerable right-skew (Figure 19B). Consequently, natural logarithm transformation was applied, and associations between log-transformed bio-ADM and invasive hemodynamic variables were evaluated using general linear models and Pearson correlation coefficients. These analyses were performed in parallel with log-transformed NT-proBNP for comparative purposes.

To determine whether bio-ADM longitudinally tracked changes in hemodynamic congestion, analyses were extended to the subgroup of patients who underwent repeat RHC 6 months after heart transplantation. Mixed-effects regression models were employed to account for the non-independence of repeated biomarker measurements within individuals. This approach enabled estimation of both overall post-transplant changes and patient-specific variation.

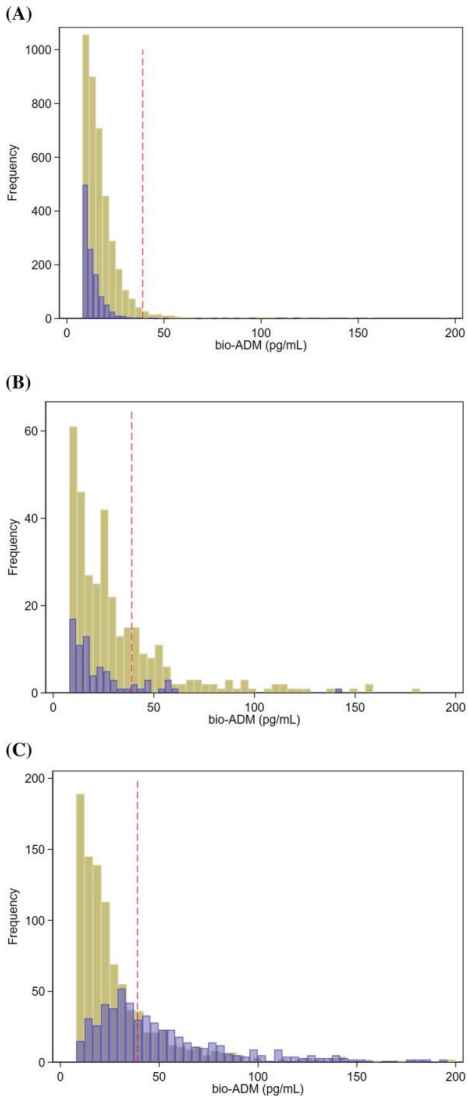

Figure 19. Bio-ADM distribution across cohorts. Distribution of bioactive adrenomedullin (bio-ADM) in the three study cohorts. For all cohorts, the dashed line indicates the upper reference threshold based on the MPP cohort (39 pg/mL), which also corresponds to the decision cut-off for elevated mRAP. (A) The MPP cohort distribution of bio-ADM in the entire cohort (n = 5060, yellow) and healthy subcohort (n = 1128, blue). Truncation of the distribution was observed at the lower limit of detection (8 pg/mL). A normal reference interval was derived from 2.5 and 97.5 percentiles through bootstrap resampling (8 [95% CI 8–8] to 39.1 [95% CI 36.5–41.8] for entire cohort, 8 [95% CI 8–8] to 29.5 95% [CI 25.6–33.7]) in the healthy subcohort. Fourteen positive outliers were excluded from the plot (max. 283 pg/mL). The proportion of subjects over the cut-off of 39 pg/mL was 2.5% in the full cohort (127 subjects) and 1.1% (12 subjects) in the healthy subcohort. (B) HF-RHC cohort. Distribution of bio-ADM at baseline in the entire cohort (n = 346, yellow) and in a subset of patients after transplantation (n = 47, blue). The proportion of patients over the cut-off of 39 pg/mL was 27.5% (95 patients) in the entire cohort, 29.8% (14 patients) before transplantation, and 19.2% (nine patients) after transplantation. (C) ADYS cohort. Distribution of bio-ADM in the entire cohort (n = 1534) (yellow) and in the subset with a final diagnosis of HF (n = 570) (blue). The proportion of patients over the cut-off of 39 pg/mL was 33.3% (510 patients) in the entire cohort and 54% (308 patients) in HF patients. Reprinted with permission.

Restricted cubic splines were used to model the relationship between bio-ADM and venous pressure measures, allowing for potential nonlinear associations while preserving model interpretability. Knots were placed at the percentiles recommended by Harrell, namely the 5th, 27.5th, 50th, 72.5th, and 95th percentiles of the congestion markers.[215] The discriminative ability of bio-ADM for elevated venous pressures was assessed using the area under the receiver operating characteristic (AUROC) curve. Cut-offs were derived based on maximization of sensitivity and specificity as well as Youden's J statistic.

Finally, bio-ADM cut-offs derived from the population-based and hemodynamic cohorts were applied in the ADYS cohort, alongside bio-ADM quartiles defined internally. As bio-ADM exhibited a skewed distribution (Figure 19C), values were natural logarithm transformed for regression analyses. Associations between bio-ADM and acute HF diagnosis, markers of congestion, and clinical outcomes were evaluated using logistic regression and Cox proportional hazards models. Model performance and AUROC values were compared with those obtained for NT-proBNP, and additional models adjusted for NT-proBNP were fitted. Differences in survival across bio-ADM thresholds were visualized using Kaplan–Meier curves. All analyses were performed using STATA SE 15 (StataCorp, College Station, TX, USA).

Paper III

Study design and Cohorts

For the third study, we included a cohort of 30 patients with HF who underwent RHC as part of their pre-transplant evaluation at Skåne University Hospital, Lund, Sweden, between August 2012 and January 2016. All patients were invited to contribute plasma samples to an ongoing biobanking initiative, the Lund Cardio-Pulmonary Register. Hemodynamic evaluation was performed using a Swan–Ganz catheter (Baxter Healthcare Corp., Santa Ana, CA, USA).

Only patients residing within the secondary-care catchment area of Skåne University Hospital, thus routinely scheduled for follow-up at the same institution, were eligible for inclusion. Alongside hemodynamic measurements, three endomyocardial biopsies were obtained concurrently. None of the patients showed evidence of graft rejection at the time of sampling.

Hemodynamic measurements

RHC was conducted in the supine position under local anaesthesia. The catheter was introduced via the right internal jugular vein using the Seldinger technique, advanced into the pulmonary artery under ultrasound and fluoroscopic guidance, and its final position verified through characteristic pressure waveforms. Pressures were recorded in the pulmonary arterial wedge position (pulmonary arterial wedge pressure, PAWP), pulmonary artery (mean pulmonary arterial pressure, mPAP), right ventricle (right ventricular diastolic pressure, RVPd), and right atrium (mean right atrial pressure, mRAP).

Cardiac output was measured by thermodilution as the mean of three determinations and indexed to body surface area to obtain the cardiac index (CI). Stroke volume index (SVI) was calculated by dividing cardiac output by heart rate. Mixed venous

oxygen saturation (SvO_2) was obtained from pulmonary artery blood samples analysed on an ABL90 Flex (Radiometer Medical ApS, Copenhagen, Denmark).

Elevated filling pressures were defined according to published criteria (mRAP >10 mmHg; PAWP >15 mmHg), whereas pulmonary hypertension was defined as mPAP >25 mmHg, in accordance with the guidelines of the European Society of Cardiology.[216] An alternative threshold of PAWP >18 mmHg was also evaluated, given its relevance in chronic HF cohorts.

Proteomic analyses

Aptamer-based proteomic profiling was performed on serial plasma samples drawn from central venous catheters. Samples were centrifuged at 2000 g for 10 minutes, aliquoted, and stored at –80 °C until analysis without prior thawing. Aliquots were randomly assigned to 96-well plates and analysed using a high-multiplex aptamer-based assay (SOMAscan, SomaLogic Inc., Boulder, CO), which relies on modified slow-off rate aptamers (SOMAmers) for protein capture and detection via oligonucleotide microarrays (Agilent Technologies, Santa Clara, CA). This platform quantified 1305 proteins across diverse biological pathways and molecular classes, including secreted proteins (47%), extracellular domains (28%), and intracellular proteins (25%). The analytical procedure is described in detail in the background section.

Statistical methods

Of the 1305 quantified proteins, 912 (70%) exhibited a skewness coefficient >2, consistent with pronounced right-skew; thus, all protein concentrations were natural log transformed. Protein levels were subsequently standardized to a mean of 0 and standard deviation of 1 to facilitate interpretability.

To identify candidate proteins reflecting cardiac hemodynamics, each of the 1305 proteins was assessed for its longitudinal association with 30 hemodynamic traits, including indices of cardiac workload and contractility (CI, SVI), left ventricular preload (PAWP) and afterload (SVRI), right ventricular preload (mRAP) and afterload (PVRI), arterial pressures (MAP, mPAP), overall cardiac output (CI), and circulatory efficiency (SvO_2). A linear mixed model was used with age, sex and protein level as fixed effects and patient as random effect intercept and slope. Statistical significance was defined using Bonferroni correction for multiple testing across 1305 proteins and 30 traits, yielding a significance threshold of $p < 1.28 \times 10^{-6}$. Linear relationships were verified by inspection of scatterplots for all significant proteins.

To derive a subset of maximally informative proteins with orthogonal contributions among those meeting Bonferroni significance criteria, a supervised machine-learning approach based on gradient-boosted decision trees with mixed effects was applied (GPBoost R package).[217] This method was selected due to its ability to

model non-linear relationships, account for repeated measurements obtained before and after heart transplantation, and incorporate random effects to accommodate within-individual correlation structures. GPBoost integrates tree-based boosting with Gaussian-process components, making it well suited for the high-dimensional proteomic dataset.

Discriminatory performance of the most informative biomarkers for hemodynamic parameters was evaluated using the area under the receiver-operating characteristic curve (AUROC). All statistical analyses were performed using R version 4.2.2 (R Foundation for Statistical Computing) or Stata/SE 15 (StataCorp, College Station, TX, USA).

Ethical aspects

All studies included in this thesis were conducted in accordance with the principles outlined in the Declaration of Helsinki, and informed consent was obtained from all participants across the eight cohorts. While such ethical compliance is often regarded as self-evident, the rapid evolution of multi-omics research and the increasing digitalisation of healthcare introduce new and complex ethical challenges that extend beyond traditional regulations that clinicians and researchers are used to work with.

As genomic sequencing and large-scale proteomic profiling become more accessible and cost-effective, the potential to longitudinally monitor molecular changes within individual patients is becoming a realistic future. However, this raises significant ethical questions concerning data ownership, privacy, and the boundaries of clinical intervention. Clear information and responsible data management are therefore essential. Participants need to understand not only the purpose of the current study, but also how their data may be stored and protected. They must also be informed about potential use in future research, including secondary analyses and long-term biobanking. Careful handling of sensitive data and transparent communication are central to protecting patient autonomy and maintaining trust in research.

Results

Paper I

Baseline characteristics and study cohort

In study I, plasma samples from three cohorts underwent proteomic analysis. The baseline characteristics for all cohorts are presented in Table 4. Participants who later developed HF (n=185) in the MDC-CC cohort, with a median follow-up of 20.2 years (range 0.36-22.2; median time to HF diagnosis 14.6 years), exhibited an adverse cardiometabolic profile at baseline, characterized by higher body mass index and blood pressure, as well as an increased prevalence of diabetes mellitus, atrial fibrillation, and previous myocardial infarction compared with those who remained free of HF (n=583). Plasma concentrations of NT-proBNP, CRP, and creatinine were elevated, indicating subclinical cardiac strain, systemic inflammation, and early renal dysfunction.

Risk estimates for incident HF of individual risk factors in the nested subcohort (n=583), based on Prentice-weighted Cox proportional hazards regression, were consistent with unweighted estimates from the full MDC-CC cohort (n=6103), confirming the robustness of the associations across the population sample.

The hospital-based cohort with manifest HF patients had a lower age at diagnosis and burden of comorbidities than the population-based incident cases from the MDC-CC. As expected, NT-proBNP levels were more than tenfold higher in manifest HF than in individuals at baseline who later developed the disease. Patients with advanced HF awaiting heart transplantation, had further lower age, burden of comorbidities and higher NT-proBNP.

