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The role of platelets in heart failure syndrome

Die Rolle von Thrombozyten beim Syndrom Herzinsuffizienz

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List of abbreviations

ACCF/AHA	American College of Cardiology Foundation/ American Heart Association
ACE	Angiotensin-converting enzyme
ARB	Angiotensin receptor blocker
ARNI	Angiotensin receptor-neprilysin inhibitor
BNP	B-type natriuretic peptide
CVRFs	Cardiovascular risk factors
E/E'	Measure of diastolic function
EF	Ejection fraction
eGFR	Estimated glomerular filtration rate
ESC	European Society of Cardiology
HF	Heart failure
HFimpEF	Heart failure with improved ejection fraction; baseline LVEF ≤40%, a ≥10% increase from baseline LVEF, and a second measurement of >40% LVEF
HFmrEF	Heart failure with mildly reduced ejection fraction, 41% to 49%
HFpEF	Heart failure with preserved ejection fraction (≥ 50%)
HFpEF borderline	Heart failure with ejection fraction between 41% to 49% (ACCF/AHA definition, 2013)
HFrEF	Heart failure with reduced ejection fraction (≤ 40%)
HR	Hazard ratio
LVEF	Left ventricular ejection fraction
MI	Myocardial infarction
MPV	Mean platelet volume
NT-proBNP	N-terminal pro-B-type natriuretic peptide
NYHA	New York Heart Association
PTH	Parathyroid hormone
RAAS	Renin-angiotensin-aldosterone system
SGLT2	Sodium-glucose co-transporter 2
TIMP-4	Metalloproteinase inhibitor 4

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English summary

This dissertation investigates the role of platelets in heart failure (HF). HF is a diverse syndrome with a high mortality rate. Platelets represent one factor in the pathophysiology of HF.

Platelet indices are routinely measured and can be described by mean platelet volume (MPV) and platelet count. Both markers help to determine the activity of platelets and are of high importance for this dissertation. MPV does not only describe the size of platelets, it has been described as a surrogate for platelet reactivity and platelet activation. In combination with leukocytes, monocytes and/or lymphocytes, platelets are important players in immune response and were associated with worse cardiac function and adverse clinical outcome.

In addition, platelets interact with several proteins and humoral messengers. Parathyroid hormone (PTH) is commonly known to be responsible for the homeostasis of calcium in the blood, but also an interaction with the heart was found. The results presented in this thesis additionally showed a relation between PTH and both platelet indices, a positive association with MPV and a negative association with platelet count, in subjects with HF. Furthermore, the analysis showed sex-specific differences for the associations of PTH with platelet indices within the HF phenotypes.

To assess platelet-related proteins, a plethora of proteins released after platelet activation, was analyzed to give further information beyond the routine markers MPV and platelet count in HF individuals. This analysis project showed important differences and specific platelet-related protein signatures for each HF phenotype. Furthermore, scores of the relevant protein signatures showed increased risk for the primary study outcome “worsening of HF” in all HF phenotypes.

Overall, this dissertation identified an important role of platelets in HF syndrome. Different aspects and interactions of platelets in HF pathophysiology were highlighted within HF phenotypes, underlining the substantial difference in clinical characteristics among HF phenotypes with reduced vs. preserved ejection fraction as well as sex-specific differences within a specific HF phenotype. Additionally, the results showed a substantial link of platelet biomarkers to worsening of HF in individuals with HF syndrome.

Deutsche Zusammenfassung

Diese Promotionsarbeit stellt die Rolle der Thrombozyten in Bezug auf das Syndrom der Herzinsuffizienz dar. Die Herzinsuffizienz zeigt eine vielseitige Pathophysiologie mit auffallend hoher Mortalitätsrate. Einen Faktor hierbei stellen die Thrombozyten dar.

Parameter zur Charakterisierung von Thrombozyten werden routinemäßig gemessen und anhand des mittleren Thrombozytenvolumens (mean platelet volume, MPV) und der Thrombozytenzahl (platelet count) beschrieben. Diese beiden Marker geben Hinweise auf die Aktivität der Thrombozyten und sind für diese Promotionsschrift von großer Bedeutung. Mit dem MPV kann nicht nur die Größe der Thrombozyten dargestellt werden, sondern MPV wurde auch als Surrogat für die Aktivität und Reaktivität der Thrombozyten beschrieben. In Kombination mit der Zahl der Leukozyten, Monozyten und/oder Lymphozyten stellen die Thrombozyten wichtige Faktoren der Immunantwort dar und waren mit einer schlechteren kardialen Funktion und nachteiligem klinischem Outcome assoziiert.

Des Weiteren interagieren die Thrombozyten mit einer Vielzahl an Proteinen und körpereigenen Botenstoffe. Das Parathormon (parathyroid hormone, PTH), welches hauptsächlich als Gegenspieler zu Vitamin D zur Calciumhomöostase im Blut beiträgt, zeigt darüber hinaus einen Einfluss auf das Herz. Diese Arbeit konnte außerdem bei Personen mit kardialer Dysfunktion Zusammenhänge zwischen PTH und den beiden analysierten Parametern der Thrombozyten - MPV und platelet count – zeigen; die Zusammenhänge verhielten sich invers. Die Assoziation zu MPV war positiv, wohingegen sich eine negative Assoziation zur Thrombozytenzahl ergab. Die Analyse ergab darüber hinaus geschlechtsspezifische Unterschiede der Zusammenhänge von PTH mit den beiden Parametern der Thrombozyten innerhalb der Phänotypen der Herzinsuffizienz.

Zur Bestimmung von den Thrombozyten zugehörigen Proteinen (platelet-related proteins) wurde eine Vielzahl von Proteinen bezüglich ihrer Expression in aktivierten Thrombozyten analysiert. Diese platelet-related proteins gaben zusätzliche Auskunft über die Rolle der Thrombozyten, welche über die Informationen, die durch MPV und platelet count gewonnen werden können, hinausgehen. Es konnten große Unterschiede und spezifische Thrombozyten-Proteinprofile für die unterschiedlichen Phänotypen der Herzinsuffizienz herausgearbeitet werden. Zusätzlich wiesen die für die jeweiligen Phänotypen selektionierten Proteine, jeweils in einem Score zusammengefasst, auf einen relevanten Zusammenhang mit dem primären Studienendpunkt „Verschlechterung der Herzinsuffizienz“ (worsening of HF) hin.

Insgesamt zeigt diese Dissertation die bedeutende und vielseitige Rolle der Thrombozyten in Bezug auf das Syndrom der Herzinsuffizienz, welche in mehreren Teilanalysen umfänglich

dargestellt werden konnte. Die verschiedenen Aspekte und Interaktionen der Thrombozyten in der Pathophysiologie der Herzinsuffizienz innerhalb der verschiedenen Phänotypen der Herzinsuffizienz wurden verdeutlicht und zeigten enorme Unterschiede im klinischen Erscheinungsbild der Herzinsuffizienz mit erhaltener und mit reduzierter Ejektionsfraktion, sowie geschlechtsspezifische Unterschiede innerhalb der einzelnen Phänotypen. Auch im Hinblick auf die Verschlechterung der Herzinsuffizienz zeigte sich ein relevanter Zusammenhang mit dem mittleren Thrombozytenvolumen und der Thrombozytenzahl, sowie einem Proteinscore, der aus den für die jeweiligen Phänotypen der Herzinsuffizienz relevanten Proteinen gebildet wurde.

General introduction

The aim of this doctoral work was to investigate the relation of platelet indices, surrogate for platelet activation, in heart failure (HF) syndrome including HF phenotypes and the association with clinical outcome. Further, the association between PTH concentrations and platelet indices was investigated sex-specifically in phenotypes of HF. In addition, circulating platelet-related proteins, measured by the targeted protein biomarker discovery approach, were characterized in patients with HF and associations with HF phenotypes and clinical endpoint were determined.

HF, according to the universal definition of the Heart Failure Society of America, Heart Failure Association of the European Society of Cardiology (ESC), Japanese Heart Failure Society, and endorsed by the Canadian Heart Failure Society, Heart Failure Association of India, Cardiac Society of Australia and New Zealand, and Chinese Heart Failure Association, was defined as "a clinical syndrome with symptoms and/or signs caused by a structural and/or functional cardiac abnormality and corroborated by elevated natriuretic peptide levels and/or objective evidence of pulmonary or systemic congestion".[1] HF phenotypes were described according to the left ventricular ejection fraction (LVEF) as heart failure with preserved ejection fraction (HFpEF) with LVEF ≥ 50%, heart failure with mildly reduced ejection fraction (HFmrEF) with LVEF 41 - 49%, referred to as "HFpEF borderline" in the 2013th guideline of the American College of Cardiology Foundation/ American Heart Association (ACCF/AHA)[2]; and heart failure with reduced ejection fraction (HFrEF) was determined as symptomatic HF with LVEF ≤ 40%. Additionally, in the 2021 universal heart failure guideline, the phenotype heart failure with improved ejection fraction (HFimpEF) with a baseline LVEF ≤40%, a ≥10 point increase from baseline LVEF, and a second measurement of LVEF >40%, was established.[1]

The myocardium of HFpEF individuals showed increased left ventricular wall thickness and/or increased left atrial size due to increased filling pressure.[3] Coronary microvascular endothelial inflammation was found to drive myocardial remodeling in HFpEF due to several comorbidities affecting the heart.[4] This proinflammatory state caused structural and functional alterations of the myocardium, such as stiff cardiomyocytes and interstitial fibrosis, leading to concentric cardiac remodeling, and should be considered in future HFpEF treatment strategies.[4] HFrEF, on the other hand, is mainly driven by ischemia, infection and toxicity resulting in loss of cardiomyocytes through necrosis, apoptosis, and autophagy and can be described by eccentric cardiac remodeling with longitudinal and transversal LV hypertrophy.[4]

HF is classified into different stages according to severity and progression of the syndrome as shown in **Table 1**.

Table 1. Description of the ACCF/AHA stages of HF adapted from Bozkurt, 2021[1]

ACCF/ AHA stages of HF	Description
A, At risk for HF	Without current or prior symptoms or signs of HF and without structural cardiac changes or elevated biomarkers of heart disease
B, Pre-HF	Without current or prior symptoms or signs of HF but evidence of one of the following: structural heart disease, abnormal cardiac function, elevated natriuretic peptide levels
C, HF	With current or prior symptoms and/or signs of HF caused by a structural and/or functional cardiac abnormality
D, Advanced HF	Severe symptoms and/or signs of HF at rest, recurrent hospitalizations despite guideline-directed management and therapy, refractory or intolerant to guideline-directed management and therapy; requiring advanced therapies such as consideration for transplantation, mechanical circulatory support, or palliative care

The New York Heart Association (NYHA) provided further information about symptom severity and functional capacity by ranking symptomatic HF into functional classes I-IV (**Table 2**).

Table 2. Description of the NYHA functional classification adapted from McDonagh, 2021[5]

NYHA functional classification	Description
I	No limitation in physical activity. Ordinary physical activity does not cause symptoms of HF.
II	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in symptoms of HF.
III	Marked limitations of physical activity. Comfortable at rest, but less than ordinary activity results in symptoms of HF.
IV	Unable to carry on any physical activity without discomfort. Symptoms at rest can be present.

Nevertheless, there are major differences between HF phenotypes and further research is required to improve knowledge of HF characteristics and pathophysiology for differentiated

therapy and management of HF subjects. For individuals with HFrEF, several treatment options are available: Neuro-hormonal antagonists, such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), angiotensin receptor-neprilysin inhibitors (ARNIs), mineralocorticoid receptor blockers, sodium-glucose co-transporter 2 (SGLT2) inhibitors, diuretics, and beta blockers have been revealed to reduce mortality and morbidity in HFrEF individuals.[3] ACE inhibitors, mineralocorticoid receptor blockers, and beta blockers have been shown to have additional anti-inflammatory properties.[6] Until recently, none of these drugs or interventions have substantially improved the prognosis of HFpEF patients in large clinical trials.[7, 8] But in 2021, the EMPEROR-Preserved study found a reduction in the combined risk of cardiovascular death or hospitalization in HF patients with LVEF >40% receiving Empagliflozin in addition to usual therapy.[9] These results showed optimism for the future as until then mortality rates of HFpEF individuals were similar to that of certain malignancies, such as colorectal cancer in males or breast cancer in females.[10, 11] To reduce the patients´ overall burden and improve their quality of life, the key cardiovascular risk factors (CVRFs), comorbidities, and symptoms were the main target of treatment in HFpEF patients.[2, 3] However, this approach was merely a symptomatic relief that did not address the causal pathophysiological target. Identifying novel actors in HF pathophysiology is of great importance to improve the risk stratification and management of HF syndrome. Therefore, a universal definition of HF was established to standardize HF phenotypes and classification for future clinical studies and investigations.[1]

As one approach, the phenotype HFmrEF was classified between reduced and preserved EF with 41-49% LVEF over the past decade, and with the latest update in 2021, those individuals with LVEF improvement of at least ten points increase from a baseline LVEF below 40% and a second measurement of LVEF >40% were classified as HFimpEF.[1] Both HF phenotypes showed a distinct response to medical treatment in clinical trials compared to HFrEF and HFpEF phenotypes or were excluded from previous studies as shown in Table 2 and Table 3 in the position paper of Bozkurt et al., 2021.[1]

For a better understanding of HF pathophysiology and to analyze open questions regarding HF syndrome, the MyoVasc study was established at the University Medical Center of the Johannes Gutenberg-University in Mainz, Germany. This study is an investigator-initiated, prospective cohort study with 3,289 individuals aged 35 to 84 years enrolled at baseline from January 2013 to April 2018. All participants underwent a highly standardized five-hour examination at the study center that included physical examination, echocardiography, and a complete laboratory investigation including extensive biobanking of biomaterial such as blood samples from several time points (baseline and follow up after two, four, six, and eight years) and of multiple qualities, e.g., serum and citrated plasma, for further analyses such as platelet function testing. All procedures were performed by qualified staff according to standard

operating procedures. The aim of the MyoVasc study was to investigate the development and progression of HF syndrome, phenotypes of this heterogeneous syndrome, and the interactions among phenotypes with the vasculature regarding their impact on the course of HF.[12]

This doctoral thesis aimed to investigate the role of platelets in HF within this comprehensive cohort of well-characterized HF individuals from the MyoVasc study.

Platelets play a key role in hemostasis and thrombosis, and there were several indications for a role of platelets in HF pathophysiology as well.[13, 14] Due to the remodeling of the left ventricle, blood flow might be impaired in HF.[15] This impairment is suggested to cause a state of stasis with increased hypercoagulability and endothelial injury leading to further platelet activation. In thrombosis, this phenomenon is well-known as Virchow´s triad.[15, 16] It has been long of interest whether and to what extent platelet function is associated with the failing heart and whether platelets might be useful as biomarkers for HF severity and/or applicable as therapeutic targets in HF patients. In HF subjects with sinus rhythm, antithrombotic and antiplatelet agents were not found to be beneficial.[2, 3] Nevertheless, a link between platelets and outcome in HF phenotypes has not been fully excluded.[17, 18] Platelets not only play a key role in thrombosis and hemostasis, but also in inflammation.[19] They release a plethora of different proinflammatory cytokines and chemokines in response to their activation from α-granules, dense-granules, and cytoplasm leading to systemic inflammation.[19] Additionally, platelets present surface proteins at activation, that can interact with immune cells, modulate leukocyte recruitment, and the activation of vascular endothelium. It is of particular interest to evaluate the platelet role, as an inflammatory and immune cell, in HF phenotypes, as HFpEF and HFrEF present with different inflammatory characteristics.[4]

Platelet state can be characterized by measuring mean platelet volume (MPV) and platelet count. Both are non-expensive and easily available routine markers that provide important information about platelet activation and can be associated with several cardiovascular diseases.[20, 21] Increased platelet activation was associated with several risk factors for cardiovascular diseases and HF, such as diabetes mellitus, arterial hypertension, hypercholesterinemia, obesity, and smoking.[20] In addition, differences in the presentation of platelet indices between men and women have been observed.[22] In females, higher MPV was linked to oral contraceptives and menstrual bleeding, whereas in males, higher MPV was associated with older age, smoking, hypertension, and high glucose levels. Additionally, a worse survival was found in men with elevated levels of MPV.[22]

In this doctoral thesis, the platelet phenotypes in HF patients, depicted from platelet indices, were characterized, and platelet characteristics were precisely distinguished between HFpEF and HFrEF phenotypes. The relation of platelets with leukocytes, lymphocytes, and monocytes

were investigated as platelet-to-leukocyte ratio, platelet-to-lymphocyte ratio, and platelet-to-monocyte ratio, as novel markers of inflammation that may contribute to a deeper understanding of the role of platelets as part of the inflammatory and immune response in HF individuals with HF, including the relation to cardiac function and clinical outcome of HF.

Endogenous hormones, particularly PTH and Vitamin D, have been linked to cardiac function.[23] PTH interacts directly with the heart, but an indirect pathway via interaction with the renin-angiotensin-aldosterone system (RAAS) has also been discussed.[24] In addition, PTH has been shown to interact with platelets.[25] Therefore, the second chapter of this dissertation investigated the association between platelet indices and PTH in HF subjects. In addition to the variation in HF phenotypes, further sex-specific differences within HF phenotypes were assessed.

Furthermore, the role of plasma proteins related to activated platelets was investigated. Proximity extension assay (PEA) technology by Olink Proteomics, Uppsala, Sweden, was used to assess proteins in the plasma of HF subjects from the MyoVasc study. As platelets release their rich content into the circulation[26], a detailed literature research using the search term “platelet activation” was conducted and a selection of measured proteins that were released by activated platelets were defined as “platelet-related proteins” and further investigated in HF phenotypes and with regard to clinical outcome. The selected proteins were increased in response to platelet activation and analyzed to gain a deeper understanding of the role of platelets as part of the immune response in the pathophysiology of HF phenotypes. A platelet-related protein signature specific for each HF phenotype was identified. By defining a score of the platelet-related protein signatures, important associations with clinical outcome for each HF phenotype were elaborated.

The clinical outcome “worsening of HF” was defined as a composite of cardiac death, hospitalization due to worsening of HF, and the transition from asymptomatic to symptomatic HF.[12] In the first part of this doctoral thesis, platelet indices, i.e. MPV and platelet count as well as platelet-to-lymphocyte, platelet-to-monocyte, and platelet-to-leukocyte ratios, were analyzed as independent variables regarding outcome, whereas for a deeper insight into the role of platelets in HF outcome, a score of platelet-related proteins was defined with relevant proteins for each HF phenotype to determine the relationship with clinical outcome. In addition to the different roles in HF phenotypes, the role of platelets in outcome was of clinical relevance and may provide important information for future research.

Publication: The impact of platelet indices on clinical outcome in heart failure: results from the MyoVasc study

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ORIGINAL RESEARCH ARTICLE

The impact of platelet indices on clinical outcome in heart failure: results from the MyoVasc study

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Abstract

Aims Platelet indices have been associated with traditional cardiovascular risk factors, cardiovascular diseases and all-cause mortality. This study aimed to investigate the role of platelet count, mean platelet volume (MPV) and platelet-to-leukocyte ratio, including platelet-to-monocyte and platelet-to-lymphocyte ratio with cardiac function, heart failure (HF) phenotypes and clinical outcome, worsening of HF.

Methods and results Univariate and multivariable linear and Cox regression analyses were used to investigate the associations between platelet indices, cardiac function and worsening of HF in 3250 subjects enrolled in the MyoVasc study. Higher MPV, lower platelet count, lower platelet-to-leukocyte and platelet-to-monocyte ratios have been associated with reduced left ventricular ejection fraction (beta estimate $[\beta]_{\text{MPV [fL]}} = -0.05$ [−0.09; −0.02], $\beta_{\text{platelet count (}\times 10^9/\text{L)}} = 3.4$ [1.2; 5.6], $\beta_{\text{platelet-to-leukocyte ratio}} = 1.4$ [1.1; 1.8], $\beta_{\text{platelet-to-monocyte ratio}} = 28$ [20; 36]) and increased E/E' ratio ($\beta_{\text{MPV [fL]}} = 0.04$ [0.003; 0.07], $\beta_{\text{platelet count (}\times 10^9/\text{L)}} = -3.1$ [−5.3; −0.92], $\beta_{\text{platelet-to-leukocyte ratio}} = -0.83$ [−1.2; −0.46], $\beta_{\text{platelet-to-monocyte ratio}} = -20$ [−28; −12]), independent of age and sex. Cox regression demonstrated an increased risk for worsening of HF in subjects with MPV > 75th percentile (hazard ratio [HR] = 1.47 [1.16; 1.87]), platelet count < 25th percentile (HR = 1.36 [1.07; 1.74]), platelet-to-leukocyte < 25th percentile (HR = 1.53 [1.20; 1.95]), platelet-to-monocyte < 25th percentile (HR = 1.38 [1.08; 1.77]) and platelet-to-lymphocyte > 75th percentile (HR = 1.50 [1.17; 1.93]) ratios, independent of potential confounders. MPV > 75th percentile and platelet count < 25th percentile were strongly related to outcome in HFpEF vs. HFrEF (*P* for difference = 0.040). Platelet-to-leukocyte ratios were associated with worse outcome in both HF phenotypes, without a significant difference between HFpEF and HFrEF.

Conclusions Platelet indices are linked with worse cardiac function and adverse clinical outcome, independent of subjects' underlying cardiovascular profile. This study emphasizes their important value to provide additional information on pathophysiology and risk stratification in HF syndrome.

Keywords Heart failure; Mean platelet volume; Platelet count; HFrEF; HFpEF; Worsening of heart failure

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Introduction

Heart failure (HF) is a major public health problem affecting more than 23 million individuals worldwide.[1] A recent large epidemiological study, including 3 million individuals from Germany with at least two documented HF-related diagnoses, demonstrated a prevalence of 3.96% and an incidence of 655 new cases per 100,000 persons at risk for HF in Germany

only.[2] HF is a complex clinical syndrome including unspecific symptoms like shortness of breath and peripheral oedema and thus requires further invasive and non-invasive diagnostic tools.[1] The current HF classification is based on left ventricular ejection fraction (LVEF) into (1) HF with preserved ejection fraction (HFpEF) with signs and symptoms of HF and diastolic abnormalities on echocardiography, (2) HFpEF borderline or HF with mid-range ejection fraction (HFmrEF) with EF of 41–49% and (3) HF with reduced ejection fraction (HFrEF) with EF $\leq$ 40%.[3,4] Particularly for HFpEF, considerable uncertainty remains regarding its pathogenesis, diagnosis and optimal therapeutic approach.[5] Endothelial dysfunction, inflammation, cardiomyocyte dysfunction and myocardial fibrosis have been implicated as key factors in the development of HF.[6,7]

Platelet activation has been described in patients with congestive HF as increased whole blood aggregation, higher mean platelet volume (MPV) and higher expression of platelet bound and soluble P-selectin.[8] Platelet markers including MPV have been associated with traditional cardiovascular risk factors (CVRFs) such as arterial hypertension, diabetes mellitus, obesity, hypercholesterolaemia and smoking, often concomitantly present in HF subjects.[9–11] Platelets have an important role as mediators of inflammation, particularly via their interaction with leukocytes.[12] In addition, platelet-to-leukocyte ratios, including platelet-to-monocyte and platelet-to-lymphocyte ratios, have been suggested as novel biomarkers to assess systemic inflammation in various conditions.[13,14] Platelet indices and their significance have not yet been comprehensively explored in individuals with HF. This study aimed to investigate the relation of MPV, platelet count and platelet-to-leukocyte ratios with parameters of cardiac function, HF phenotypes and clinical outcome in the MyoVasc study, a cohort of individuals with HF.

Methods

Study sample

MyoVasc is a large epidemiological, prospective, cohort study at the University Medical Center of the Johannes Gutenberg-University Mainz in Germany conceptualized to investigate pathophysiology, diagnostics, clinical course and treatment of HF.[15] Information about inclusion and exclusion criteria of the MyoVasc study were provided in the Supporting Information. Baseline examination of the *n* = 3289 MyoVasc study participants took place between January 2013 and April 2018. All participants, aged from 35 to 84 years, underwent a comprehensive, highly standardized clinical investigation at the MyoVasc study centre. Platelet indices, measured in fresh blood samples within the routine laboratory at baseline examination, were available in 3250 individuals; *n* = 294 were controls with normal echocardiographic function (*Figure S1*). Written informed consent was obtained from all study participants prior to entering the study. The study complies with the principles outlined in the Declaration of Helsinki, Good Clinical Practice and Good Epidemiological Practice. An approval from the responsible ethics committee (reference number 837.319.12 (8420-F)) and data safety commissioner was obtained in 2012, before study initiation. The MyoVasc study is registered at http://clinicaltrials.gov (identifier: NCT04064450).