Protein characteristics and pathway representation

In total, 1305 proteins were analysed, were 50% of proteins on the assay were annotated as secreted in the Human protein Atlas (HPA), 30% as intracellular, and the remainder as membrane bound. The representation of established biological pathways by the 1305 proteins was explored based on functional annotations in the biological process component of the Gene Ontology[218] project database (v2.1, accessed on April 12, 2018). Nearly half, 5527 (46%) of the total of 11897 biological processes included at least one of the 1305 proteins on the aptamer-based assay.

Table 4. Baseline characteristics.

Baseline characteristics					
	Full MDC-CC (n=6103)	Nested MDC-CC subcohort (n=583)	Incident MDC-CC cases (n=185)	Manifest HF (n=85)	Heart transplant recipients (n=30)
Baseline age (years)	57.48 (5.93)	56.97 (5.76)	61.25 (4.76)	65.79 (12.04)	51.14 (9.97)
Age at diagnosis (years)	74.75 (6.93)	-	74.71 (6.93)	-	-
Male sex (n[%])	2572 (42.14%)	237 (40.58%)	106 (56.99%)	65 (77.38%)	23 (76.7%)
BMI (kg/m2)	25.77 (3.99)	25.42 (3.69)	27.76 (4.49)	27.66 (4.49)	26.52(4.40)
SBP (mmHg)	141.38(19.08)	138.53 (18.11)	149.31(19.90)	123.85(20.80)	100.20(13.56)
DBP (mmHg)	86.98(9.46)	85.52(9.00)	89.96(9.47)	74.90(11.95)	71.55(9.96)
Antihypertensive treatment (n[%])	1115(18.27)	86(14.73)	77(41.40)	-	-
LDL cholesterol (mmol/L)	4.16(0.99)	4.12(0.97)	4.15(1.01)	-	-
History of MI (n[%])	102(1.67)	9(1.54)	19(10.22)	15(17.85)	3(10%)
History of MI at diagnosis (n[%])	79(25.16)	-	43(23.12)	-	-
History of AF at baseline (n[%])	58(0.95)	3(0.51)	9(4.84)	33(39.29)	14(46.67)
History of MI at diagnosis (n[%])	145(44.34)	-	87(46.77)	-	-
History of diabetes (n[%])	547(8.96)	53(9.08)	45(24.32)	12(14.29)	3(10)
Current smoking (n[%])	1,620(27.99)	159(28.24)	49(28.00)	7(8.33)	0
NT-proBNP pg/mL	61.00(34.00-112.43)	60.00(32.14-100.52)	98.23(52.14-212.01)	1036.00(474.00-2922.00)	3997(2682.75-4929.75)
CRP mg/L	1.15(1.07-1.32)	1.14(1.06-1.28)	1.25(1.12-1.62)	-	-
Creatinine (μmol/L)	84.76(16.31)	84.41(13.10)	86.09(17.19)	107.14(34.42)	117.07(47.00)

Characteristics of participants in each of the five study cohorts: (i) the cardiovascular cohort of the population-based Malmö Diet and Cancer Study (MDC-CC), (ii) a random nested subcohort from the MDC-CC for proteomic analysis without prevalent or incident heart failure (HF), (iii) cases with incident heart failure (HF) and plasma available from the MDC-CC, (iv) manifest HF outpatients from the nurse-led HF management program at Skane University Hospital, and (iv) patients with advanced HF who subsequently underwent heart transplantation at Skane University Hospital. Information on a number of parameters was unavailable in the manifest HF cohorts. Continuous variables are presented as mean and standard deviation or median and interquartile range, and categorical variables as count and proportion *BMI* body mass index, *CRP* C-reactive protein, *DBP* diastolic blood pressure, *LDL* low-density lipoprotein cholesterol, *MI* myocardial infarction, *NT-proBNP* N-terminal pro-B-type natriuretic peptide, *SBP* systolic blood pressure

Plasma proteins in HF development

Proteomic profiling in the population-based cohort identified multiple plasma proteins associated with future HF development. Established biomarkers including NT-proBNP, CRP, ST2, troponin T, and TNFα were significantly elevated among individuals who later developed HF, in agreement with previous studies based on conventional immunoassays. Galectin-3 concentration was also nominally higher with similar effects as in previous studies.[168,194,219,220] Among the matrix metalloproteinases, only MMP7 and MMP12 were associated with incident HF, while no significant associations were observed for their inhibitors (TIMPs).

Sixteen proteins on the panel passed the Bonferroni-adjusted significance threshold ($p < 3.8 \times 10^{-5}$), several showing effect magnitudes comparable to NT-proBNP. Among the identified proteins, 12 were secreted and 11 displayed tissue-enriched expression. The strongest association was observed for complement component C5a, followed by C9, both representing activation of the complement cascade. The matricellular protein thrombospondin-2 (TSP2) was also prominently associated with HF and correlated with several matrix metalloproteinases (MMP2, MMP7, MMP12, MMP13, MMP17) and the inhibitor TIMP2, indicating a link to extracellular matrix remodelling. In contrast, TSP2 was inversely correlated with MMP1 and TIMP3. Four additional proteins reflected immune system activity (CRP, CXCL13, IL-1RA, and IL-18R), while protein C and tissue plasminogen activator (tPA) were associated with plasma coagulation pathways. Six intracellular or membrane-bound proteins, gelsolin, carbonic anhydrase 13 (CA13), contactin-1, DUSP3, PRKACA, and UNC5H4, were also significantly associated with incident HF.

After adjustment for established cardiovascular risk factors, most associations remained directionally consistent, though precision was reduced; only NT-proBNP, CXCL13, and the complement components remained significant after Bonferroni correction. Additional adjustment for renal function yielded minimal changes in effect estimates. When analyses were restricted to non-ischemic HF, all associations persisted except for CRP, which was further attenuated.

Comparison with patients with established HF demonstrated marked differences in plasma protein concentrations relative to the general population, consistent with the direction of effect observed in incident cases. All proteins except the Netrin receptor UNC5D were significantly altered in patients with manifest HF, indicating molecular changes from early disease development to advanced HF.

Complement and coagulation systems in HF development

The extensive coverage of the aptamer-based proteomic platform enabled comprehensive characterization of circulating components within the complement and coagulation cascades. Marked associations were observed between incident HF and several complement proteins, notably C5a and C9, indicating activation of the

complement cascade among individuals at risk of developing HF. Consistent directionality and low p values were evident across effector and amplification stages of the complement system shown in figure 20, including elevated concentrations of C3a, C5a, C5b–C6, and other terminal complement components (C7, C8, C9).

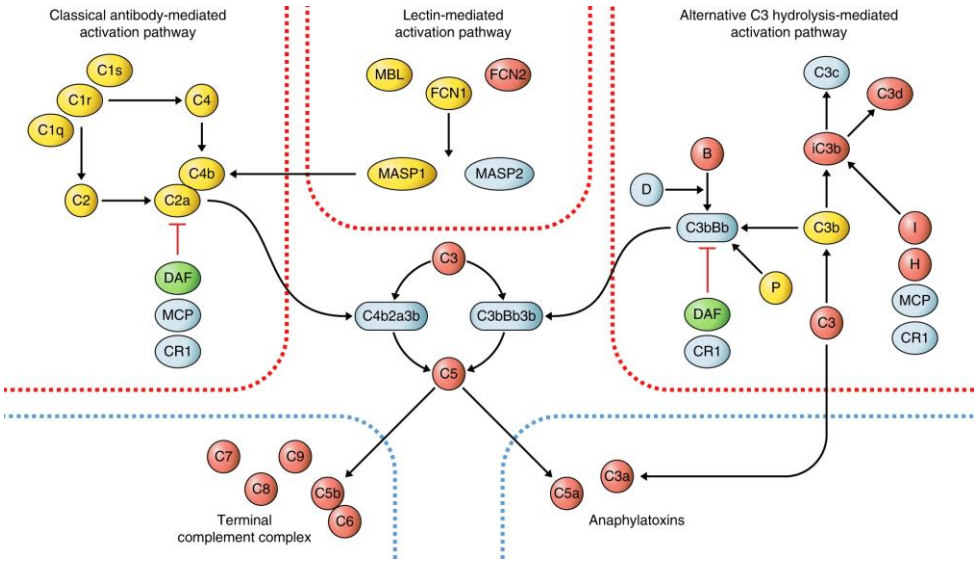

Figure 20. Association of complement system component with development of heart failure. Diagram of protein interactions in the complement system based on a published review. The three activation pathways (red borders) lead to activation of complement factor 3 which amplifies the signal via feedback loops and further activates the two common effector pathways: the anaphylatoxins and the terminal complement complex (blue dashed borders). Directionality of significant associations ($p < 0.05$) with incident heart failure (HF) from Cox proportional hazards regression models adjusting for age and sex (n = 583 population-representative controls, 185 cases) is indicated for each component with colour codes, where red indicates higher concentration with incident HF, green lower concentrations, and yellow no significant association. White colour indicates that the component was not present on the aptamer-based proteomics platform. Reprinted with permission.

In contrast, no associations were observed for proteins belonging to the classical or lectin-mediated activation pathways. Increased levels of mediators in the alternative pathway (C3 and factor B), as well as C3b degradation products (iC3b, C3d) and their regulatory factors (I and H), were accompanied by reduced levels of decay-accelerating factor (DAF), a negative regulator of C3bBb formation. Collectively, these findings are consistent with enhanced activity of the alternative complement pathway in the pathogenesis of HF.

To further identify perturbations within the coagulation system, potential confounding by anticoagulant therapy, warfarin, reported by seven participants, all of whom later developed HF was evaluated. As expected, warfarin users displayed

significantly reduced concentrations of vitamin K-dependent coagulation factors II, VII, IX, and X, as well as proteins C and S. Exclusion of these individuals substantially attenuated the association between protein C and HF risk (HR = 0.66, p = 0.002), whereas the relationship with tissue plasminogen activator (tPA) remained robust (HR = 1.56, $p = 1 \times 10^{-4}$).

Comprehensive analysis of the coagulation cascade revealed additional associations, including lower levels of antithrombin III and higher concentrations of factor IX and tissue factor pathway inhibitor (TFPI) (Figure 21), as well as increased von Willebrand factor (central to platelet activation) and nominal associations with higher tissue factor and lower factor VII. The observed pattern points to coordinated dysregulation of key regulatory components as tPA, TFPI, antithrombin III, and protein C, suggesting a procoagulant shift characterised by enhanced activity of the tissue factor-initiated coagulation pathway.

Together, the proteomic findings indicate concerted activation of both the complement and coagulation systems in early HF development, highlighting intertwined inflammatory and haemostatic processes that may contribute to myocardial remodelling and progressive cardiac dysfunction.

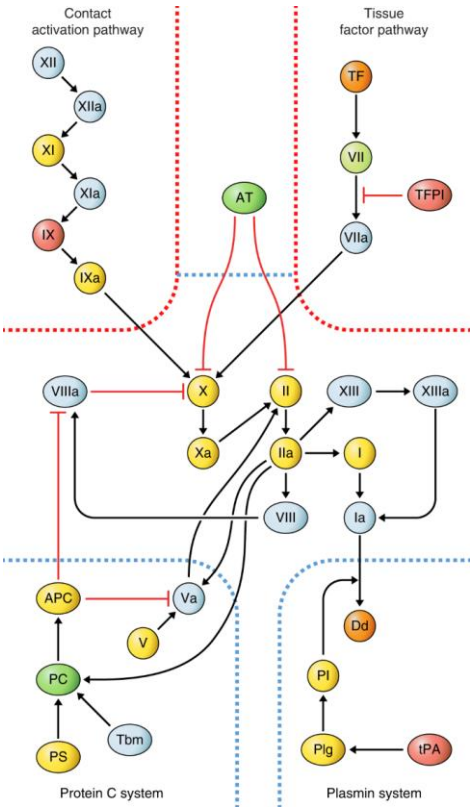

Figure 21. Association of coagulation system components with development of heart failure. Diagram of protein interactions in the coagulation system based on a published review. The two activation pathways (red borders) lead to activation of factor X which further activates II (thrombin) and subsequently fibrin and XIII, which form the blood clot. The blood clot is digested into d-dimers by the plasmin system, and three additional systems (blue dashed borders) negatively regulate clotting (protein C, AT, TFPI). Directionality of nominally significant associations ($p < 0.05$) with incident heart failure (HF) from Cox proportional hazards regression models adjusting for age and sex (n = 583 population-representative controls, 185 cases) is indicated for each component with colour codes, where red indicates higher concentration with incident HF, green lower concentrations, and yellow no significant association. Nominally significant associations ($p < 0.10$) are indicated in orange for higher (two components) and mint green for lower (one component) concentration. All associations were examined only after exclusion of subjects on oral anticoagulation therapy (warfarin). White colour indicates that the component was not present on the aptamer-based proteomics platform. APC activated protein C, AT antithrombin III, Dd d-dimer, PC protein C, Pl plasmin, Plg plasminogen, PS protein S, Tbm thrombomodulin, TFPI tissue factor pathway inhibitor, TF tissue factor. Reprinted with permission.

Plasma Proteins in Manifest Heart Failure

Proteomic profiling identified extensive alterations in circulating proteins among patients with manifest HF compared with population-based controls from the MDC-CC cohort. As expected, established HF biomarkers, including NT-proBNP, CRP, ST2, galectin-3, troponin T, TNF-α, and renin, were significantly elevated along with several matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs).

The analysing of 1305 plasma proteins revealed a pronounced excess of associations with HF, with 421 proteins (32%) surpassing the Bonferroni-adjusted significance threshold ($p < 3.8 \times 10^{-5}$). Approximately half (56%) of these proteins were reduced in HF, predominantly intracellular or membrane-bound proteins with broad tissue distribution, while proteins elevated in HF were mainly secreted and tissue-enriched (61%), many with plausible roles in HF pathophysiology. Replication in an independent HF cohort (BioVU) demonstrated strong external validity, with correlated effect estimates (Pearson's $r = 0.54$) and concordant directionality in 75% of shared proteins. Gene-set enrichment analysis of the 421 associated proteins revealed activation of multiple biological pathways.

Molecular changes during advanced HF and reversal after transplantation

To further test the association of proteins with HF as opposed to comorbidities, serial measurements from 30 advanced HF patients before and 6 months after heart transplantation were compared. Distribution of the 16 proteins associated with HF development before transplantation was comparable to the HF outpatient cohort, although many proteins displayed marked further deviation from the general population. A total of 12 of the 16 proteins (75%) changed in directional consistency with the risk estimate for incident HF towards the general population, supporting origin in the heart or in other tissues in response to HF, rather than comorbid conditions. Of the other four proteins, CXCL13 increased markedly, contactin-1 decreased, tPA increased and DUS3 did not change. Of the 421 proteins associated with manifest HF, 158 (38%) changed significantly after heart transplantation ($p < 0.05$) towards levels observed in the general population (Figure 22).

Tissue and Cellular Origin of Plasma Proteins

To determine the cardiac contribution to circulating proteins, plasma concentrations from the coronary sinus and peripheral veins were compared in six patients with hypertrophic obstructive cardiomyopathy. Six of sixteen proteins (C5a, gelsolin, TSP2, tPA, IL-18R, and UNC5D) showed higher coronary sinus levels, suggesting cardiac release. Analyses of public transcriptomic and proteomic datasets (GTEx, HPA, Tabula Muris, and cardiac mass spectrometry) confirmed these findings, demonstrating concordant cardiac expression for NT-proBNP, gelsolin, TSP2, and IL-18R, whereas CRP, C9, protein C, and C5 were primarily liver-derived.

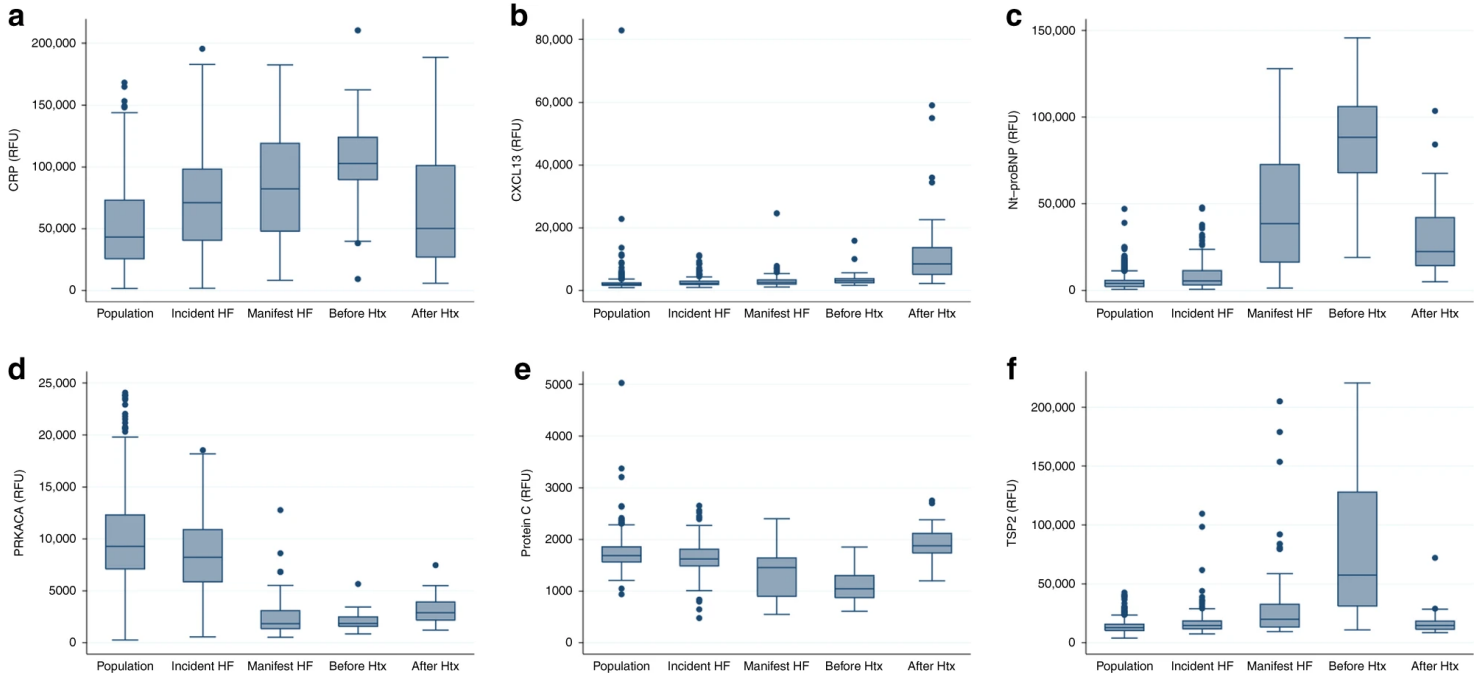

Figure 22. Plasma protein abundance across heart failure stages. Box plots depicting distribution of (a) CRP, (b) CXCL13, (c) NT-proBNP, (d) PRKACA, (e) Protein C, and (f) TSP2 across cohorts representing different stages of heart failure. In addition to association with incident heart failure, these six proteins changed markedly (>50%) after heart transplantation (HTx). Cohorts include individual human subjects from a population-based cohort who did not develop heart failure during follow-up ("Population", n = 583), from the population-based cohort who did develop heart failure ("Incident HF", n = 185), subjects with manifest heart failure ("Manifest HF", n = 84), and serial measures from subjects with advanced heart failure before and after heart transplantation ("Before Htx" and "After Htx", n = 30 for both). All except CXCL13 changed in directional consistency with the risk estimate for incident HF towards the general population. The box center line indicates the median, box bounds represent 25th and 75th percentiles, and the whiskers are the most extreme values within 1.5x the interquartile range from the nearer quartile. RFU relative fluorescence units. Reprinted with permission.

Spatial transcriptomics of failing human myocardium revealed distinct cellular origins: NT-proBNP expression colocalised with cardiomyocyte markers, gelsolin with vascular markers, and TSP2 with fibroblast markers. These observations, supported by data from the Human Protein Atlas and mouse single-cell cardiac transcriptomes, indicate that several circulating proteins, particularly NT-proBNP, TSP2, and gelsolin, derive partly from specific cardiac cell types, reflecting diverse pathophysiological processes in heart failure.

Genetic Associations with Plasma Proteins

Genome-wide association analyses of 16 candidate proteins in 1421 individuals identified significant pQTLs ($p < 3 \times 10^{-9}$) at 11 loci for nine proteins, with replication in an independent cohort (n = 759) confirming additional cis-regulatory variants for five proteins. Most lead variants explained modest proportions of variance (1–6%), except for IL-18R1 (37%), TSP2 (13%), and protein C (15%). Thirteen proteins exhibited cis-pQTLs, while C5a, contactin-1, and protein C also displayed trans-associations with biologically plausible loci, CFH, TMEM8A, and PROCR, respectively. Several cis-loci contained functional missense variants (e.g., IL1F10, C9, PLAT, GSN, E2F5), and three loci (C5a trans-locus, contactin-1, and gelsolin) represented novel associations. Expression QTL analyses confirmed that most cis-pQTLs influenced gene expression, including cardiac expression for NT-proBNP, TSP2, and IL-18R. All trans-pQTLs colocalised with eQTLs for their regulatory genes, reinforcing mechanistic plausibility.

Paper II

Bioactive adrenomedullin in the community

Baseline characteristics of the community-based MPP cohort (n = 5060) and the healthy subcohort (n = 1128) are shown in Table 5. The median bio-ADM plasma concentration was 14 pg/mL (interquartile range [IQR] 10–19 pg/mL), and the reference range was 8–39 pg/mL (95% confidence interval [CI] for upper limit 36–42 pg/mL) in the full cohort and 8–30 pg/mL (95% CI 26–34 pg/mL) in the healthy subcohort.

Clinical covariates of bio-ADM from regression analysis showed strongest associations with measures of body size (BMI, r = 0.41; and waist circumference, r = 0.38) followed by triglycerides (r = 0.26) and eGFR (r = −0.22). The ranges of BMI and eGFR were 15.8–54.5 kg/m^2 and 6.8–120.9 mL/min/1.72 m^2, and 1103 (22%) subjects had a BMI > 30 kg/m^2, 120 (2%) subjects BMI < 20 kg/m^2, 60 (1%) subjects eGFR <30 mL/min/1.72 m^2, and 1648 (33%) subjects eGFR 30–60 mL/min/1.72 m^2. Subjects with BMI > 30 kg/m^2 had a median bio-ADM of 19 pg/mL (IQR = 14–25 pg/mL), median bio-ADM of 10 pg/mL (IQR = 8–13 pg/mL)

for BMI <20 kg/m^2, median bio-ADM of 21 pg/mL (IQR = 15–31 pg/mL) for eGFR <30 mL/min/1.72 m^2, and median bio-ADM of 16 (IQR = 12–21) for eGFR of 30–60 mL/min/1.72 m^2 indicating small bio-ADM differences by major clinically relevant strata for these correlates.

Bioactive adrenomedullin and central hemodynamic

Baseline characteristics of the 346 patients included in the HF-RHC cohort are presented in Table 5. Overall, central hemodynamics reflected a hypokinetic circulatory state, with a mean cardiac index of 2.32 L/min/m² and a mean mixed venous oxygen saturation (SvO_2) of 61%. Venous filling pressures were mildly elevated at the group level, both in the pulmonary circulation, as indicated by a mean pulmonary artery wedge pressure (PAWP) of 16 mmHg, and in the systemic venous circulation, with a mean right atrial pressure (mRAP) of 10 mmHg. However, a substantial proportion of patients exhibited markedly elevated pressures, with 179 individuals (54%) presenting with PAWP > 15 mmHg and 136 individuals (41%) with mRAP > 10 mmHg. Most patients with elevated mRAP also had elevated PAWP (n = 119). Among patients with elevated mRAP but normal PAWP (<15 mmHg), pulmonary hypertension was present in 76%. Overall mean pulmonary artery pressure (mPAP) was mildly elevated, with a median value of 27 mmHg (IQR 18–35). NT-ProBNP was markedly elevated, with a median of 2582 pg/mL.

Table 5. Baseline characteristics of study cohorts.