Assessment of cardiac structure and function

Resting two-dimensional transthoracic echocardiograms were performed according to recommendations of the American and European Society of Echocardiography using an iE33 echocardiography system (Royal Philips Electronics, Amsterdam, The Netherlands).[16] The mitral inflow velocity pattern was recorded from the apical four-chamber view with the pulsed-wave Doppler sample volume positioned at the tips of the mitral valve leaflets during diastole in expiration. Peak early (E-wave) and late (A-wave) diastolic filling velocities were measured, and their ratio (E/A) was calculated. The lateral mitral annular early diastolic velocity (E') was measured by spectral tissue Doppler imaging, and the E/E' ratio determined. LVEF was calculated by measurement according to Simpson from the apical four-chamber view.

Laboratory assessment

Venous blood sampling for the present analysis on platelet indices was performed by using tripotassium ethylenediaminetetraacetic acid (K3-EDTA) tubes. Platelet and leukocyte counts, including monocyte and lymphocyte counts, and MPV were automatically determined within 30–90 min after blood withdrawal on an ADVIA 120 Hematology System (Siemens, Erlangen, Germany) in the central laboratory of the Institute for Clinical Chemistry and Laboratory Medicine, University Medical Center Mainz, Germany.

Data assessment and statistical analysis

HF phenotypes were defined according to established echocardiographic criteria as follows: (i) no cardiac dysfunction: LVEF $\geq$ 55%, E/A $\geq$ 0.75, E/E' $<$ 10 and $DT_E \geq 140$; (ii) preserved ejection fraction (PEF): LVEF $\geq$ 50% and one of the following: (E/A $<$ 0.75 and E/E' $<$ 10), (E/A $\geq$ 0.75 and E/E' $\geq$ 10 and $DT_E \geq 140$ ms) or (E/A $>$ 2 and E/E' $\geq$ 10 and $DT_E < 140$ ms); (iii) reduced ejection fraction (REF): LVE $\leq$ 40%.[4,17] Individuals with LVEF of 41–49% were not considered for this study. Symptomatic HF was defined in patients with echocardiographic findings as stated in (ii) or (iii) who reported at least one of the following: New York

Heart Association (NYHA) functional class $\geq$ II; (bilateral ankle swelling OR rales OR nocturia) AND N-terminal pro-B-type natriuretic peptide (NT-proBNP) $>$ 125 pg/mL; NYHA Class I AND NT-proBNP $>$ 125 pg/mL AND HF medication. HFpEF was defined as symptomatic HF with PEF, and HFrEF was defined as symptomatic HF with REF.

According to these criteria, the analysis sample comprised *n* = 2111 individuals with PEF, *n* = 637 with HFpEF. *n* = 844 individuals were subjects with REF; *n* = 341 were diagnosed as HFrEF and *n* = 397 as HFpEF borderline (*Figure S1*). HFpEF borderline individuals and not classifiable individuals (*n* = 343), with HF symptoms and PEF but without diastolic dysfunction, were excluded for those analysis where HFpEF vs. HFrEF was compared.

Study outcome was defined as worsening of HF, a composite of transition from asymptomatic to symptomatic HF and cardiac death in asymptomatic HF individuals as well as a composite of hospitalization due to worsening of HF and cardiac death in symptomatic HF individuals.[15]

Statistical analysis was performed after data quality control including a review for completeness and plausibility performed by the data management unit. Clinical characteristics of the study sample were described according to quartiles of MPV and platelet count. Additionally, clinical characteristics were presented for the total analysis sample, HFpEF and HFrEF individuals. Normally distributed values were described by using mean ± standard deviation. Categorical variables were expressed as absolute and relative frequencies. MPV, platelet count and platelet-to-leukocytes ratios were assessed by univariate and multivariable linear regression models adjusted for age, sex, cardiovascular risk profile and cancer or age, sex, systolic and diastolic cardiac function (by LVEF and E/E' ratio, respectively) as well as plus antithrombotic medication (ATC B01). Beta estimates for LVEF (%) and E/E' ratio were presented per 1 standard deviation (SD) of the trait. In addition, the distribution of LVEF (%) and E/E' ratio per increasing MPV (fL) or per increasing platelet count (10^9/L) were depicted as scatter plots. The cardiovascular risk profile comprises CVRFs and cardiovascular diseases (CVDs) as described in the Supporting Information. The distributions of CVD per increasing MPV (fL) or per increasing platelet count (10^9/L) were depicted as boxplots. Outcome data on worsening of HF were depicted as cumulative incidence plots for quartiles of MPV, platelet count, platelet-to-leukocyte ratio, platelet-to-monocyte ratio and platelet-to-lymphocyte ratio with Grey's test for differences between curves, respectively. A forest plot depicted the relation between platelet indices and worsening of HF, calculated by Cox regression analyses with hazard ratio (HR) and 95% confidence interval (CI) and adjusted for age and sex and additionally for the cardiovascular risk profile and cancer. The difference in worsening of HF between HFrEF and HFpEF was depicted by a cumulative incidence plot. Cox regression analyses were calculated to determine the role of platelet indices in clinical outcome within the phenotypes independent of CVRFs and cancer as well as to determine differences for the roles of platelet indices in HFrEF vs. HFpEF. Furthermore, the roles of antithrombotic agents (ATC B01) and history of cancer on the clinical outcome, worsening of HF, were analysed.

Because of the explorative character of the analysis, a significance threshold was not defined for *P*-values. The *P*-value should be interpreted as continuous measure of statistical evidence. All statistical analyses were performed using R Version 3.6.0 software (http://www.r-project.org).

Results

Clinical characteristics of study participants

Clinical characteristics of the study sample at baseline are reported according to quartiles of MPV and platelet count in *Table 1* and *Table S1*. Increasing MPV quartiles were going along with increasing frequencies of individuals with diabetes mellitus, obesity and atrial fibrillation (AF) and history of cancer. Proportions of subjects with REF and HFrEF increased along with increasing MPV quartiles, whereas proportions of subjects with PEF and HFpEF decreased with higher MPV quartiles with the highest prevalence in the lowest MPV quartile (MPV $\leq$ 7.7 fL). Myocardial infarction (MI), coronary artery disease (CAD) and peripheral artery disease (PAD) showed a U-shape-like distribution with the highest proportions in both lowest and highest MPV quartiles.

Individuals in the lowest quartile of platelet count ($\leq 186 \times 10^9$/L) were older, more male with higher prevalence of diabetes mellitus and dyslipidaemia (*Table S1*). In addition, MI, CAD, AF, PAD and venous thromboembolism (VTE) were more prevalent in the lowest platelet quartile. The highest frequencies of individuals with history of cancer, chronic kidney disease (CKD) and chronic liver disease (CLD) were present in individuals from the lowest platelet count quartile. Additionally, the distribution of CVD and co-morbidities were depicted in *Figure S2A* for increasing MPV (fL) and in *Figure S2B* for increasing platelet count (10^9/L). The intake of antithrombotic agents (B01) was highest in the lowest platelet count quartile (74.1%) compared with quartiles with higher platelet count with a frequency of antithrombotic intake of less than 60%. The number of individuals with PEF showed an increasing trend with higher platelet counts, whereas subjects with REF showed the opposite relation with a higher proportion of individuals with REF in the lowest quartile of platelet count.

Overall, the analysis sample was 64.6 ± 11.1 years old and included 1184 (36.4%) females (*Table S2*). Antithrombotic agents were reported in 1993 (61.3%) individuals. Comparing HF phenotypes, HFpEF vs. HFrEF, HFpEF subjects were older

Table 1 Clinical characteristics of the study sample according to MPV quartiles (n = 3250)

	≤25%	>25–50%	>50–75%	>75%
MPV	≤7.7 fL	>7.7–8.2 fL	>8.2–8.7 fL	>8.7 fL
Number	838	852	772	788
Age (years)	64.2 ± 11.2	63.8 ± 11.3	64.9 ± 10.8	65.5 ± 11.0
Sex (female)	291 (34.7%)	335 (39.3%)	288 (37.3%)	270 (34.3%)
CVRFs				
Arterial hypertension	586 (69.9%)	606 (71.1%)	567 (73.4%)	578 (73.4%)
Diabetes mellitus	170 (20.3%)	169 (19.8%)	186 (24.1%)	206 (26.1%)
Smoking	99 (11.8%)	126 (14.8%)	97 (12.6%)	110 (14.0%)
Obesity	229 (27.3%)	262 (30.8%)	259 (33.5%)	263 (33.4%)
Dyslipidaemia	580 (69.2%)	563 (66.1%)	531 (68.8%)	552 (70.1%)
Family history of MI/stroke	196 (23.4%)	208 (24.5%)	160 (20.7%)	179 (22.7%)
CVDs				
MI	227 (27.1%)	169 (19.8%)	161 (20.9%)	217 (27.5%)
Stroke	73 (8.7%)	65 (7.6%)	70 (9.1%)	68 (8.6%)
Coronary artery disease	334 (39.9%)	290 (34.0%)	265 (34.3%)	333 (42.3%)
Atrial fibrillation	178 (21.2%)	167 (19.6%)	183 (23.7%)	222 (28.2%)
PAD	63 (7.5%)	38 (4.5%)	47 (6.1%)	67 (8.5%)
VTE	73 (8.7%)	68 (8.0%)	71 (9.2%)	63 (8.0%)
Co-morbidities				
History of cancer	122 (14.6%)	119 (14.0%)	126 (16.3%)	154 (19.5%)
Chronic kidney disease	126 (15.0%)	126 (14.8%)	147 (19.0%)	149 (18.9%)
Chronic liver disease	58 (6.9%)	77 (9.0%)	62 (8.0%)	81 (10.3%)
Cardiac function and HF phenotypes				
LVEF (%)	55.0 ± 10.6	55.3 ± 10.8	54.9 ± 10.9	53.2 ± 11.7
E/E'	8.35 (6.40/11.07)	8.02 (6.18/10.71)	8.47 (6.56/11.28)	8.58 (6.51/11.63)
PEF	636 (75.9%)	650 (76.4%)	574 (74.4%)	545 (69.2%)
REF	202 (24.1%)	201 (23.6%)	198 (25.6%)	243 (30.8%)
HFpEF	162 (19.3%)	168 (19.7%)	155 (20.1%)	152 (19.3%)
HFrEF	77 (9.2%)	78 (9.2%)	78 (10.1%)	108 (13.7%)
Medication				
Antithrombotic agents (B01)	525 (62.6%)	493 (57.9%)	444 (57.5%)	531 (67.4%)

CVDs, cardiovascular diseases; CVRFs, cardiovascular risk factors; HFpEF, heart failure with preserved ejection fraction (LVEF ≥ 50%); HFrEF, heart failure with reduced ejection fraction (LVEF ≤ 40%); LVEF, left ventricular ejection fraction; MI, myocardial infarction; MPV, mean platelet volume; PAD, peripheral artery disease; PEF, preserved ejection fraction; REF, reduced ejection fraction; VTE, venous thromboembolism.

(70.7 ± 8.1 vs. 66.3 ± 10.5 years) and more females (305 [47.9%] vs. 50 [14.7%]). HFrEF individuals had more often dyslipidaemia, MI, CAD and AF but less often arterial hypertension and VTE. MPV (8.44 ± 1.00 vs. 8.28 ± 0.85 fL) and E/E' (12.39 [8.28/18.03] vs. 11.10 [8.59/13.919]) were higher in HFrEF, whereas platelet count (203.0 [167.0/245.3] vs. 222.0 [182.0/267.0]) and LVEF (31.5 ± 6.1 vs. 58.5 ± 5.6) were lower in HFrEF compared with HFpEF. Inflammatory markers such as fibrinogen and leukocyte count were lower in HFpEF compared with HFrEF.