	Population (MPP) n=5060	Healthy sub-cohort (MPP) n=1128	Hemodynamic cohort (HF-RHC) n=346	Heart transplant (HF-RHC) n=47	Dyspnea (ADYS) n=1534	Decompensated HF (ADYS) n=570
Age (years)	68.6 (67.1–74.7)	67.7 (66.0–70.6)	57.3 (14.3)	53.4 (10.8)	74.4 (62.2–84.1)	81.5 (73.4–88.2)
Sex (female)	1467 (29)	353 (31)	108 (31)	10 (21)	856 (56)	279 (49)
Body mass index (kg/m^2)	27.2 (24.4–29.4)	23.0 (23.7–27.5)	26.0 (23.0–29.4)	25.4 (22.0–29.0)	25.5 (22.3–30.0)	26.6 (23.1–30.8)
Blood pressure (mmHg)						
Systolic	146.0 (20.8)	142.7 (19.5)	118.2 (22.0)	142.2 (15.6)	145.7 (27.9)	143.4 (30.3)
Diastolic	83.5 (10.8)	82.9(9.9)	74.8 (12.1)	90.1 (10.9)	81.8 (15.7)	80.0 (16.8)
Heart rate (bpm)	70.6 (12.5)	71.4(11.9)	75.3 (14.7)	78.8 (8.8)	91.0 (21.5)	90.2 (21.8)
SaO2 (%)	N/A	N/A	95.4 (93.6–96.8)	96.4 (95.4–97.9)	95.0 (90.0–98.0)	93.0 (88.0–96.0)
Current smoking	955 (19)	0 (0)	19 (5)	0 (0)	268 (17)	68 (12)
Medical History						
Diabetes	808 (16)	0(0)	32(9)	13(28)	275(18)	161(28)
Hypertension	2021 (40)	0(0)	45(13)	8(17)	632(41)	315(56)
Myocardial infaction	345 (7)	0(0)	10(3)	1(2)	445(29)	305(54)
Atrial fibrillation	284 (6)	0(0)	17(5)	1(2)	462(30)	329(58)
HF	89(2)	0(0)	346(100)	47(100)	516(34)	570(100)
COPD or asthma	N/A	N/A	N/A	N/A	614(40)	233(41)
Medication						
ACE/ARB	878(17)	0(0)	115 (75%)	18(51)	456(30)	274(49)
Beta-blocker	1158(23)	0(0)	138 (90%)	34(87)	658(43)	407(72)
Aldosterone antagonist	143(3)	0(0)	98 (64%)	20(57)	124(8)	85(15)
Loop diuretic	307(6)	0(0)	77(78%)	32(97)	561(37)	398(70)
Plasma biomarkers						
eGFR (mL/min/1.73m^2)	65.9 (55.3–77.3)	74.1 (66.66–83.1)	69.0 (50.9–89.7)	46.2 (35.7–74.2)	72.9 (49.1–90.9)	52.6 (37.3–72.9)
NT-proBNP(pg/mL)	N/A	N/A	2581.5 (907.5–5051.5)	2189.0 (868-7102)	815.6 (141.3–3721)	3810 (1617–8590)
Bio-ADM (pg/mL)	14.0 (10.4–19.1)	11.5 (8.8–15.0)	24.9 (14.2–40.9)	19.5 (11.9–30.4)	28.0 (16.8–49.5)	42.7 (28.2–71.1)
NYHA class						
I	N/A	N/A	8(5.8)	-	N/A	86(15.2)
II			37(27.0)	1(4.8)		211(37.0)
III			82(60.0)	18(85.7)		111(19.5)
IV			10(7.3)	2(9.5)		157(27.5)

Distributions of quantitative variables are presented as mean with standard deviation or median with interquartile range, as appropriate. For categorical variables, numbers and percentage of total are presented. For heart transplant recipients, data refer to before transplantation.

In the subset of 47 patients that underwent transplantation, cardiac function improved after transplantation and pressures declined towards normal reference values. The distributions of bio-ADM in HF patients and the subset who obtained a heart transplant are depicted in Figure 18B. Bio-ADM was most strongly correlated with mRAP (r = 0.6, Figure 22A), followed by SvO_2 (r = 0.5), PAWP (r = 0.4, Figure 22B), RVPd (r = 0.4) and mPAP (r = 0.4).

In contrast, NT-proBNP was most strongly correlated with SvO2 (r = 0.6), followed by PAWP (r = 0.5), mPAP (r = 0.4), and SVI (r = 0.4). In the subgroup of patients with heart failure who underwent heart transplantation, circulating bio-ADM concentrations declined significantly over time, decreasing from a median of 28 pg/mL (IQR 18.46–42.80) at baseline to 20 pg/mL (IQR 11.89–30.43) following transplantation ($P < 0.001$). This reduction in bio-ADM was accompanied by a pronounced normalization of systemic venous pressures, with median mean right atrial pressure (mRAP) decreasing from 11 mmHg to 3 mmHg ($P < 0.001$).

Changes in bio-ADM were most strongly associated with concurrent changes in mRAP (r = 0.64), followed by mixed venous oxygen saturation (SvO_2; r = 0.60) and right ventricular end-diastolic pressure (RVPd; r = 0.59). Consistent with these univariable associations, multivariable-adjusted repeated-measures regression analyses demonstrated a significant decline in bio-ADM after transplantation that paralleled improvements in central hemodynamics, including reductions in venous filling pressures and enhancements in systemic oxygen delivery.

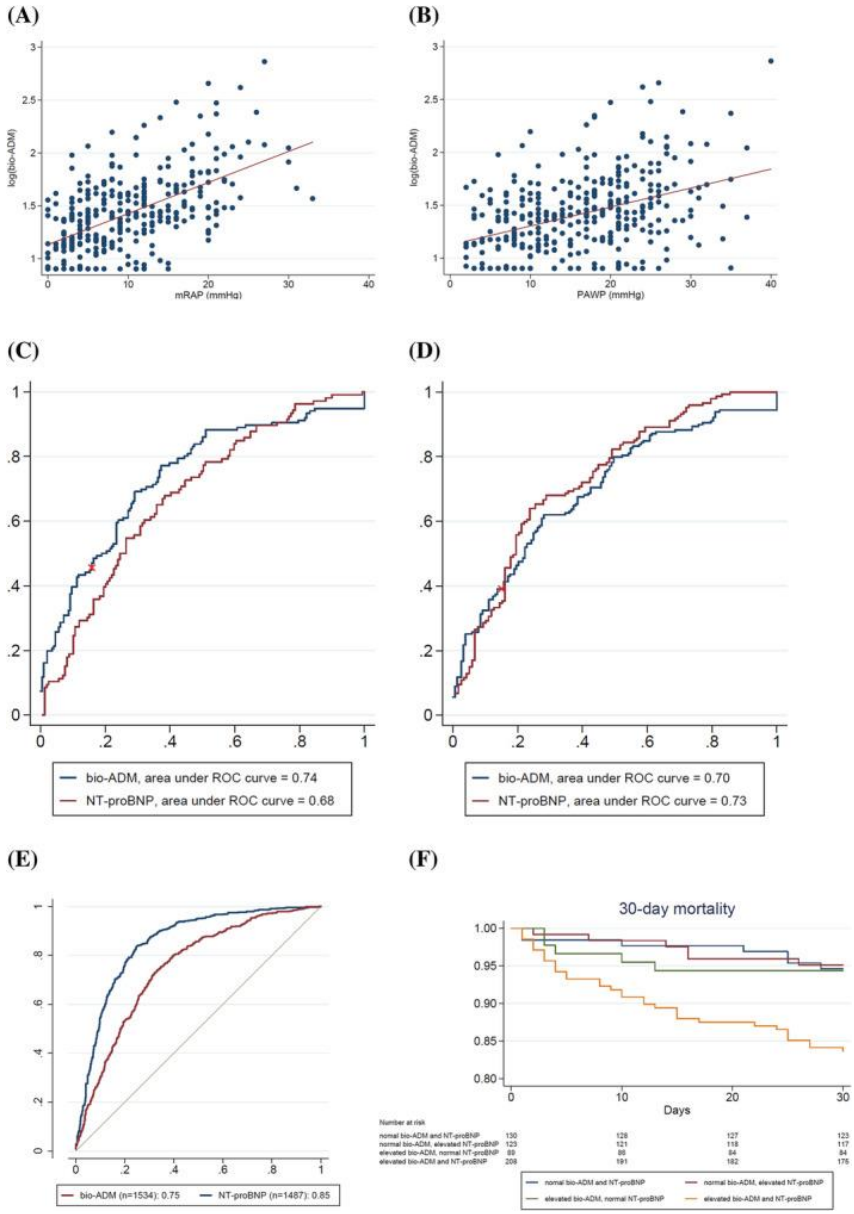

Figure 23. Association of bio-ADM with measures of congestion and outcomes. (A, B) Scatter plots depicting the relation of bio-ADM with mean right atrial pressure (mRAP) and pulmonary arterial wedge pressure (PAWP) in patients with heart failure from the HF-RHC cohort (n = 332). (C, D) Receiver-operating characteristic curves showing discrimination of elevated mRAP (>10 mmHg) and PAWP (>15 mmHg) with bio-ADM (n = 332) and NT-proBNP (n = 265). Cut-off 39 pg/mL indicated. (E) ROC curve showing discrimination of final heart failure diagnosis with NT-proBNP and bio-ADM in patients with acute dyspnoea from the ADYS cohort (n = 1534). (F) Kaplan–Meier curve illustrating 30-day mortality in decompensated heart failure patients (n = 570) with elevated bio-ADM (>39 pg/mL) and NT-proBNP (>1045 pg/mL). Reprinted with permission.

Table 6. Association of bio-ADM with measures of congestion, decompensated heart failure and outcome in dyspnea patients.

	Bio-ADM linear model	NT-proBNP linear model	Bio-ADM in NT-proBNP-adjusted model	Bio-ADM decision cutoff (>39 pg/mL)	Bio-ADM decision cutoff (>39 pg/mL) NT-proBNP-adjusted
HF diagnosis (n=570)	2.33 (2.03-2.69)	4.52 (3.71-5.51)	1.60 (1.36-1.88)	3.61 (2.82-4.63)	1.83 (1.37-2.43)
Congestion measures:					
Chest X-ray findings:					
Dilated vessels (n=47)	2.21 (1.55-3.16)	5.68 (3.21-10.05)	1.16 (0.74-1.82)	3.73 (1.91-7.29)	1.13 (0.50-2.54)
Pulmonary infiltrate (n=155)	1.41 (1.10-1.81)	1.46 (1.08-1.98)	1.26 (0.96-1.66)	1.57 (0.99-2.48)	1.23 (0.75-2.02)
Pleural effusion (n=220)	2.06 (1.63-2.61)	3.10 (2.27-4.23)	1.55 (1.20-2.00)	3.17 (2.10-4.79)	1.87 (1.19-2.95)
In-hospital diuretics (n=417)	2.06 (1.79-2.36)	3.06 (2.55-3.68)	1.57 (1.34-1.83)	3.41 (2.65-4.39)	2.11 (1.60-2.80)
Continuous positive airway pressure (n=23)	1.62 (1.11-2.36)	1.91 (1.05-3.49)	1.19 (0.73-1.94)	2.74 (1.16-6.46)	1.47 (0.55-3.93)
Hospitalization* (n=439)	2.11 (1.63-2.72)	2.04 (1.49-2.79)	1.90 (1.46-2.47)	3.06 (2.01-4.66)	2.50 (1.61-3.89)
Death at 30 days* (n=53)	1.75 (1.36-2.25)	2.47 (1.55-3.93)	1.52 (1.16-1.99)	2.91 (1.55-5.45)	2.24 (1.17-4.43)
Death at 90 days* (n=112)	1.61 (1.34-1.94)	2.35 (1.70-3.25)	1.41 (1.17-1.72)	2.05 (1.37-3.06)	1.61 (1.06-2.43)

Association of bioactive adrenomedullin (bio-ADM) with heart failure (HF) diagnosis (n = 570) and measures of congestion in patients seeking an emergency department for acute dyspnea (ADYS cohort n = 1534). Associations are tested in logistic or Cox regression models per standard deviation of log-transformed bio-ADM and Amino-terminal pro-B-type natriuretic peptide (NT-proBNP), or based on a decision cut-off for bio-ADM. Odds ratios and hazard ratios (mortality) are presented, with 95% confidence intervals. All models were age- and sex-adjusted. *Hospitalization and mortality (n = 53 deaths) was analysed in the patient subset with a HF diagnosis (n = 570).

Discriminatory performance for elevated systemic venous pressure was superior for bio-ADM compared with NT-proBNP, with an area under the receiver operating characteristic curve (AUROC) of 0.74 (95% CI 0.67–0.79) versus 0.68 (95% CI 0.61–0.75), respectively. In contrast, elevated pulmonary artery wedge pressure (PAWP) was more accurately identified by NT-proBNP than by bio-ADM (AUROC 0.73 [95% CI 0.68–0.79] vs. 0.70 [95% CI 0.64–0.75]), as illustrated in Figure 22C and 22D. Evaluation of several candidate decision thresholds demonstrated that the upper population reference limit derived from the Malmö Preventive Project (MPP; 39 pg/mL) provided optimal sensitivity while maintaining

specificity above 0.80 for the detection of both elevated mRAP and PAWP (Figure 22C and 22D).

Restricted cubic spline analyses supported a linear relationship between bio-ADM and filling pressures and corroborated 39 pg/mL as the optimal threshold for identifying elevated right atrial and pulmonary wedge pressures. This threshold also achieved approximately 80% specificity for identifying PAWP values exceeding 18 mmHg.

Bioactive adrenomedullin in acute dyspnoea

The ADYS cohort comprised 1534 patients presenting with acute dyspnoea, of whom 871 (57%) required hospital admission. Heart failure (HF) was diagnosed in 570 patients, including 516 individuals (91%) with a prior history of HF and 54 patients (9%) receiving a first HF diagnosis. Among patients with established HF, echocardiographic assessment was available in 390 cases (76%), revealing reduced LVEF <50% in 196 patients (50%). In contrast, echocardiography was performed during hospitalization in only seven patients (13%) with newly diagnosed HF, of whom four exhibited reduced ejection fraction. Overall, 439 patients (77%) with HF were hospitalized. Baseline characteristics for the full ADYS cohort and the HF subgroup are presented in Table 5. The distribution of bio-ADM concentrations in the overall cohort and among patients with HF is shown in Figure 18C. Bio-ADM was independently associated with a diagnosis of HF after adjustment for NT-proBNP and associated with radiologic measures of pulmonary congestion, including vascular redistribution, interstitial oedema, and pleural effusion, as well as with therapeutic indicators of congestion, such as administration of in-hospital diuretics and use of continuous positive airway pressure (CPAP). Following adjustment for NT-proBNP, independent associations remained significant for pleural effusion and in-hospital diuretic therapy shown in Table 6. Although NT-proBNP demonstrated superior discriminatory performance for HF diagnosis compared with bio-ADM (AUROC 0.85 [95% CI 0.83–0.87] vs. 0.75 [95% CI 0.73–0.77]), bio-ADM provided complementary diagnostic information (Figure 22E).

Among patients with HF, elevated bio-ADM was also independently associated with the need for hospitalisation and with mortality at both 30 and 90 days, even after adjustment for NT-proBNP (Table 6). A graded relationship between bio-ADM quartiles and mortality risk was observed. Within 30 days, four deaths occurred in the lowest quartile (≤28 pg/mL), compared with 35 deaths in the highest quartile (≥71 pg/mL), with the proposed threshold of 39 pg/mL corresponding to the second quartile. Patients exhibiting elevations of bio-ADM (>39 pg/mL) and NT-proBNP (>1045 pg/mL) experienced the poorest outcomes, whereas those with elevations in only one biomarker or with both biomarkers below the respective thresholds had more favourable prognosis (Figure 22F). An elevated bio-ADM concentration above 39 pg/mL was associated with a threefold increased odd of HF

diagnosis, higher likelihood of radiographic pulmonary congestion, need for in-hospital diuretics and CPAP in acute dyspnoea. Furthermore, in HF patients, this threshold conferred a two-fold increased risk for mortality at 30 days (Table 6).

Paper III

Invasive measurements of 30 central hemodynamic parameters from patients with advanced heart failure were obtained at two time points: prior to and six months following heart transplantation (Table 7). Measurements were obtained by RHC. The pairwise correlation of hemodynamic measures are shown below in Figure 24.

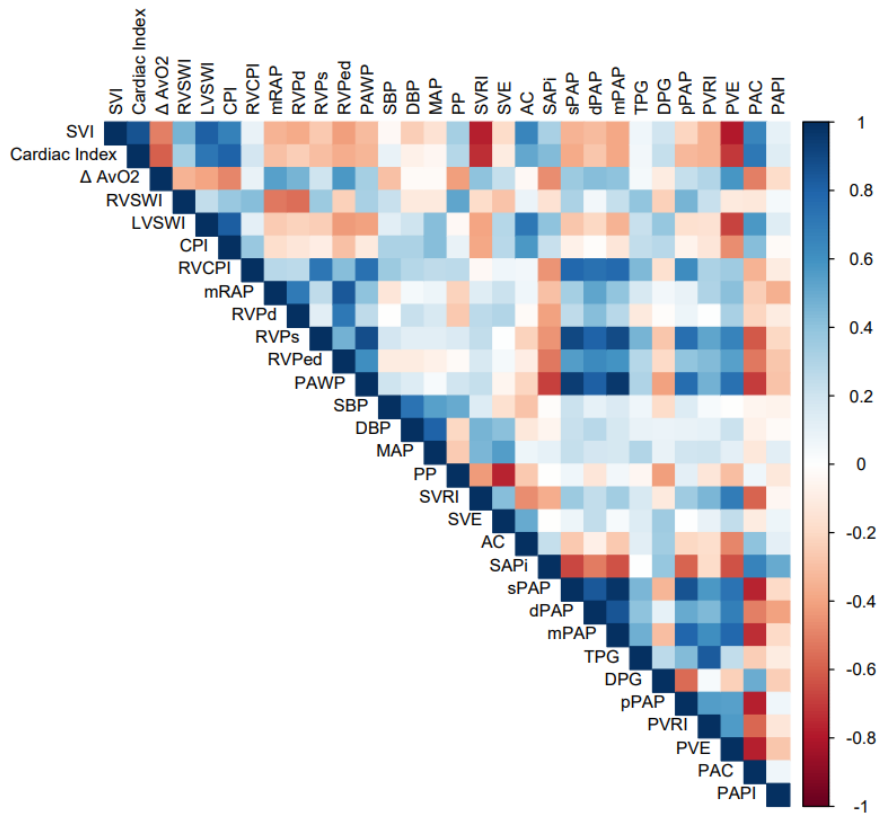

Figure 24. Correlation of hemodynamic parameters.

Table 7. Hemodynamic phenotypes and yield of protein associations.

Phenotype	Acronym	Unit	Derivation	Physiological interpretation	Difference	Median Distribution Pre /post Thx	Yield
Cardiac output and work							
Stroke volume index	SVI	mL / m^2	CI / heart rate / BSA	Body surface-averaged output per stroke	52%	24/38	32
Cardiac index	CI	L/min/m^2	Thermodilution	Body surface-averaged output per minute	76%	2/3	86
Arteriovenous difference in oxygen tension	ΔAVO_2	%	(SaO_2-SvO_2) x plasma hemoglobin x 1.34	Peripheral oxygen consumption, measure of systemic circulatory transit time	-43%	74/44	102
Right ventricular stroke work index	RVSWI	mmHg mL / m^2	(mPAP-mRAP) x SVI	RV workload	13%	378/490	1
Left ventricular stroke work index	LVSWI	mmHg mL / m^2	(MAP-PAWP) x SVI	LV workload	143%	1467/3619	104
Cardiac power index	CPI	W / m^2	MAP x CI / 451	LV workload	129%	0/1	112
RV cardiac power index	RVCPI	W / m^2	mPAP x CI /451	RV workload	-12%	0/0	0
Right heart pressures							
Mean right atrial pressure	mRAP	mmHg	Multilumen PA catheter	RV filling pressure	-84%	14/2	137
Right ventricular pressure, diastole	RVPd	mmHg	Multilumen PA catheter	RV filling pressure	-116%	6/-1	73
Right ventricular pressure, systole	RVPs	mmHg	Multilumen PA catheter	RV filling pressure	-34%	41/29	21
Right ventricular pressure, end-diastolic	RVPed	mmHg	Multilumen PA catheter	RV filling pressure	-74%	14/5	78
Left heart pressures							
Pulmonary arterial wedge pressure	PAWP	mmHg	Multilumen PA catheter	LV filling pressure	-72%	20/7	84
Systemic arterial function							
Systolic arterial pressure	SBP	mmHg	Brachial cuff	LV afterload	41%	99/139	96

Diastolic arterial pressure	DBP	mmHg	Brachial cuff	LV afterload	25%	70/90	55
Mean arterial pressure	MAP	mmHg	DBP + ((SBP – DBP) / 3)	LV afterload	22%	81/105	55
Pulse pressure	PP	mmHg	SBP – DBP	LV output	82%	30/49	63
Systemic vascular resistance index	SVRI	dyne s / cm^5 / m^2	80 x (MAP – mRAP) / CI	Static component of LV afterload	-17%	3236/2677	0
Systemic vascular elastance	SVE	mmHg / mL	MAP/SV	LV afterload and ventricle-arterial coupling	-24%	3/2	8
Arterial compliance / capacitance	AC	mL / mm Hg	SV/PP	Pulsatile component of LV afterload	-10%	2/1	0
Systemic arterial pulsatility index	SAPi	mmHg	(SBP-DBP) / PAWP	Index of ventricle-arterial coupling	445%	1/7	9
Pulmonary arterial function							
Systolic pulmonary arterial pressure	sPAP	mmHg	Multilumen PA catheter	RV afterload	-40%	43/25	25
Diastolic pulmonary arterial pressure	dPAP	mmHg	Multilumen PA catheter	RV afterload	-65%	24/9	76
Mean pulmonary arterial pressure	mPAP	mmHg	Multilumen PA catheter	RV afterload	-46%	31/16	42
Transpulmonary gradient	TPG	mmHg	mPAP-PAWP	Pulmonary vascular resistance	0%	9/9	0
Diastolic transpulmonary pressure gradient	DPG	mmHg	dPAP-PAWP	Pulmonary vascular resistance	-45%	2/3	0
Pulmonary arterial pulse pressure	pPAP	mmHg	sPAP-dPAP	RV output	-10%	18/15	0
Pulmonary vascular resistance index	PVRI	dyne s / cm^5 / m^2	80 x TPG/CI	Static component of RV afterload	-46%	391/234	1
Pulmonary vascular elastance	PVE	mmHg / mL	mPAP/SV	RV afterload and ventricle-pulmonary vascular coupling	-60%	1/0	15
Pulmonary arterial compliance / capacitance	PAC	mL / mm Hg	SV/pPAP	Pulsatile component of RV afterload	86%	3/5	3
Pulmonary artery pulsatility index	PAPI	mmHg	(SPAP-DPAP) / mRAP	Index of ventricle-arterial coupling	383%	1/6	26

In total, 201 proteins meeting our Bonferroni-adjusted significance threshold ($p<1.28x10-6$) for at least one hemodynamic parameter were identified. Yield is the number of associated proteins.

Blood samples was obtained simultaneously as measurements from the pulmonary artery, for analysis of 1305 proteins using an aptamer-based affinity capture method as described previously.[221] Plasma proteins were tested for association with each hemodynamic measure over time in repeated measures models.