Relation between platelet indices and cardiac function

As presented in *Table 2*, the linear regression analysis for MPV showed a negative association with LVEF (beta estimate, β = − 0.07, 95% of CI [−0.09; −0.04]), which remained in the multivariable model adjusted for age and sex (β = − 0.07 [−0.10; −0.04]). The detailed distribution of LVEF is presented in *Figure S3A*. Differently, the same analysis for the platelet count presented with a positive association in univariate model (β = 9.0 [7.0; 11.1]) and adjusted for age and sex with β = 4.5 (2.4; 6.5) to LVEF, additionally presented in *Figure S3B*. Platelet-to-leukocyte ratio (β = 2.4 [2.1; 2.8]) and platelet-to-monocyte ratio (β = 59 [52; 67]) also showed a positive association to LVEF in univariate models and after adjustment for age and sex ($\beta_{\text{platelet-to-leukocyte-ratio}}$ = 1.7 [1.4; 2.0] and $\beta_{\text{platelet-to-monocyte-ratio}}$ = 34 [27; 42]). The analysis between platelet indices and the diastolic function parameter expressed as E/E' ratio, presented with a positive association for MPV ($\beta_{\text{unadjusted}}$ = 0.06 [0.03; 0.09] and $\beta_{\text{adjusted for age and sex}}$ = 0.05 [0.02; 0.08]), more in detail depicted in *Figure S3C*, but negative associations for platelet count and E/E' ($\beta_{\text{unadjusted}}$ = −5.9 [−7.9; −3.8] and $\beta_{\text{adjusted for age and sex}}$ = −4.2 [−6.3; −2.1]), as presented in *Figure S3D*, platelet-to-leukocyte ratio ($\beta_{\text{unadjusted}}$ = −1.4 [−1.8; −1.1] and $\beta_{\text{adjusted for age and sex}}$ = −1.3 [−1.6; −0.93]) and platelet-to-monocyte ratio ($\beta_{\text{unadjusted}}$ = −34 [−42; −26] and $\beta_{\text{adjusted for age and sex}}$ = −29 [−36; −21]). All observed associations remained relevant, when the models were further adjusted for both systolic and diastolic function, LVEF and E/E' ratio, respectively, and further for antithrombotic medication (ATC code: B01). Platelet-to-lymphocyte ratio showed a positive association

Table 2 Relation between platelet indices and cardiac function

		MPV (fL)		Platelet count ($\times 10^9$/L)		Platelet-to-leukocyte ratio		Platelet-to-monocyte ratio		Platelet-to-lymphocyte ratio	
		β (95% CI)	*P*-value	β (95% CI)	*P*-value	β (95% CI)	*P*-value	β (95% CI)	*P*-value	β (95% CI)	*P*-value
LVEF (%)	Unadjusted	−0.07 (−0.09; −0.04)	<0.0001	9.0 (7.0; 11.1)	<0.0001	2.4 (2.1; 2.8)	<0.0001	59 (52; 67)	<0.0001	2.43 (0.34; 4.51)	0.023
	Age + sex adjusted	−0.07 (−0.10; −0.04)	<0.0001	4.5 (2.4; 6.5)	<0.0001	1.7 (1.4; 2.0)	<0.0001	34 (27; 42)	<0.0001	2.1 (−0.09; 4.2)	0.060
	+ adjusted for E/E' ratio	−0.05 (−0.09; −0.02)	0.00090	3.4 (1.2; 5.6)	0.002	1.4 (1.1; 1.8)	<0.0001	28 (20; 36)	<0.0001	2.1 (−0.21; 4.4)	0.075
	+ adjusted for antithrombotic agents (B01)	−0.05 (−0.08; −0.02)	0.0015	3.6 (1.4; 5.8)	0.0013	1.3 (0.92; 1.7)	<0.0001	24 (16; 32)	<0.0001	1.6 (−0.68; 4.0)	0.16
E/E' ratio	Unadjusted	0.06 (0.03; 0.09)	<0.0001	−5.9 (−7.9; −3.8)	<0.0001	−1.4 (−1.8; −1.1)	<0.0001	−34 (−42; −26)	<0.0001	1.45 (−0.64; 3.55)	0.17
	Age + sex adjusted	0.05 (0.02; 0.08)	0.00074	−4.2 (−6.3; −2.1)	<0.0001	−1.3 (−1.6; −0.93)	<0.0001	−29 (−36; −21)	<0.0001	−0.27 (−2.5; 1.9)	0.81
	+ adjusted for LVEF	0.04 (0.003; 0.07)	0.030	−3.1 (−5.3; −0.92)	0.0054	−0.83 (−1.2; −0.46)	<0.0001	−20 (−28; −12)	<0.0001	0.39 (−1.9; 2.7)	0.74
	+ adjusted for antithrombotic agents (B01)	0.04 (0.002; 0.07)	0.037	−3.3 (−5.5; −1.1)	0.0038	−0.71 (−1.1; −0.34)	0.00019	−17 (−25; −8.6)	<0.0001	0.73 (−1.6; 3.1)	0.54

Univariate linear regression model (unadjusted) and multivariable linear regression models in $n = 3249$ individuals for the association between MPV, platelet count, platelet-to-leukocyte ratio, platelet-to-monocyte ratio or platelet-to-lymphocyte ratio as dependent variables and left ventricular ejection fraction (LVEF) or diastolic dysfunction (E/E') as independent variables. Results are presented as beta estimates (β) for change per 1 standard deviation (SD) in LVEF (%) or E/E' ratio.
MPV, mean platelet volume.

with LVEF in the univariate model, which was lost after adjusting for age and sex. No associations were observed with E/E' ratio.

Platelet indices and clinical outcome

A total of 298 events were registered for worsening of HF during the follow-up period with a median follow-up time of 2.24 years (interquartile range: 1.18–3.97 years). As shown in *Figure 1A*, the highest quartile (Q4) of MPV (MPV > 8.7 fL, shown in *Table S3*) was associated with the highest cumulative incidence for worsening of HF compared with Q1–Q3, *P*-value < 0.0001.

Subjects within the lowest quartiles of platelet count (platelets < 186 × 10^9/L, *Figure 1B*), platelet-to-leukocyte ratio (platelet-to-leukocyte ratio < 25.8, *Figure 1C*) and platelet-to-monocyte ratio (platelet-monocyte ratio < 410, *Figure 1D*) showed a higher cumulative incidence for worsening of HF compared with subjects with higher platelet counts or platelet ratios ($P\text{-value}_{\text{platelet count}} = 0.00012$, $P\text{-values}_{\text{platelet-to-leukocyte and platelet-to-monocyte ratios}} < 0.0001$, respectively). Inversely, the highest quartile of platelet-to-lymphocyte ratio was associated with a higher cumulative incidence for worsening of HF with *P*-value = 0.0021 (*Figure 1E*).

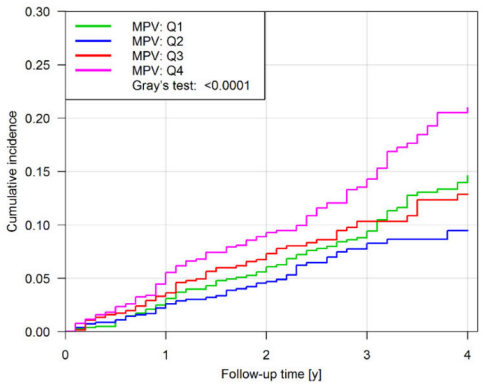
Cox regression analysis confirmed the worse outcome in subjects within the highest quartile of MPV in a model adjusted for age and sex (HR = 1.60, [95% CI: 1.26; 2.03]) and also after further adjustment for the cardiovascular risk profile and cancer (HR = 1.47, [1.16; 1.87]), as depicted in *Figure 2*. Likewise, platelet-to-lymphocyte ratio > 75th percentile (HR = 1.50 [1.17; 1.93]) as well as levels below the 25th percentile of platelet count (HR = 1.36 [1.07; 1.74]), platelet-to-leukocyte ratio (HR = 1.53 [1.20; 1.95]) and platelet-to-monocyte ratio (HR = 1.38 [1.07; 1.77]) were associated with lower survival independent of age, sex, cardiovascular risk profile and cancer.

Relation of platelet indices and outcome in HF phenotypes

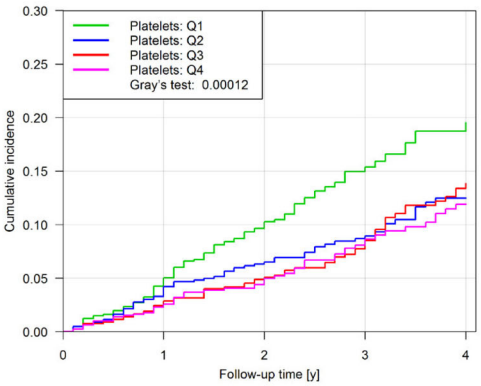
Looking into HF phenotypes, a higher incidence for worsening of HF was found among HFrEF individuals compared with HFpEF (*P* < 0.0001, *Figure S4*). However, the effect of MPV > 75th percentile was stronger in HFpEF (HR = 1.99 [1.22; 3.24]) than in HFrEF individuals (HR = 1.03 [0.69; 1.54]) independent of age and sex (*P* for difference = 0.043) and remained after further adjustment for CVRFs and cancer (*P* for difference = 0.040; HR [HFrEF] = 0.97 [0.64; 1.47] and HR [HFpEF] = 1.90 [1.15; 3.12]) as presented in *Table 3*. Similarly, the effect of platelet count differed between HFrEF and HFpEF independent of age, sex, CVRFs and cancer (*P* for difference = 0.0022) with a higher risk for worse outcome in

Figure 1 Presented are cumulative incidence plots for worsening of HF in the study sample (n = 3220) with a median follow-up time of 2.24 years (interquartile range: 1.18–3.97 years) according to quartiles of MPV (A), platelet count (B), platelet-to-leukocyte ratio (C), platelet-to-monocyte ratio (D) and platelet-to-lymphocyte ratio (E).

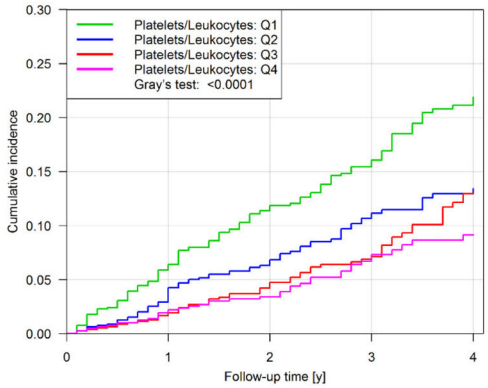
A. Worsening of HF according to MPV quartiles



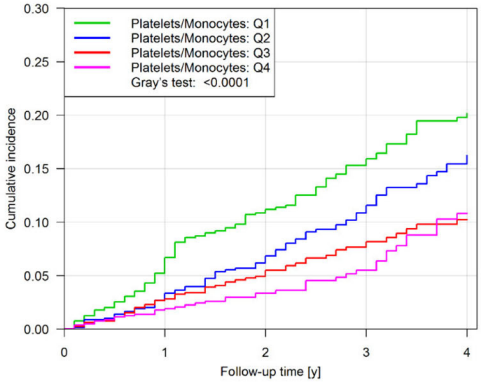
B. Worsening of HF according to platelet count quartiles



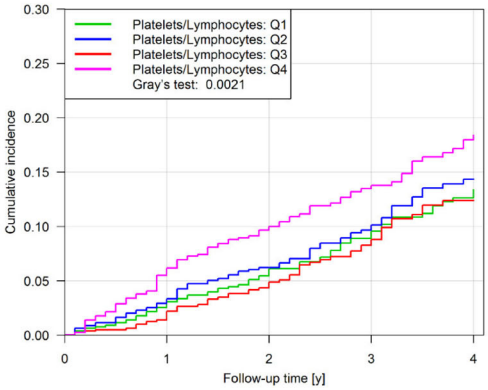
C. Worsening of HF according to platelet-to-leukocyte ratio quartiles



D. Worsening of HF according to platelet-to-monocyte ratio quartiles



E. Worsening of HF according to platelet-to-lymphocyte ratio quartiles



HFpEF (HR = 2.30 [1.42; 3.74]) compared with HFrEF (HR = 0.85 [0.56; 1.31]).

Platelet-to-leukocyte and platelet-to-monocyte ratios of the lowest quartile and platelet-to-lymphocyte ratio above the 75th percentile did not show relevant different effects in HFrEF compared with HFpEF phenotype independent of age, sex, CVRFs and cancer. Whereas the effects on worse outcome of platelet-to-leukocyte and platelet-to-monocyte ratios were higher among HFpEF phenotype, for platelet-to-lymphocyte ratio, the association to outcome was stronger in HFrEF phenotype. *Table S4* presented the model additionally adjusted for antithrombotic medication (ATC code: B01).