In total, 201 proteins were identified meeting our Bonferroni-adjusted significance threshold ($p<1.28x10^{-6}$) for at least one hemodynamic parameter shown in Table 7. The largest number of protein associations (>100) were observed for mean right atrial pressure (mRAP), cardiac power index (CPI), left ventricular stroke work index (LVSWI), and arteriovenous difference in oxygen tension (ΔAVO_2) followed by systolic blood pressure (SBP), cardiac index (CI), and pulmonary arterial wedge pressure (PAWP, >80) (Table 7). No proteins were significantly associated with 6 of the hemodynamic measures which however changed little after transplantation: right ventricular pressure index (RVCPI, -12% after transplantation), systemic vascular resistance (SVRI, -17%), arterial compliance (AC, -11%), transpulmonary gradient (TPG, 0%), diastolic transpulmonary pressure gradient (DPG, -45%), pulmonary artery pulse pressure (pPAP, -10%) (Table 7). Of the 201 proteins, 167 (83%) associated with >1 hemodynamic measure.

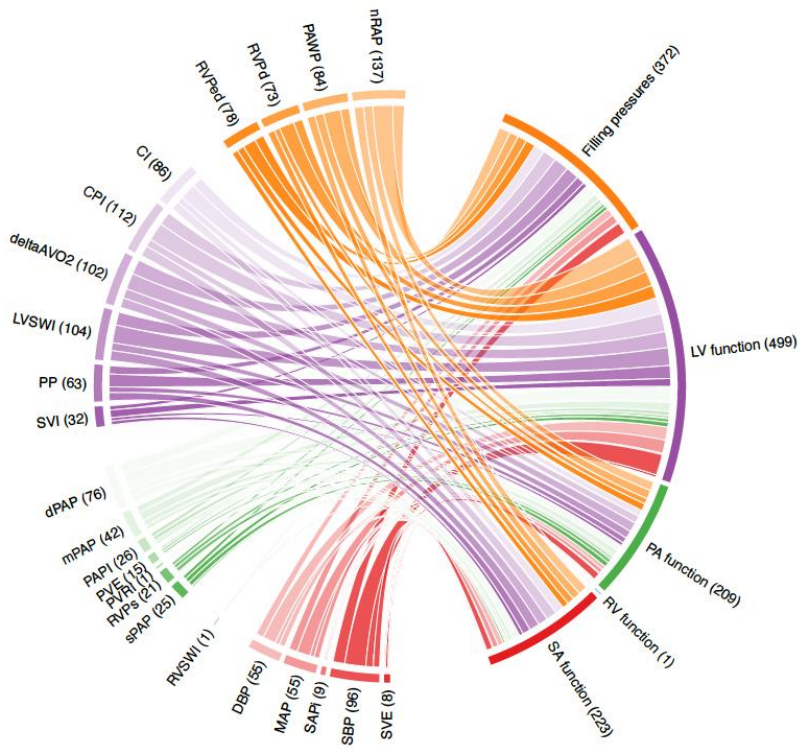

Figure 25. Shared protein association between hemodynamic parameters

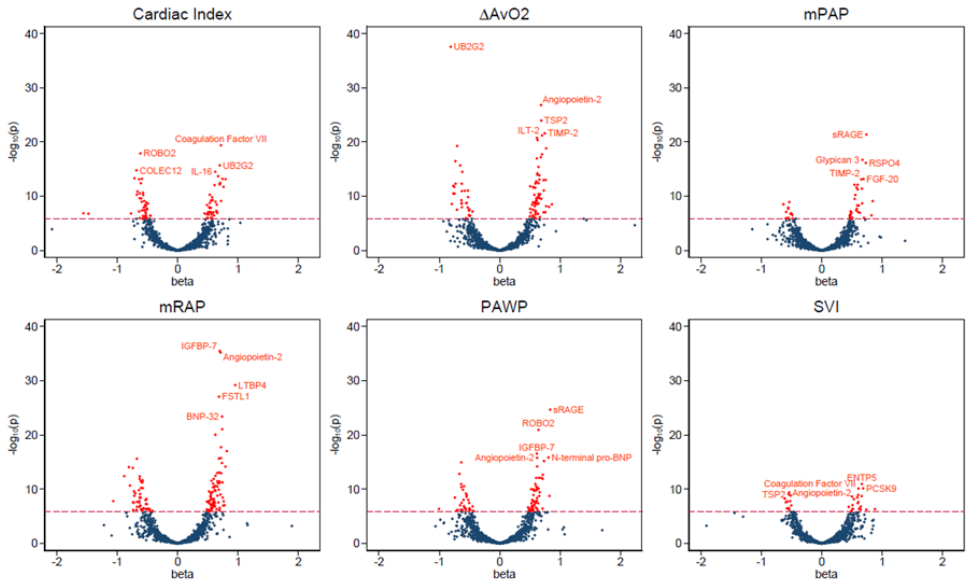

Figure 26. Proteins associating with key clinical hemodynamic parameters over time, measured before and after heart transplant. mPAP, mean pulmonary artery pressure; mRAP, mean right arterial pressure; PAWP, pulmonary artery wedge pressure; PVRI, pulmonary vascular resistance index; SVI, stroke volume index; SVRI, systemic vascular resistance index.

Next, to identify the most informative proteins for a set of the 6 most clinically relevant hemodynamic measures (CI, SVI, ΔAVO_2, PAWP, mRAP, mPAP), a supervised machine learning model was applied to rank proteins by variable importance: gradient boosted decision tree models with mixed effects and 8-fold cross validation, which is robust to collinearity and non-linear effects, and works well with small samples with large feature numbers. However, the method does not provide information on orthogonal effects, so highly correlated variables representing strong signals will all be highly ranked, representing redundancy in the variable selection. The discriminatory performance of the most informative variables for each measure in identifying clinically significant cut-offs was high, as shown in Figure 27.

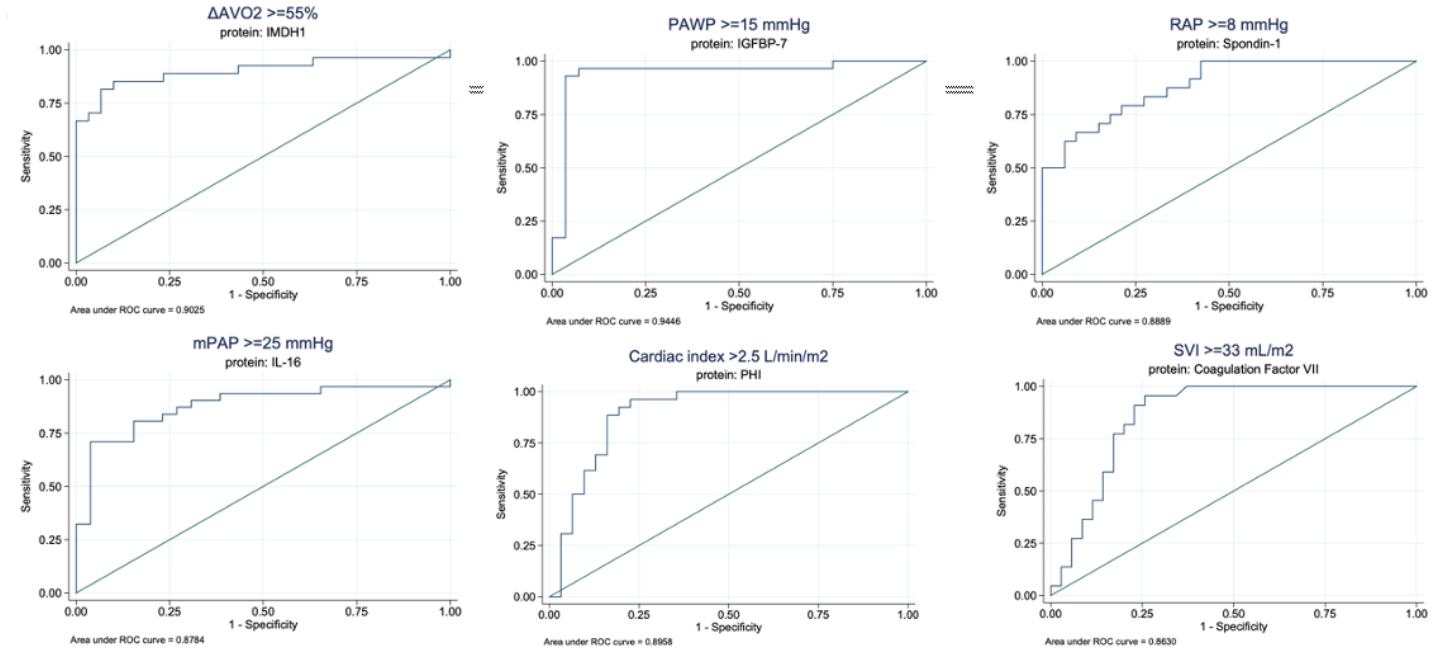

Figure 27. Discriminatory performance of most informative proteins for clinically relevant cutoffs in key hemodynamic parameters. ROC. receiver operating characteristic. IMDHI, inosine monophosphate dehydrogenase 1. IGFBP-7, insulin growth factor binding protein-7. PHI, peptide histidine isoleucine. IL-16, interleukin-16.

Discussion

Paper I

The first study in this thesis provides a systematic characterisation of the circulating plasma proteome across distinct clinical stages of heart failure. The results demonstrate that disease progression is accompanied by profound and coordinated alterations in multiple biological systems. Rather than reflecting isolated myocardial dysfunction, the observed proteomic changes indicate that heart failure represents a multisystem disorder involving inflammatory activation, extracellular matrix remodelling, endothelial and vascular dysregulation, metabolic perturbations, and altered proteostasis. The study was also designed to study molecular patterns before and after heart transplantation where activity in several of the pathways declined after transplantation, indicating that protein perturbations were induced by the failing heart rather than comorbid conditions.

A key finding is that proteomic signatures develop gradually in a stage-dependent manner, suggesting that circulating molecular pathways are differentially engaged as heart failure progresses from early to advanced disease. Early stages are characterised by subtle yet consistent alterations in proteins related to immune modulation and vascular homeostasis, CRP, CXCL13, IL-1RA, IL-18R, TNFα, C5, C9 whereas more advanced stages display pronounced changes in markers of fibrosis, tissue remodelling, neurohormonal activation, and systemic inflammation like ST2, galectin-3, CRP, renin, MMPs and TIMPS. This observation supports the concept that circulating proteins capture both compensatory and maladaptive processes over the course of the disease.

This, together with results highlighted in the background section, suggests that clinical classifications of heart failure severity are likely paralleled by distinct molecular phenotypes. This suggests that plasma proteomics can provide complementary information beyond conventional biomarkers, which often reflect only a narrow spectrum of pathophysiological mechanisms. The range of affected pathways observed in this work highlights the limitation of single-marker approaches and underscores the value of multiplexed proteomic profiling for improved phenotypic resolution.

Furthermore, several proteins identified as strongly associated with disease severity are involved in extracellular matrix organisation and tissue remodelling, reinforcing

the central role of structural myocardial and vascular changes in heart failure progression. These findings align with the conceptual view that maladaptive remodelling is not only a consequence of hemodynamic stress, but an active and sustained biological process reflected in the circulation. Similarly, the importance of inflammatory and immune related proteins suggests a persistent low-grade inflammatory state that may contribute to disease progression rather than simply representing a downstream effect.

Taken together, the results supports the thesis that heart failure progression may be driven by an interplay between hemodynamic stress, systemic inflammation, vascular dysfunction, and maladaptive tissue remodelling.[222] The proteomic profiles identified in this thesis may therefore provide mechanistic insight into disease biology while also offering a potential foundation for future biomarker development and patient stratification.

Limitations

Several limitations of this work warrant consideration.

First, the predominantly cross-sectional design with independent cohorts limits the ability to postulate temporal relationships or causality between proteomic alterations and disease progression. While clear stage-dependent differences were observed, it cannot be determined whether specific proteomic changes precede clinical deterioration or arise as a consequence of established disease. Longitudinal sampling is needed to better distinguish causal drivers from markers that reflect disease severity. In addition, complementary approaches are required to assess the causality of the identified pathways.

Second, the proteomic platform used, while enabling high-throughput and broad protein coverage, is inherently constrained by assay sensitivity and specificity. Not all circulating proteins are equally represented, and low-abundance or highly transient proteins may be under detected. In addition, measured protein concentrations reflect relative abundance rather than absolute quantification, which may limit direct biological interpretation. However, extensive assessment of cross-reactivity with homologous proteins has been conducted previously, with limited off-target reactivity observed for most proteins and no cross-reactivity observed for any of the 16 proteins associated with HF development. We reported correlation with dual-binder immunoassays for all nine of the 16 proteins that were available on both platforms. Preanalytical factors, sampling condition, sample tubes and biobanking conditions were standardised in each of the cohorts. However, there were differences in storage times and fasting conditions in the different cohorts that could affect the results.