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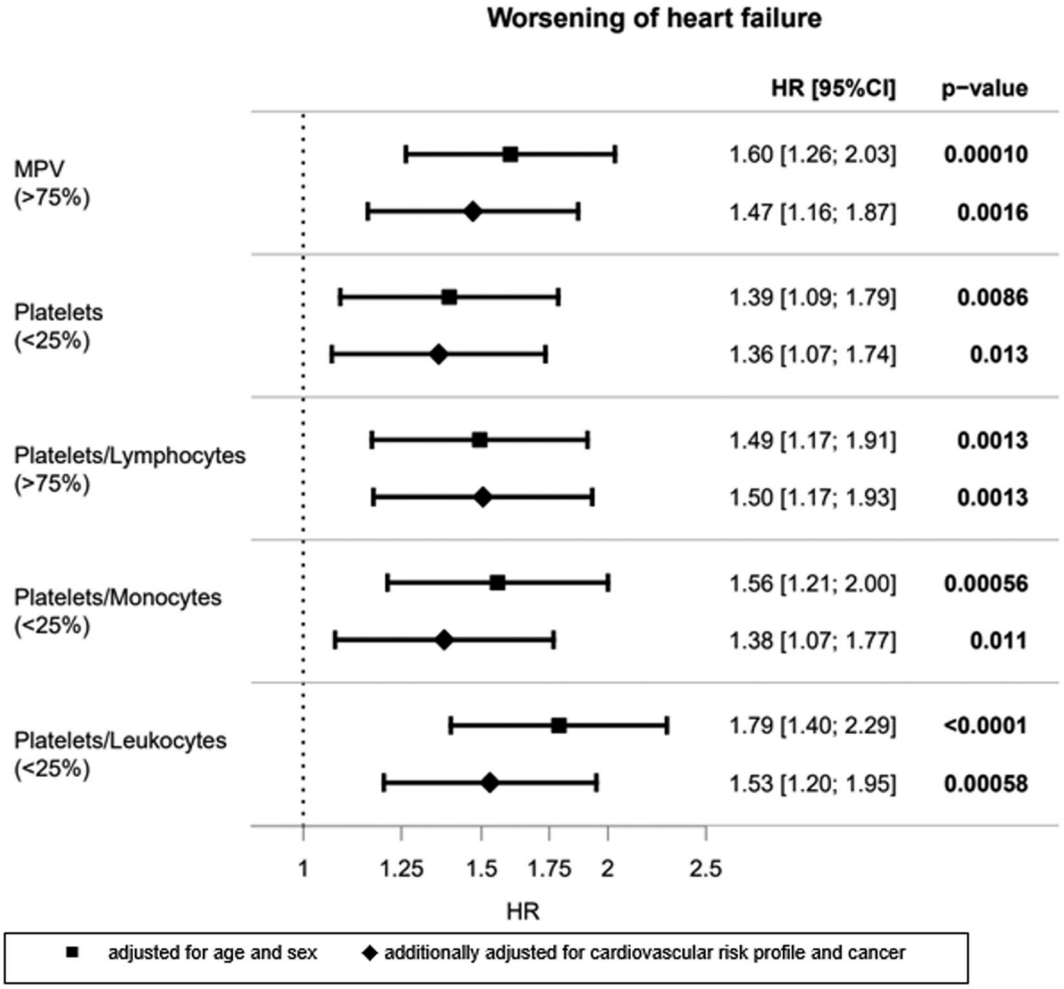
Figure 2 Forest plot presenting the association of MPV > 75th percentile, platelet count < 25th percentile, platelet-to-leukocyte ratio < 25th percentile, platelet-to-monocyte ratio < 25th percentile and platelet-to-lymphocyte ratio > 75th percentile and worsening of HF with hazard ratios (HRs) with 95% confidence interval (CI), adjusted for age and sex and additionally adjusted for CVRFs and cancer in *n* = 3188 individuals (298 events); Cardiovascular risk factors (CVRFs) are arterial hypertension, diabetes mellitus, smoking, obesity, dyslipidaemia, family history of myocardial infarction/stroke, myocardial infarction, stroke, coronary artery disease, atrial fibrillation, peripheral artery disease and venous thromboembolism; MPV, mean platelet volume.



The intake of antithrombotic agents did not substantially change the associations between platelet indices and risk for worsening of HF.

In addition, to investigate if cancer history modifies the association with worsening of HF, an analysis excluding subjects with cancer history was performed in comparison with the whole sample that included subjects with cancer history. The subgroup without cancer history with MPV > 75th percentile and platelet-to-lymphocyte ratio > 75th percentile showed a higher risk for worsening of HF compared with the complete sample but lower risk for worsening of HF with platelet count < 25th, platelet-to-leukocyte ratio < 25th percentile or platelet-to-monocyte ratio < 25th percentile independent of age, sex, antithrombotic agents, CVRFs and co-morbidities (*Table S5*).

Discussion

This study investigated several platelet indices like MPV, platelet count and platelet- to-leukocyte ratio, including platelet-to-monocyte and platelet-to-lymphocyte ratio, in relation to cardiac function and clinical outcome in HF individuals. Higher levels of MPV were associated with reduced LVEF, a measure of systolic dysfunction, and increased E/E', a measure of diastolic dysfunction, independent of age and sex. In the same line, with opposite direction only, were the findings for the relation between platelet count and cardiac function measurements. The highest MPV quartile and the lowest quartile of platelet count were characterized by worse cardiovascular risk profile with higher frequencies of diabetes mellitus, CAD, AF, CKD and CLD. Higher MPV, a potential

Table 3 Relation between platelet indices and worsening of HF in HF phenotypes

		Adjusted for age and sex			Additionally adjusted for CVRFs and cancer		
		HR (95% CI)	*P*-value	*P*-value for difference HFrEF vs. HFpEF	HR (95% CI)	*P*-value	*P*-value for difference HFrEF vs. HFpEF
MPV (fL) > 75th percentile	HFrEF	1.02 (0.68; 1.52)	0.94		0.97 (0.64; 1.47)	0.90	
	HFpEF	1.94 (1.19; 3.16)	0.0080		1.90 (1.15; 3.12)	0.012	
	HFrEF vs. HFpEF	0.52 (0.28; 0.99)	-	0.043	0.51 (0.27; 0.97)	-	0.040
Platelet count < 25th percentile	HFrEF	0.80 (0.52; 1.21)	0.29		0.85 (0.56; 1.31)	0.46	
	HFpEF	2.28 (1.39; 3.75)	0.0011		2.30 (1.42; 3.74)	0.00076	
	HFrEF vs. HFpEF	0.35 (0.19 0.66)	-	0.0011	0.37 (0.20; 0.70)	-	0.0022
Platelet-to-leukocyte ratio < 25th percentile	HFrEF	1.33 (0.90; 1.97)	0.16		1.41 (0.94; 2.10)	0.093	
	HFpEF	1.88 (1.14; 3.12)	0.014		1.73 (1.05; 2.86)	0.032	
	HFrEF vs. HFpEF	0.71 (0.38; 1.30)	-	0.26	0.81 (0.44; 1.50)	-	0.51
Platelet-to-monocyte ratio < 25th percentile	HFrEF	1.03 (0.69; 1.53)	0.88		1.11 (0.74; 1.67)	0.61	
	HFpEF	1.86 (1.12; 3.10)	0.016		1.84 (1.10; 3.07)	0.020	
	HFrEF vs. HFpEF	0.55 (0.30; 1.03)	-	0.063	0.61 (0.32; 1.14)	-	0.12
Platelet-to-lymphocyte ratio > 75th percentile	HFrEF	2.73 (1.84; 4.05)	<0.0001		2.65 (1.78; 3.95)	<0.0001	
	HFpEF	1.60 (0.97; 2.63)	0.066		1.56 (0.94; 2.60)	0.085	
	HFrEF vs. HFpEF	1.71 (0.91; 3.23)	-	0.099	1.70 (0.89; 3.23)	-	0.11

Cox regression analysis in $n = 951$ individuals for the association between mean platelet volume (MPV) > 75th percentile, platelet count < 25th percentile, platelet-to-leukocyte ratio < 25th percentile, platelet-to-monocyte ratio < 25th percentile or platelet-to-lymphocyte ratio > 75th percentile and worsening of HF ($n = 174$ events). Results are presented as hazard ratios (HRs) with 95% confidence interval (CI). In addition, differences for the effects of platelet indices for HFrEF vs. HFpEF were calculated. Cardiovascular risk factors (CVRFs) are arterial hypertension, diabetes mellitus, smoking, obesity, dyslipidaemia and family history of myocardial infarction/stroke.

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marker of platelet activation,[18,19] has previously been associated with traditional CVRFs and CVDs, particularly with diabetes mellitus, obesity and AF.[9–11,20,21] High levels of MPV have also been described in the setting of HF.[22] In a large adult population-based cohort, the relation between higher MPV and increased all-cause mortality was independent of traditional CVRFs. However, this relation was lost after adjusting for CVDs including HF, suggesting for a possible role of HF in the association between MPV and total mortality.[9] MPV has been reported to be associated with higher thrombin generation potential assessed in presence of platelets, particularly among individuals at risk for CVDs.[23] In addition, higher MPV was correlated with a higher percentage of platelets expressing surface P-selectin, another recognized marker of platelet activation.[3,8,20,23]

The present results support an important role of platelets in HF pathophysiology and HF-related outcome in both HF phenotypes. The overall incidence of worsening of HF was higher among HFrEF compared with HFpEF, but with respect to platelet indices, higher MPV and lower platelet count showed a stronger effect on worse outcome in HFpEF phenotype. CVRFs and cancer did not substantially change the association between platelet indices and clinical outcome, even though the cardiovascular risk profile and laboratory parameters differed between HF phenotypes and co-morbidities have been shown to modulate platelet activation.[9,11,24,25] The risk for worsening of HF remained higher independent of intake of antithrombotic agents. Individuals without cancer history with higher MPV and/or higher platelet-to-lymphocyte ratio had even higher risk for worsening of HF compared with the total analysis sample including subjects with cancer history. This finding could potentially speak for the benefits of regular, closer follow-up of cancer patients for developing cardiovascular complication with particular consideration for the cardiovascular toxicities from cancer treatment.[26] In addition to the underlying cardiovascular risk profile, HF specific features such as haemodynamic and vascular changes including cardiac remodelling could also have an impact on platelet characteristics.[22]

Platelets are recognized mediators of inflammation, particularly through their interaction with leukocytes and endothelial cells.[27–29] Increased release of cytokines and catecholamines observed in severe HF has been associated with platelet activation and higher levels of MPV.[22] Platelet ratios to leukocytes, to monocytes and particularly to lymphocytes have been reported as novel markers of inflammation and were linked to total mortality.[13] This study demonstrated that both platelet-to leukocyte and platelet-to-monocyte ratios have important associations to cardiac function parameters such as LVEF and E/E' that remained independent of age, sex and antithrombotic agents. However, a role of age and/or sex was observed for the associations to cardiac function parameters. Lower ratios were associated with worse systolic and diastolic function. Differently, a positive trend between platelet-to-lymphocyte ratio and LVEF, but no relation to E/E' ratio, has been also observed in models adjusted for age and sex.

Higher MPV has been associated with increased mortality after MI, a strong risk factor for HFrEF,[5] whereas *lower* platelet count has been associated with increased risk of total, cancer and non-cardiovascular/non-cancer mortality but was unrelated to cardiovascular mortality.[9,11] Interestingly, this study showed that higher MPV and lower platelet count were more related to clinical outcome in HFpEF compared with HFrEF independent of CVRFs and cancer. For platelet-to-leukocyte ratios, including platelet-to-monocyte and platelet-to-lymphocyte ratios, no differences for the risk prediction of worsening of HF have been found between HFpEF and HFrEF. Lower platelet-to-leukocyte ratio and platelet-to-monocyte ratio showed an important trend towards worse clinical outcome particularly for HFpEF phenotype, as observed for MPV and platelet count. Increased leukocyte count has been associated with adverse clinical outcome in HFpEF subjects.[30] In this study, fibrinogen levels and leukocyte count were observed higher in HFrEF individuals compared with HFpEF. Lower platelet-to-leukocyte ratios resulting from higher leukocyte counts contribute to a proinflammatory state in HF that may promote activation of platelets and coagulation system in both phenotypes. An activation of the unspecific immune response in individuals with worse cardiac function could be anticipated, as C-reactive protein (CRP), fibrinogen and leukocyte count were higher in both symptomatic HF phenotypes compared with the rest of the analysis sample. Furthermore, due to the release of a plethora of inflammatory mediators by activated platelets, the inflammatory state in HF individuals could be further potentiated.[31]

Higher platelet-to-lymphocyte ratio showed a stronger trend for worsening of HF among HFrEF subjects compared with HFpEF, independent of the underlying cardiovascular risk profile. Recent studies in acute HF individuals reported different results for the association of platelet-to-lymphocyte ratio and long-term mortality as independent predictor of outcome in acute HF.[13,14,32] In this study, within the highest quartile of platelet-to-lymphocyte ratio, HFrEF individuals showed a 2.65-fold increased risk and HFpEF individuals 1.56-fold increased risk for worsening of HF, indicating an important role for high platelet-to-lymphocyte ratio as a biomarker of clinical outcome related to reasons other than worse systolic and diastolic function.

Strengths and limitations

The major strength of this study is the comprehensive, highly standardized clinical investigation and follow-up of a large sample of individuals with HF syndrome. However, there are some limitations that should be considered: Despite the observed important links between platelet indices and HF, this study was not design to investigate a causal relationship.

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Furthermore, the lack of detailed information on the type and stage of cancer prevented us to investigate more in details the role of cancer history on the association with platelet indices and HF outcome. Further mechanistic studies are warranted to clarify the role of platelets as cause or result of HF pathophysiology and their role in the HF-related pathological response. Nevertheless, platelet indices were associated with measures of systolic and diastolic function, as well as with clinical outcome in HF individuals. According to the guidelines, HF is divided into three phenotypes: HFpEF, HFpEF borderline and HFrEF.[4] This study analysed only HF phenotypes with preserved and REF but excluded individuals with EF of 41–49%. The role of platelets in HFpEF borderline individuals needs to be further investigated as this phenotype presented with partial characteristics of HFpEF and some HFrEF properties.[4]

Conclusion

In conclusion, this study supports a role for platelets in the pathogenesis of HF demonstrating an important link to the clinical outcome in HFpEF and HFrEF phenotypes. Better characterization of platelet function is warranted to increase the knowledge on platelet-related molecular mechanisms involved in HF-related inflammation, especially in HFpEF phenotype, as well as to understand further if these biomarkers help to identify HF patients at risk for worse clinical outcome.

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Conflict of interest

The remaining authors declare no competing interests.

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Supporting information

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

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Supplement Material: The impact of platelet indices on clinical outcome in heart failure: results from the MyoVasc study

Link to supporting information of the original publication:

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Part A. Supplemental Methods

Inclusion and exclusion criteria MyoVasc study

Inclusion criteria for the MyoVasc study, as recently reported were the following[12]:

- Main inclusion criteria: suffering of any type of asymptomatic or symptomatic HF; HF is defined as: (a) diagnosed by a physician, or (b) assessed according to criteria of international HF guidelines, or (c) defined by cardiac dysfunction;
- Further inclusion criteria: age 35 to 84 years, sufficient knowledge of the German language to understand study documents and computer-assisted interviews.

Patients with normal cardiac structure and function were included in the study, if they were treated with HF medication (e.g. ACE-inhibitors, AT1-receptor-blockers, ß-blockers, aldosterone-antagonists, diuretics) and had a history of echocardiographically documented cardiac dysfunction. Normal cardiac function is defined as follows: LVEF ≥ 55%, E/A ≥ 0.75, E/E´< 10, and DTE ≥ 140 ms.

Exclusion criteria were: (a) acute endocarditis, myocarditis or pericarditis within the last six months prior to inclusion; (b) acute myocardial infarction within the last three months prior to inclusion (in the case of non-ST-segment-elevation-myocardial infarction) or within the last four months prior to inclusion (in the case of ST-segment-elevation myocardial infarction); (c) acute infectious disease; (d) acute decompensated HF; (e) inability to give written consent.

Patients at all stages of HF were eligible for study enrolment to the HF cohort.

Assessment of cardiovascular risk factors and cardiovascular diseases

Cardiovascular risk factors are determined as:

- Diabetes mellitus: HbA1c ≥ 6.5% or blood glucose level ≥ 126 mg/dL at baseline examination after an overnight fast or at least 8 hours or a blood glucose level of ≥ 200 mg/dL at baseline examination after a fasting period > 5 hours or diagnosed diabetes mellitus by a physician or intake of antidiabetic medication;

- Arterial hypertension: blood pressure >140/90 mmHg or diagnose by a physician or intake of antihypertensive medication;

- Smoking: statement of current smoking;

- Dyslipidemia: low density lipoprotein/ high density lipoprotein > 3.5 and/ or triglycerides level > 150 mg/dL or diagnose by a physician intake of lipid modifying medication;

- Obesity: body-mass index (BMI) ≥ 30.0kg/m^2;

- Family histories of myocardial infarction and stroke are defined as myocardial infarction and/or stroke of male first-degree relatives until the age of 60 years or female first-degree relatives until the age of 65 years.

Cardiovascular diseases and comorbidities are self-reported and include myocardial infarction (MI), stroke, chronic obstructive pulmonary disease (COPD), pulmonary embolism (PE), venous thromboembolism (VTE), deep vein thrombosis (DVT), coronary artery disease (CAD), peripheral artery disease (PAD), chronic kidney disease, chronic liver disease, cancer, arthritis, atrial fibrillation (AF), and congestive heart failure (CHF).

Part B. Supplemental Tables

Table S1. Characteristics of the study sample according to quartiles of platelet count (N=3,250)

	≤ 25%	**> 25%-50%**	**> 50%-75%**	**> 75%**
Platelet count	≤ 186 x10^9/L	> 186 x10^9/L – 223 x10^9/L	> 223 x10^9/L – 263 x10^9/L	> 263 x10^9/L
Number	826	822	798	804
Age (years)	67.7 ± 10.1	64.7 ± 11.4	64.1 ± 10.9	61.8 ± 11.1
Sex (female)	150 (18.2%)	270 (32.8%)	337 (42.2%)	427 (53.1%)
CVRFs				
Arterial hypertension	619 (74.9%)	589 (71.7%)	559 (70.1%)	570 (70.9%)
Diabetes mellitus	220 (26.6%)	163 (19.8%)	187 (23.4%)	161 (20.0%)
Smoking	96 (11.6%)	109 (13.3%)	92 (11.5%)	135 (16.8%)
Obesity	273 (33.1%)	228 (27.7%)	253 (31.7%)	259 (32.2%)
Dyslipidemia	614 (74.3%)	544 (66.2%)	534 (66.9%)	534 (66.4%)
Family history of MI/stroke	172 (20.8%)	194 (23.6%)	182 (22.8%)	195 (24.3%)
CVDs				
MI	237 (28.7%)	193 (23.5%)	172 (21.6%)	172 (21.5%)
Stroke	74 (9.0%)	63 (7.7%)	68 (8.5%)	71 (8.8%)
Coronary artery disease	387 (46.9%)	316 (38.4%)	264 (33.1%)	255 (31.7%)

Atrial fibrillation	260 (31.5%)	193 (23.5%)	164 (20.6%)	133 (16.5%)
PAD	68 (8.2%)	50 (6.1%)	44 (5.5%)	53 (6.6%)
VTE	94 (11.4%)	58 (7.1%)	60 (7.5%)	63 (7.8%)
Comorbidities				
History of cancer	163 (19.7%)	128 (15.6%)	119 (14.9%)	111 (13.8%)
Chronic kidney disease	179 (21.7%)	131 (15.9%)	127 (15.9%)	111 (13.8%)
Chronic liver disease	103 (12.5%)	57 (6.9%)	61 (7.7%)	57 (7.1%)
Cardiac function and HF phenotypes				
LVEF (%)	51.9 ± 11.4	55.0 ± 11.0	55.6 ± 10.8	56.1 ± 10.5
E/E`	8.83 (6.68/12.46)	8.23 (6.22/10.87)	8.29 (6.33/10.99)	8.08 (6.27/10.49)
PEF	533 (64.5%)	619 (75.4%)	613 (76.8%)	640 (79.6%)
REF	293 (35.5%)	202 (24.6%)	185 (23.2%)	164 (20.4%)
HFpEF	172 (20.8%)	152 (18.5%)	148 (18.5%)	165 (20.5%)
HFrEF	123 (14.9%)	91 (11.1%)	67 (8.4%)	60 (7.5%)
Medication				
Antithrombotic agents (B01)	612 (74.1%)	488 (59.4%)	452 (56.6%)	441 (54.9%)

CVRFs: Cardiovascular risk factors; MI: Myocardial infarction; CVDs: Cardiovascular diseases; PAD: Peripheral artery disease; VTE: Venous thromboembolism; LVEF: Left ventricular ejection fraction; PEF: Preserved ejection fraction; REF: Reduced ejection; HFpEF: Heart failure with preserved ejection fraction ≥ 50% LVEF; HFrEF: Heart failure with reduced ejection fraction (LVEF ≤ 40%)

Table S2. Characteristics of study participants (N= 3,250) and according to HFpEF (N= 637) and HFrEF (N= 341)

	Total analysis sample	**HFpEF**	**HFrEF**	**p-value***
Number	3,250	637	341	**-**
Age (years)	64.6 ± 11.1	70.7 ± 8.1	66.3 ± 10.5	**<0.0001**
Sex (female)	1184 (36.4%)	305 (47.9%)	50 (14.7%)	**<0.0001**
CVRFs				
Arterial hypertension	2337 (71.9%)	553 (86.8%)	258 (75.7%)	**<0.0001**
Diabetes mellitus	731 (22.5%)	194 (30.5%)	119 (34.9%)	0.17
Smoking	432 (13.3%)	66 (10.4%)	59 (17.3%)	**0.0025**
Obesity	1013 (31.2%)	256 (40.2%)	117 (34.3%)	0.073
Dyslipidemia	2226 (68.5%)	483 (75.8%)	288 (84.5%)	**0.0018**
Family history of MI/stroke	743 (22.9%)	141 (22.1%)	94 (27.6%)	0.059
CVD				
MI	774 (23.8%)	157 (24.6%)	148 (43.4%)	**<0.0001**
Stroke	276 (8.5%)	72 (11.3%)	39 (11.4%)	1.00
Coronary artery disease	1222 (37.6%)	281 (44.1%)	198 (58.1%)	**<0.0001**
Atrial fibrillation	750 (23.1%)	214 (33.6%)	143 (41.9%)	**0.012**
PAD	215 (6.6%)	60 (9.4%)	43 (12.6%)	0.13
VTE	275 (8.5%)	84 (13.2%)	30 (8.8%)	**0.047**
Comorbidities				
History of cancer	521 (16.0%)	137 (21.5%)	57 (16.7%)	0.078
Chronic kidney disease	548 (16.9%)	147 (23.1%)	104 (30.5%)	**0.014**

Chronic liver disease	278 (8.6%)	70 (11.0%)	29 (8.5%)	0.27
Laboratory parameter				
MPV (fL)	8.27 ± 0.86	8.28 ± 0.85	8.44 ± 1.00	**0.013**
Platelet count (10^9/L)	227.6 ± 61.9	222.0 (182.0/267.0)	203.0 (167.0/245.3)	**<0.0001**
LVEF (%)	54.6 ± 11.0	58.5 ± 5.6	31.5 ± 6.1	**<0.0001**
E/E`	8.36 (6.39/11.19)	11.10 (8.59/13.91)	12.39 (8.28/18.03)	**0.00041**
CRP (mg/L)	1.80 (0.88/3.80)	2.40 (1.20/5.30)	2.70 (1.30/5.40)	0.30
Fibrinogen (mg/dL)	323 (275/381)	359 ± 89	383 ± 96	**0.00013**
Leukocytes (10^9/L)	6.98 (5.91/8.32)	7.21 (5.99/8.66)	7.57 (6.51/9.13)	**0.00067**
Medication				
Antithrombotic **agents (B01)**	1993 (61.3%)	494 (77.6%)	294 (86.2%)	0.0012

P-value was calculated for difference between HFpEF vs. HFrEF. HFpEF: Heart failure with preserved ejection fraction ≥ 50%; HFrEF: Heart failure with reduced ejection fraction ≤ 40%; CVRFs: Cardiovascular risk factors; MI: Myocardial infarction; CVD: Cardiovascular disease; PAD: Peripheral artery disease; VTE: Venous thromboembolism; MPV: Mean platelet volume; LVEF: Left ventricular ejection fraction; CRP: C-reactive protein; monocytes and lymphocytes were counted as percentages (%) of leukocyte count.

Table S3. Quartiles of platelet indices

	Q1	Q2	Q3	Q4
MPV (fL)	< 7.69	7.69 – 8.19	8.20 – 8.70	> 8.70
Platelet count (x10^9/L)	< 186	186 - 223	224 - 263	> 263
Platelet-to-leukocyte ratio	< 25.8	25.8 – 31.6	31.7 – 38.3	> 38.3
Platelet-to-monocyte ratio	< 410	410-531	532 - 691	> 691
Platelet-to-lymphocyte ratio	< 103	103 - 131	132 - 170	> 170

For outcome analysis platelet indices were divided into quartiles. MPV: mean platelet volume.