We also recognise that that the patients from whom coronary sinus samples were drawn all represented a more distinct pathophysiological entity, hypertrophic

obstructive cardiomyopathy, that may not in all aspects generalise to the heart failure in general.

Finally, circulating protein levels are influenced by both cardiac and extra-cardiac factors, including renal function, hepatic metabolism, systemic inflammation, and comorbid conditions. Although extensive clinical characterisation and statistical adjustments were applied, residual confounding cannot be fully excluded.

Strengths

A major strength of this study is the integration of proteomic data with detailed clinical and phenotypic information, allowing molecular findings to be interpreted in the context of disease severity and clinical presentation. This combined approach enhances biological plausibility and facilitates translational relevance, as identified proteins and pathways can be directly related to clinically meaningful disease stages.

The analytical approach taken in this work, including rigorous statistical modelling and correction for multiple testing, and validation strengthens the robustness of the findings. The consistency of observed proteomic patterns across disease stages further supports the validity of the results in differentiating biomarkers that represent mediators of HF rather than consequences.

Finally, by identifying coordinated changes across multiple biological pathways, this study offers a framework for future work aimed at refining heart failure phenotyping, improving prognostic assessment, and exploring new therapeutic targets. This study demonstrates the potential of plasma proteomics to contribute meaningfully to both mechanistic understanding and therapeutic innovation in heart failure.

Paper II

The second study in the thesis builds upon and extends our work on bio-ADM as a biomarker of venous congestion in HF, demonstrating that bio-ADM tracks with invasively measured systemic venous pressures and is independently associated with key clinical outcomes. By establishing reference intervals from a large community cohort and validating its relation to central hemodynamics and clinical prognosis, the study characterises bio-ADM not only as a correlate of congestion but also as a potential tool to enhance diagnostic and prognostic assessment in decompensated HF. Our findings, showing a relatively strong association of bio-ADM with systemic congestion, mRAP, than with pulmonary congestion, PAWP, that complement existing evidence that bio-ADM reflects vascular and endothelial stress more specifically than traditional biomarkers such as NT-proBNP, which have a stronger relation to cardiac stretch and pulmonary pressures.[223]

These results contribute to the conceptualisation of congestion as a multifaceted pathophysiological process rather than a single hemodynamic phenomenon. Traditional reliance on natriuretic peptides for congestion assessment is limited by their predominant sensitivity to intracardiac stretch and pulmonary pressures rather than venous capacitance and systemic volume distribution. By contrast, bio-ADM, appears to reflect the integrated impact of vascular leakage, endothelial dysfunction, and intravascular fluid redistribution. This is consistent with mechanistic insights that ADM is synthesied by endothelial and smooth muscle cells and functions to preserve vascular barrier integrity and modulate vasodilation, processes that are directly disrupted in HF-associated congestion.[141]

Importantly, our results align with and extend findings from large observational HF cohorts in which bio-ADM has been associated with clinical markers of congestion, including oedema, orthopnoea, jugular venous distension, and increased mortality or rehospitalization risk. In the BIOSTAT-CHF cohort, for example, bio-ADM emerged as one of the strongest predictors of clinical congestion scores and was independently associated with adverse outcomes, supporting its potential role in guiding decongestive strategies.[209] Moreover, emerging data from the STRONG-HF trial indicate that bio-ADM correlates with residual congestion status and longer-term outcomes, providing further evidence that dynamic changes in bio-ADM may capture clinical responses to therapy and residual risk not fully captured by NT-proBNP alone.[223]

Collectively, this body of evidence suggests that bio-ADM could enrich the current biomarker repertoire by offering complementary information on vascular and fluid status, particularly systemic venous congestion, which is poorly quantified by conventional measures. This biomarker may therefore help differentiate between congestion phenotypes that have distinct prognostic and therapeutic implications, an area of increasing interest as precision medicine approaches are applied to HF management.

Limitations

Despite the strengths of the study, several important limitations merit consideration. First, the primary analyses were cross-sectional in nature with respect to the relationship between bio-ADM and hemodynamic measures. While strong associations were observed, causality cannot be determined, and longitudinal changes in bio-ADM relative to dynamic shifts in congestion or clinical status require further validation in prospective cohorts. The temporal alteration of congestion biomarkers in relation to therapeutic interventions remains an active area of investigation, and more frequent longitudinal sampling could help clarify whether bio-ADM changes precede or follow clinical decongestion.

Second, while bio-ADM added additional information beyond NT-proBNP, the magnitude of improvement in discrimination was modest. This suggests that

although bio-ADM captures distinct aspects of congestion, its standalone utility may be limited without integration into multimarker panels or clinical algorithms. For example, the AUROC for elevated mean right atrial pressure was 0.74 for bio-ADM versus 0.68 for NT-proBNP, indicating moderate predictive performance that may be improved by combining biomarkers with clinical and imaging data.

Third, pre-analytical variability may have influenced biomarker concentrations across cohorts: storage times differed, fasting status varied, and sampling sites included central venous and peripheral blood. Although the bio-ADM assay has proven stability across freeze-thaw cycles,[206] the effect of sampling conditions remains uncertain. Although adjustments for major covariates, medication effects also may confound results. For example, ARNi are known to increase adrenomedullin levels. Even though none of the study participants received ARNi, this could limit data generalisability to today and future treatment eras.

Finally, while associations with mortality and HF diagnosis were robust, residual confounding from comorbid conditions (e.g., renal dysfunction, systemic inflammation) that also influence biomarker levels cannot be fully excluded. The interdependence of congestive pathways and systemic organ dysfunction in HF underscores the challenge of separating disease mechanisms from biomarkers that may reflect multiple overlapping biological processes. Although our results suggest prognostic utility, our cohort design was observational; causality or utility in guiding therapy remains to be proven in prospective interventional trials.

Strengths

A key strength of this work is the strong pathophysiological validation of bio-ADM as a marker of systemic venous congestion. By directly relating circulating bio-ADM levels to invasively measured central venous pressures, particularly mRAP, the study addresses an important limitation of some existing congestion biomarkers like NT-pro BNP, which primarily reflect myocardial stretch or pulmonary pressures rather than venous and vascular stress.

Another major strength is the integrated study design combining population-based reference data, invasive hemodynamics, and clinical outcomes within a single study. By deriving a “normal” bio-ADM interval (8–39 pg/mL) in an elderly cohort, we provide benchmark thresholds suited to the demographic most at risk of HF. This is an important advance, as prior smaller studies (e.g., cohorts of 88 or 200 subjects) lacked robust representativeness.[206] The identification and validation of a clinically interpretable decision threshold for bio-ADM (39 pg/mL) further enhance translational relevance. This cut-off was consistently supported by population reference data, spline analyses, invasive hemodynamic measures, and clinical outcome associations, providing a practical framework for future clinical application.

The use of a highly specific immunoassay with low cross-reaction, measuring the biologically active form of adrenomedullin represents an additional methodological strength, given the peptide's complex processing, small size and short half-life (22 min).

Bio-ADM was shown to provide information complementary to NT-proBNP. While natriuretic peptides remain superior for detecting intracardiac pressure overload and pulmonary congestion, bio-ADM preferentially reflects systemic venous congestion and vascular dysfunction. This supports a multimarker approach to congestion assessment and aligns with precision-medicine strategies in heart failure. Moreover, these findings provide a biomarker rationale for emerging vascular-targeted therapies, including ongoing clinical evaluation of adrenomedullin modulation with agents such as Adrecizumab, a monoclonal antibody targetin adrenomedullin.

Paper III

In the third study, we applied large-scale, affinity-based plasma proteomics in patients with advanced HF undergoing serial RHC before and after heart transplantation and coupled proteomic data to variability in central hemodynamic measures. We identified a broad panel of circulating proteins that reflect central hemodynamic parameters across multiple physiological domains, including filling pressures, ventricular function, and vascular tone. These findings highlight the potential of several proteomic biomarkers to serve as non-invasive indicators of central cardiovascular function in HF and support their use in precision monitoring and therapeutic decision-making.

Altered central hemodynamics are central to HF pathophysiology and prognosis, yet direct measurement through RHC is an invasive procedure. Current biomarker tools, including natriuretic peptides and ST2, offer only limited granularity in mapping specific hemodynamic derangements. Our study addresses this gap by demonstrating that a substantial proportion of plasma proteins are associated with key hemodynamic measures, often spanning across multiple domains. These associations persisted across serial time points, reinforcing their potential clinical utility.

We noted that several of the most informative proteins in our dataset have an established role as biomarkers or in pathophysiology, such as NT-proBNP, known to correlate with elevated filling pressures and impaired left ventricular function, which showed strong association with PAWP also shown in paper II.[224-226] Similarly, angiotensinogen, a component of RAAS was associated with vascular function measures, including systolic and diastolic blood pressure.[227] These expected findings provide support for our method for biomarker discovery.

Other findings of note included Adrenomedullin, previously described in this section and in paper II. The observed association between adrenomedullin levels and AC in our data support its proposed role in maintaining vascular homeostasis. Elevated adrenomedullin may reflect compensatory response to increased vascular stiffness, aiming to preserve endothelial function. These findings align with previous data linking adrenomedullin as a biomarker for vascular function.

Another particularly notable finding was Inosine monophosphate dehydrogenase 1 (IMPDH1) which plays a critical role in cellular energy metabolism and proliferation.[228] HF is characterized by chronic hypoperfusion and metabolic stress, accompanied by increased tissue oxygen extraction (elevated ΔAVO_2) and activation of nucleotide biosynthetic pathways. Parallel changes in IMPDH1 expression and ΔAVO_2 after heart transplantation may therefore reflect interaction between purine metabolism, energy demand, and oxygen utilization. Furthermore, IL-16, associated with mPAP in our analysis has previously been linked to myocardial fibrosis and LV dysfunction.[229] The finding of both expected and novel biologically plausible associations underscore the opportunities with our platform for discovery of previously unrecognised biomarkers involved in hemodynamic regulation. However, we recognise that these associations need to be validated in independent cohorts.

By leveraging heart transplantation as a natural, physiological intervention, one that results in marked normalisation of cardiac pressures and outputs, we were able to capture proteomic changes that reflect dynamic shifts in central cardiovascular function. This longitudinal design strengthens the validity of our biomarker associations and allows for more robust identification of proteins that track with hemodynamic recovery or persistence of dysfunction. Interestingly, six hemodynamic parameters, primarily those related to systemic arterial and RV function, changed little after heart transplant and showed no significant protein associations. Most identified proteins were associated with multiple parameters, and nearly three-quarters spanned several functional domains. These overlapping associations reflect the integrative and systemic nature of HF pathophysiology, where filling pressures, cardiac output, and vascular resistance interact in complex, nonlinear ways. This observation once agin supports a multimarker panels approach rather than reliance on single markers.

The ability to non-invasively monitor central hemodynamics could transform the management of HF. In current clinical practice, treatment decisions are often based on indirect surrogates such as weight, urine output, and symptoms. Our findings suggest that plasma proteomics could offer a non-invasive, reproducible, quantitative tool to more precisely guide therapy, especially in advanced HF where hemodynamic monitoring is critical.

Limitations

In this study, several limitations merit consideration. First, the sample size of this proteomic study was limited to 30 patients with advanced HF undergoing heart transplantation, which may limit generalisability to earlier disease stages or ambulatory settings. Importantly, the findings will need to be validated in larger more heterogenous cohorts.

Second, while the use of a high-throughput proteomic platform enabled broad coverage, quantitative accuracy varies across analytes, and our findings require confirmation with a targeted method which precisely quantifies absolute plasma concentrations with high reproducibility.

Third, although our design allowed for temporal analysis, causal relations of the identified biomarkers with hemodynamic parameters cannot be inferred from the observed associations. Lastly, some level of confounding by factors specific to the post-transplant phase such as immunosuppressive therapy cannot be ruled out, despite the substantial reversal of hemodynamic derangements.