Table S4. Relation between platelet indices and worsening of HF in HF phenotypes with additional adjustment for Antithrombotic medication (ATC code: B01)

		adjusted for age, sex, CVRFs and cancer			additionally adjusted for antithrombotic agents (B01)		
		HR (95% CI)	**p-value**	**p-value for difference HFrEF vs. HFpEF**	**HR (95% CI)**	**p-value**	**p-value for difference HFrEF vs. HFpEF**
MPV (fL) >75th percentile	HFrEF	0.97 (0.64; 1.47)	0.90		0.97 (0.64; 1.47)	0.89	
	HFpEF	1.90 (1.15; 3.12)	**0.012**		1.84 (1.12; 3.03)	**0.017**	
	HFrEF vs. HFpEF	0.51 (0.27; 0.97)	**-**	**0.040**	0.53 (0.28; 1.00)	**-**	0.050
Platelet count < 25th percentile	HFrEF	0.85 (0.56; 1.31)	0.46		0.84 (0.55; 1.29)	0.42	
	HFpEF	2.30 (1.42; 3.74)	**0.00076**		2.27 (1.40, 3.68)	**0.00089**	
	HFrEF vs. HFpEF	0.37 (0.20; 0.70)	**-**	**0.0022**	0.37 (0.20; 0.70)	**-**	**0.0021**
Platelet-to-leukocyte ratio < 25th percentile	HFrEF	1.41 (0.94; 2.10)	0.093		1.44 (0.96; 2.16)	0.078	
	HFpEF	1.73 (1.05; 2.86)	**0.032**		1.73 (1.05; 2.84)	**0.031**	
	HFrEF vs. HFpEF	0.81 (0.44; 1.50)	-	0.51	0.83 (0.45; 1.54)	-	0.56
Platelet-to-monocyte ratio < 25th percentile	HFrEF	1.11 (0.74; 1.67)	0.61		1.13 (0.75; 1.70)	0.55	
	HFpEF	1.84 (1.10; 3.07)	**0.020**		1.83 (1.10, 3.04)	**0.020**	
	HFrEF vs. HFpEF	0.61 (0.32; 1.14)	-	0.12	0.62 (0.33; 1.16)	-	0.14
Platelet-to-lymphocyte ratio > 75th percentile	HFrEF	2.65 (1.78; 3.95)	**<0.0001**		2.64 (1.78; 3.93)	**<0.0001**	
	HFpEF	1.56 (0.94; 2.60)	0.085		1.52 (0.91; 2.54)	0.11	
	HFrEF vs. HFpEF	1.70 (0.89; 3.23)	-	0.11	1.73 (0.72; 1.50)	-	0.85

Cox regression analysis in N= 950 individuals for the association between mean platelet volume (MPV) > 75th percentile, platelet count < 25th percentile, platelet-to-leukocyte ratio < 25th percentile, platelet-to-monocyte ratio < 25th percentile or platelet-to-lymphocyte ratio >75th percentile and worsening of HF (N= 174 events). Results are presented as hazard ratios (HRs) with 95% confidence interval (CI). In addition, differences for the effects of platelet indices for HFrEF vs. HFpEF were calculated. Cardiovascular risk factors (CVRFs) are arterial hypertension, diabetes mellitus, smoking, obesity, dyslipidemia, family history of myocardial infarction/stroke.

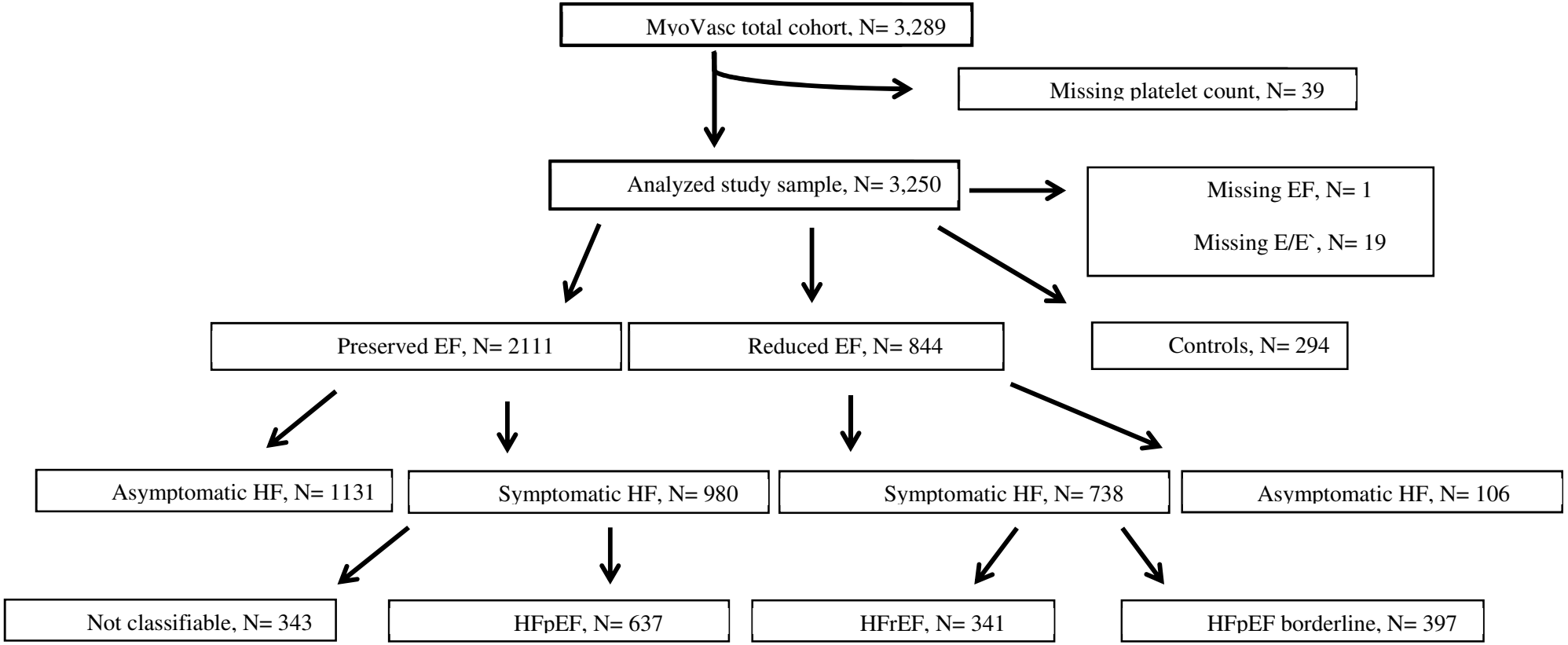
Table S5. Relation between platelet indices and worsening of HF in total analysis sample and after excluding individuals with history of cancer

		adjusted for age, sex and antithrombotic agents		additionally adjusted for CVRFs and comorbidities	
		HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**
MPV (fL) >75th percentile	total sample	1.55 (1.22; 1.96)	**0.00032**	1.47 (1.16; 1.87)	**0.0015**
	w/o cancer history	1.62 (1.23; 2.14)	**0.00056**	1.53 (1.16; 2.01)	**0.0029**
Platelet count < 25th percentile	total sample	1.35 (1.05; 1.73)	**0.018**	1.34 (1.05; 1.71)	**0.018**
	w/o cancer history	1.16 (0.87; 1.55)	0.31	1.16 (0.87; 1.55)	0.32
Platelet-to-leukocyte ratio < 25th percentile	total sample	1.67 (1.31; 2.14)	**<0.0001**	1.62 (1.19; 1.93)	**0.00075**
	w/o cancer history	1.64 (1.24; 2.18)	**0.00054**	1.46 (1.11; 1.94)	**0.0073**
Platelet-to-monocyte ratio < 25th percentile	total sample	1.47 (1.15; 1.89)	**0.0061**	1.38 (1.08;1.77)	**0.010**
	w/o cancer history	1.37 (1.03; 1.83)	**0.033**	1.27 (0.95; 1.70)	0.11
Platelet-to-lymphocyte ratio > 75th percentile	total sample	1.49 (1.16; 1.91)	**0.0015**	1.52 (1.18; 1.95)	**0.0010**
	w/o cancer history	1.56 (1.17; 2.07)	**0.0023**	1.61 (1.21; 2.15)	**0.0013**

Cox regression analysis in the total analysis sample, N= 3188 and in individuals without cancer history, N= 2676, for the association between mean platelet volume (MPV) > 75th percentile, platelet count < 25th percentile, platelet-to-leukocyte ratio < 25th percentile, platelet-to-monocyte ratio < 25th percentile or platelet-to-lymphocyte ratio >75th percentile and worsening of HF (N= 298 events in the total analysis sample and N= 223 for individuals without cancer history). Results are presented as hazard ratios (HRs) with 95% confidence interval (CI). Antithrombotic agents are assessed as ATC-code B01; cardiovascular risk factors (CVRFs) are arterial hypertension, diabetes mellitus, smoking, obesity, dyslipidemia, family history of myocardial infarction/stroke; comorbidities are myocardial infarction, stroke, chronic obstructive pulmonary disease, pulmonary embolism, venous thromboembolism, deep vein thrombosis, coronary artery disease, peripheral artery disease, chronic kidney disease, chronic liver disease, arthritis, atrial fibrillation, and congestive heart failure.

Part C. Supplemental Figures

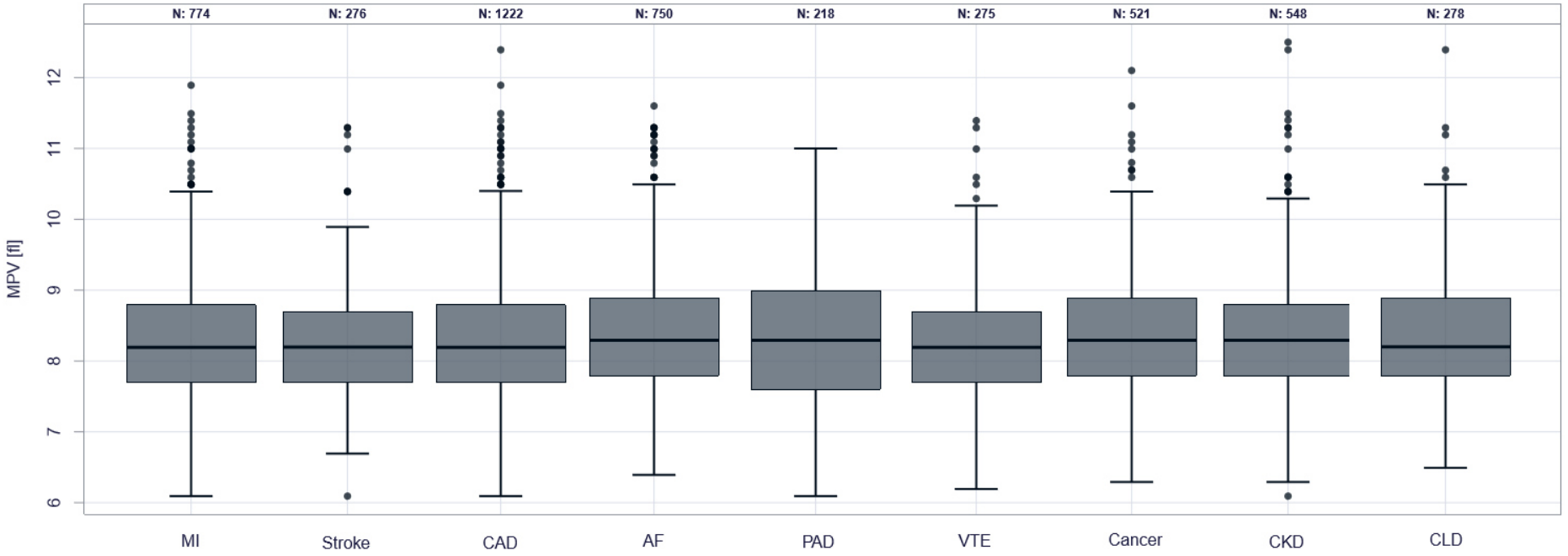
Supplemental Figure S1. Derivation of the analysis sample