To overcome such limitations, we are currently working to develop an assay with absolute protein quantification for application in independent clinical validation cohorts with serial sampling.

Strengths

This study has several notable strengths. First, the integration of serial invasive hemodynamic measurements with plasma proteomics with longitudinal design, where each patient serving as their own control before and after transplantation, substantially reduces inter-individual variability and strengthens causal inference regarding associations between hemodynamics and proteomics.

Second, the use of comprehensive affinity-based proteomic profiling enabled simultaneous assessment of over 1300 circulating proteins spanning diverse biological pathways, facilitating both hypothesis-driven validation of known biomarkers and discovery of novel candidates.

Third, the application of mixed-effects models and machine learning approaches allowed robust handling of repeated measures, small sample size, and high-dimensional data, while minimising the risk of overfitting. Finally, the simultaneous acquisition of blood samples and invasive hemodynamic measurements ensured tight temporal coupling between biological and physiological data.

Conclusions

Collectively, the studies included in this thesis provide complementary perspectives on biomarkers in HF and highlights the difficulties in interpretation of biomarker associations with HF due to the heterogeneity of this condition where multiple organ systems may be affected.

Study-specific conclusions:

Paper I — This study provides a broad proteomic view of the HF syndrome, describing the full spectrum of molecular patterns spanning from more than a decade before incident HF to advanced end-stage HF and reversal of HF after transplantation. Through comprehensive and fine-grained statistical analyses, controlling for tissue origin and integrating GWAS data, we have identified candidate proteins that may play a key role in the molecular mechanisms underlying heart failure and warrant further examination.

Paper II — Bio-ADM tracks with mRAP and associates with measures of systemic congestion and with mortality in decompensated heart failure independently from NT-proBNP. Our findings support utility of Bio-ADM as a biomarker of systemic venous congestion in heart failure and nominate a decision threshold.

Paper III — This study identifies a panel of plasma proteins associated with central hemodynamic parameters in HF, offering a potential non-invasive strategy for phenotyping and monitoring of cardiovascular function. These findings represent a step toward the integration of precision proteomics into HF care and provide a range of protein associations for future translational efforts to refine biomarker-guided management strategies.

Future perspectives

HF is an increasing health problem globally with high prevalence, morbidity and mortality, calling for innovative approaches to treatment and diagnostics. Promising advances in multi-omics technologies offer exciting new opportunities to detect subclinical disease, assess patient risk more accurately, and enable non-invasive monitoring. Alongside this, multi-omics approaches, including lipidomics, metabolomics, proteomics, and genomics, provide a deeper, more comprehensive understanding of HF. These tools support the discovery of novel biomarkers, potential drug targets, and the development of personalised treatment.

To move identified biomarkers forward towards clinical utility, biomarkers need to be tested and validated across broad and diverse patient populations. HF is a highly variable condition that differs across age, ethnicity, and subtypes such as HFrEF and HFpEF. Long-term studies with extended follow-up are essential to understand how well these biomarkers predict clinical outcomes like hospitalisation and mortality, and how they can guide treatment decisions. Collaborative efforts like the HOMAGE project,[230] a European research initiative that included both observational studies and a randomised clinical trial to investigate HF, demonstrate how pooling data and resources can speed up progress. Expanding such initiatives to include underrepresented populations would deepen our understanding of HF across different healthcare environments.

Innovations in point-of-care (POC) diagnostics also play a key role in bringing multi-omics into routine care. New platforms now allow for the simultaneous measurement of multiple biomarkers like BNP and NT-proBNP, bedside or even at home. When paired with smartphone apps, these tools support remote monitoring and allow clinicians to adjust treatments quickly, which is especially valuable for managing chronic HF. However, to see widespread adoption, the focus must shift toward developing affordable and scalable technologies that work well in settings with limited resources that affects outcome.

These advances offer exciting potential for personalised care. However, several significant challenges remain to be addressed before they can be fully integrated into everyday clinical practice. Key areas that require attention include large-scale validation, ongoing technological innovation, and ensuring fair and, equally important, policy changes needed to make this advanced technology accessible to all. Governments and healthcare systems must invest in the infrastructure required

to support omics-based diagnostics, especially in low- and middle-income countries. Strong ethical and legal frameworks are also essential to protect privacy and ensure responsible use of sensitive data.

Another major opportunity lies in combining multi-omics data with artificial intelligence (AI) and machine learning (ML). These technologies can detect complex patterns in omics datasets that are beyond the reach of traditional analysis. By integrating genetic, proteomic, clinical, and lifestyle data, AI can help build precision medicine models tailored to individual patients. Cloud-based platforms can further level the playing field by giving providers in low-resource settings access to advanced data processing tools.

Lastly and probably most important, collaboration across disciplines is vital to access full potential of these tools. Essential is partnerships between researchers, clinicians, patients and industry that can help translate scientific discoveries into practical, affordable solutions with maximum benefit for the patient.

Summary in Swedish

Populärvetenskaplig sammanfattning

Hjärtsvikt är ett tillstånd som karaktäriseras av att hjärtat inte längre orkar pumpa runt blodet i kroppen tillräckligt effektivt. Det gör att kroppens organ och vävnader inte får det syre och de näringsämnen de behöver. Symtomen kommer inte sällan smygande och utgörs ofta av andfåddhet vid ansträngning eller i vila, svullna ben och anklar samt en påtaglig trötthet som inte försvinner trots vila.

Tillståndet kan försämras över tid och påverka andra viktiga organ, som njurar och lever, vilket kan leda till livshotande komplikationer. Hjärtsvikt är en vanlig orsak till upprepade sjukhusbesök, nedsatt livskvalitet och ökad dödlighet.

Under de senaste 25 åren har andelen människor som riskerar att insjukna i hjärtsvikt under sin livstid ökat, från 1 av 5 till 1 av 3-4. Idag lever omkring 64 miljoner människor i världen med hjärtsvikt. Bakom ökningen ligger bland annat ökad livslängd, men också att riskfaktorer som högt blodtryck, typ 2-diabetes och att övervikt blivit vanligare i allt yngre åldrar. Detta gör hjärtsvikt till ett växande folkhälsoproblem världen över som bidrar till ett ökat lidande men även till en samhällsekonomisk utmaning.

Stora framsteg har gjorts av utveckling av olika nya behandlingsmetoder vid hjärtsvikt. Nya läkemedel, som SGLT2-hämmare och neprilysinreceptor-hämmare, har förbättrat livskvalitet, prognos och minskat risken att läggas in på sjukhus, för många patienter med hjärtsvikt. Utöver traditionell läkemedelsbehandling, finns avancerade medicintekniska lösningar, så som inopererade defibrillatorer (ICD), pacemakers som hjälper hjärtat att slå mer effektivt och synkroniserat (CRT), och i svåra fall mekaniska hjärtpumpar eller hjärttransplantation.

Trots dessa framsteg är dödligheten i hjärtsvikt fortfarande hög. Många behandlingar bygger på generella riktlinjer snarare än individanpassning. Idag får ofta alla patienter med hjärtsvikt liknande behandling, oavsett bakomliggande orsak eller individuell variation i hur kroppen svarar på behandlingen. För att förbättra vården behövs därför nya sätt att förstå och behandla hjärtsvikt, med fokus på varje enskild patients unika förutsättningar och orsak till sin hjärtsvikt.

Här kommer precisionsmedicin in i bilden. Det är ett forskningsfält som strävar efter att anpassa behandling och diagnos efter individens gener, biologi och livsstil. Ett

centralt verktyg inom detta område är biomarkörer, exempel på detta är ämnen i kroppen, ofta proteiner, som kan mätas i blodet som kan ge information om sjukdomstyp, förväntad sjukdomsutveckling, risk och hur väl en viss behandling fungerar för den enskilda patienten.

Genom att kartlägga biomarkörer i olika stadier av hjärtsvikt hoppas man kunna hitta nya ledtrådar till hur sjukdomen fungerar på cellnivå. Det kan i sin tur leda till nya, mer träffsäkra behandlingsmetoder. Biomarkörer kan också hjälpa läkare att tidigare upptäcka vilka patienter som löper störst risk att drabbas av hjärtsvikt, försämras i sin sjukdom samt att skräddarsy behandlingen utifrån resultaten.

Syftet med denna avhandling är att bidra med pusselbitar, för att öka förståelsen för vilka biomarkörer som kan vara relevanta i olika stadier av hjärtsvikt, hur dessa hänger ihop med hjärtats funktion och olika konsekvenser av hjärtsvikt, och hur väl vissa biomarkörer fungerar för att ställa diagnos och förutsäga framtida risk – i jämförelse med dagens etablerade metoder.

På sikt är förhoppningen att den här typen av forskning ska bidra till en mer riktad, effektiv och individanpassad vård för personer med hjärtsvikt.

Denna avhandling innefattar tre delstudier:

I **delstudie I** har vi studerat 1305 proteiner i blodet från tre olika patientgrupper som alla representerar individer med olika stadier av hjärtsvikt. Från individer som riskerar att insjukna i hjärtsvikt, till patienter med manifest hjärtsvikt, och slutligen till individer med svår hjärtsvikt som genomgår hjärttransplantation. Totalt 16 proteiner visade sig vara starkt kopplade till hjärtsviktsinsjuknande och vi kunde följa hur vissa proteiner normaliserades efter hjärttransplantation medan andra endast ändrades något. Studien visar på en komplex bild med flera proteiner som samspelar från olika delar av kroppen och förändringarna i proteinnivåer kan mätas långt innan patienten får symtom.

I **delstudie II** undersökte vi ett protein som heter bioaktivt adrenomedullin (Bio-ADM), som har visat sig vara kopplat till övervätskning i kroppen i samband med hjärtsvikt. Vi började med att mäta halten av Bio-ADM hos en grupp friska äldre personer för att ta reda på vad som räknas som normala nivåer (referensintervall). Därefter studerade vi patienter med hjärtsvikt, särskilt de som hade tecken till vätskeansamling i vävnaderna och sökte vård akut för andfåddhet, ett vanligt och allvarligt symtom vid hjärtsvikt. Vi kunde då se att höga nivåer av Bio-ADM tydligt hängde ihop med en tendens att samla på sig vätska i kroppen.

Vi såg att patienter med förhöjda nivåer av Bio-ADM hade en högre risk att avlida inom 30 dagar från att de sökt akutmottagningen med andningssvårigheter till följd av sin hjärtsvikt. Detta pekar mot att Bio-ADM skulle kunna användas som en prognostisk biomarkör, dvs ett verktyg för att identifiera hjärtsviktspatienter med högre risk, och därmed ge dem mer skräddarsydd och snabb behandling.

I delstudie III undersökte vi om vanliga blodprov kan ge information om hur hjärtat fungerar hos patienter med hjärtsvikt. I dag krävs ofta en avancerad undersökning, så kallad hjärtkateterisering, för att mäta tryck och blodflöde i hjärtat. Vid en sådan undersökning förs en tunn slang via ett blodkärl på halsen in till hjärtat. Metoden ger mycket detaljerad information men används i dag bara i utvalda situationer, till exempel vid utredning inför hjärttransplantation.

I studien analyserade vi över 1 300 olika proteiner i blodprover från 30 patienter med svår hjärtsvikt. Proven togs i samband med hjärtkateterisering både före och efter hjärttransplantation. Vi fann att mer än 200 av dessa proteiner hade tydliga samband med tryckförhållanden i olika delar av hjärtat.

Resultaten tyder på att blodbaserade biomarkörer i framtiden skulle kunna användas för att spegla hjärtats funktion utan behov av lika avancerade och invasiva undersökningar. Detta kan på sikt göra det lättare att följa sjukdomsutveckling och anpassa behandling utifrån individuella förändringar i hjärtats funktion.

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Use of artificial intelligence (AI)

In the writing of this thesis, the author used the AI tool ChatGPT (OpenAI) for support with grammar, structure, and wording suggestions. All aspects of the scientific work, including study design, data collection, analysis, interpretation, and conclusions, were carried out independently by the author, who assumes full responsibility for the content of the thesis.

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