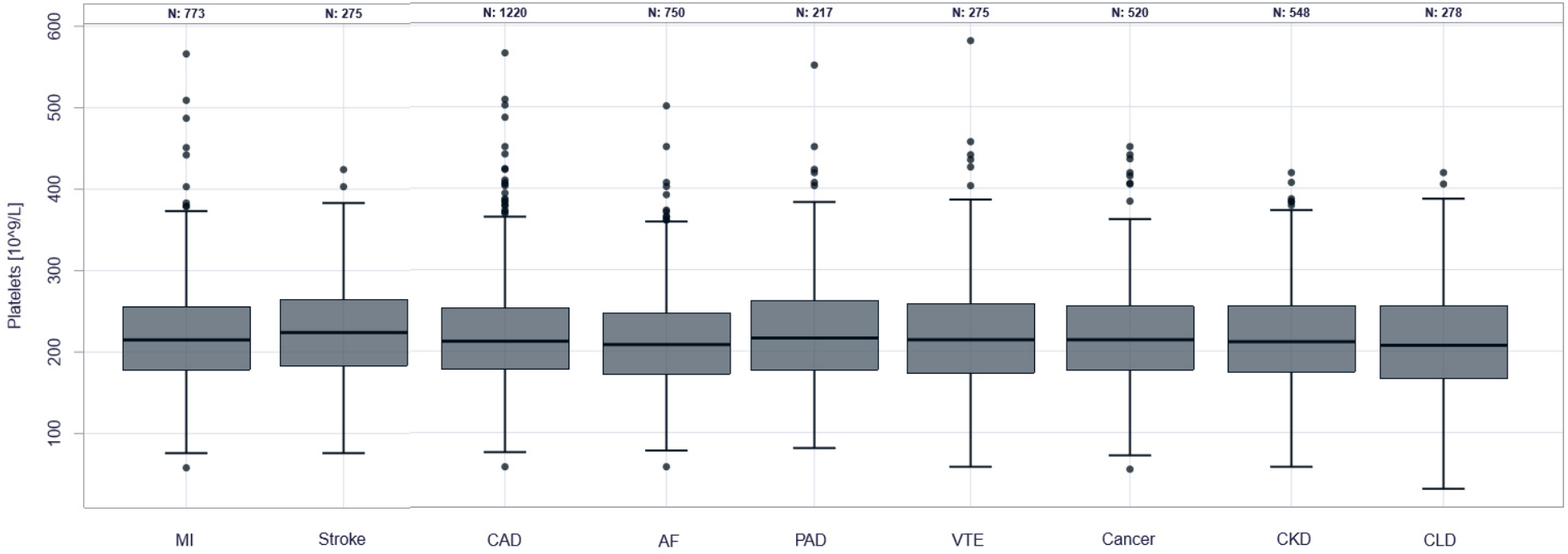
Flow chart presenting the derivation of the analysis sample based on measurements of platelet indices and ejection fraction. Outcome analyses were calculated in HFpEF and HFrEF individuals only, thereof missing information on outcome, N= 27; Controls were individuals with normal echocardiographic function; Not classifiable individuals had symptoms of HF + EF ≥ 50% but no diastolic dysfunction; Abbreviations: N: number of individuals; EF: ejection fraction; HFpEF: heart failure with preserved ejection fraction; HFpEF borderline: heart failure with LVEF 41% to 49%; HFrEF: heart failure with reduced ejection fraction

Supplemental Figure S2. Boxplots for the distribution of cardiovascular diseases and comorbidities

S2a. Distribution of comorbidities per increasing MPV (fL)



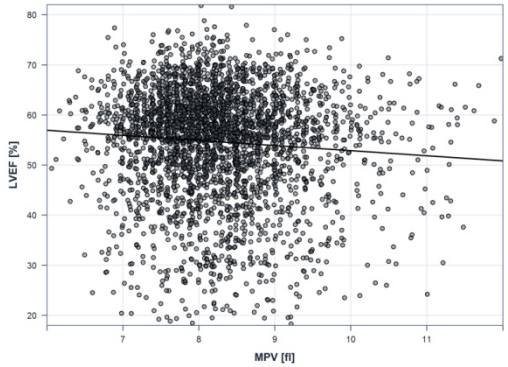
S2b. Distribution of comorbidities per increasing platelet count (10^9/L)



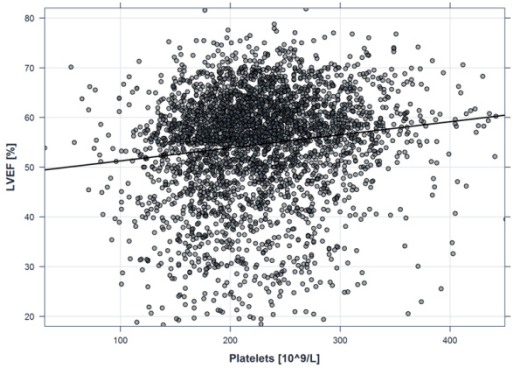
Boxplots presenting the distribution of cardiovascular diseases and comorbidities. MPV: mean platelet volume; MI: myocardial infarction; CAD: coronary artery disease; AF: atrial fibrillation; PAD: peripheral artery disease; VTE: venous thromboembolism; CKD: chronic kidney disease; CLD: chronic liver disease

Supplemental Figure S3. Scatter plots of LVEF and E/E' per increasing MPV (fL) or per increasing platelet count (10^9/L)

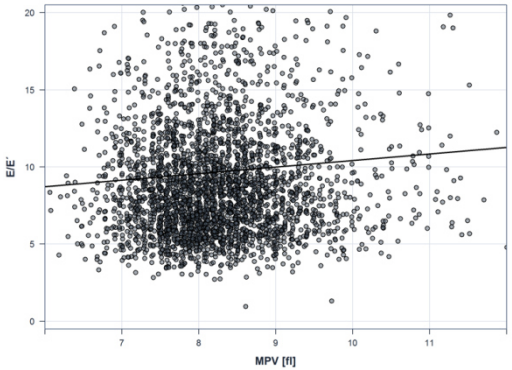
S3a. Distribution of LVEF per increasing MPV (fL)



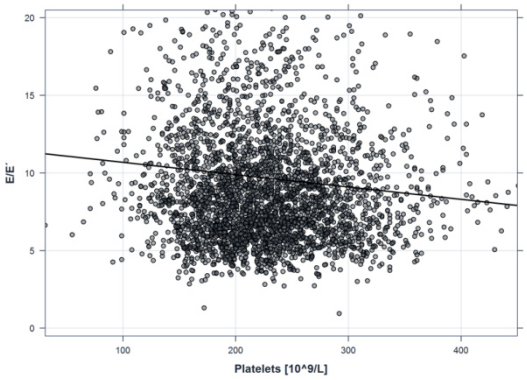
S3b. Distribution of LVEF per increasing platelet count (10^9/L)



S3c. Distribution of E/E' per increasing MPV (fL)

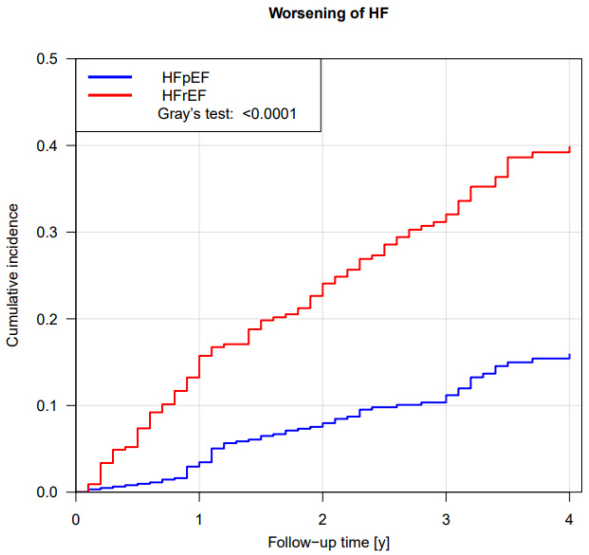


S3d. Distribution of E/E' per increasing platelet count (10^9/L)



Scatter plots showing the distribution of LVEF per increasing MPV (fL) in S3a and per increasing platelet count (10^9/L) in S3b as well as the distribution of E/E' per increasing MPV (fL) in S3c and per increasing platelet count (10^9/L) in S3d. LVEF: left ventricular ejection fraction; MPV: mean platelet volume

Supplemental Figure S4. Cumulative incidence plot for worsening of HF in HFrEF and HFpEF



Cumulative incidence plot presenting worsening of HF in HFpEF (N=637) and HFrEF (N=341) individuals with a median follow-up time of 2.51 years (interquartile range: 1.05 – 4.00 years). HFpEF: heart failure with preserved ejection fraction (LVEF ≥ 50%); HFrEF: heart failure with reduced ejection fraction (LVEF ≤ 40%)

References:

1. Gobel S, Prochaska JH, Trobs SO, Panova-Noeva M, Espinola-Klein C, Michal M, Lackner KJ, Gori T, Munzel T, Wild PS. Rationale, design and baseline characteristics of the MyoVasc study: A prospective cohort study investigating development and progression of heart failure. Eur J Prev Cardiol 2020:2047487320926438.

Publication: Sex-specific relationship between parathyroid hormone and platelet indices in phenotypes of heart failure – Results from the MyoVasc study

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Sex-Specific Relationship Between Parathyroid Hormone and Platelet Indices in Phenotypes of Heart Failure—Results From the MyoVasc Study

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Background: Heart failure (HF) is a multifactorial syndrome with pathophysiological complexities still not fully understood. Higher mean platelet volume (MPV), a potential marker of platelet activation, and high concentrations of parathyroid hormone (PTH) have been implicated in the pathogenesis of HF.

Aim: This study aims to investigate sex-specifically the association between PTH concentrations and platelet indices in phenotypes of HF.

Methods and Results: PTH and platelet indices (MPV and platelet count) were available in 1,896 participants from the MyoVasc study in Mainz, Germany. Multivariable linear regression models, adjusted for age, sex, season, vitamin D status, cardiovascular risk factors, comorbidities, estimated glomerular filtration rate, and medication, were used to assess the associations between platelet indices and PTH. The results showed distinct sex-specific associations between PTH and platelet indices. A positive association between PTH and MPV was found in females with symptomatic HF with reduced ejection fraction (HFrEF) only [$\beta = 0.60$ (0.19; 1.00)]. Platelet count was inversely associated with PTH in male HFrEF individuals [$\beta = -7.6$ (-15; -0.30)] and in both males and females with HF with preserved ejection fraction (HFpEF).

Conclusion: This study reports differential, sex-specific relationships between PTH and platelet indices in HF individuals independent of vitamin D status and clinical profile. Particularly in phenotypes of symptomatic HF, distinct associations were observed, suggesting a sex-specific mechanism involved in the interaction between PTH and platelets.

Keywords: heart failure, MPV, platelet count, parathyroid hormone, heart failure with preserved ejection fraction, heart failure with reduced ejection fraction

INTRODUCTION

Heart failure (HF) is one of the most common cardiovascular diseases (CVDs) accounting for substantial morbidity and mortality worldwide, with increasing incidence and prevalence especially among the elderly (1). As a heterogeneous condition, HF syndrome comprises predominantly two phenotypes (1). HF with preserved ejection fraction (HFpEF) is more frequent in females with cardiovascular comorbidities, whereas males with history of ischemic heart disease suffer more often from HF with reduced ejection fraction (HFrEF) (2, 3).

Recently, elevated parathyroid hormone (PTH) concentrations have been associated with all-cause and cardiovascular mortality in HF patients, suggesting a potential role for PTH in the pathogenesis and progression of HF (4, 5). PTH is physiologically released at low calcium concentrations to stimulate the synthesis of the active form of vitamin D, Calcitriol, which in turn suppresses PTH release as a negative feedback regulation of calcium homeostasis (6). Besides calcium concentrations, plasma PTH concentrations were also modulated by age and renal function (4, 7). Higher concentrations of PTH have been associated with advanced stages of HF according to categories of the New York Heart Association (NYHA) (8, 9), reduced left ventricular ejection fraction (LVEF) (8), and elevated brain natriuretic peptide (BNP) or N-terminal propeptide of BNP (NT-proBNP) (10–12). Different pathways have been proposed for the interaction of PTH with the heart. As a stimulator of hypertrophy, arrhythmia, and inflammation, PTH directly drives cardiomyocyte necrosis and thus accelerates the severity of HF (8, 11). In addition, PTH indirectly exacerbates HF by the activation of the renin–angiotensin–aldosterone system (RAAS), a key element of HF pathophysiology (13).

Platelet activation has been associated with traditional cardiovascular risk factors (CVRFs) and CVD including the HF syndrome (14, 15). Higher mean platelet volume (MPV), a potential marker of platelet activation, was reported in individuals with arterial hypertension, obesity, dyslipidemia, and diabetes mellitus (16). We have recently reported on sex-specific determinants of MPV in the general population with age, smoking, arterial hypertension, and high blood glucose concentrations linked with higher MPV in males, whereas oral contraceptives and menstrual bleeding were associated with higher MPV in females (14).

Platelet activation including higher MPV, increased whole blood aggregation tendency, and higher platelet-bound and soluble P-selectin has been associated with HF syndrome (14, 15). Positive associations between MPV and PTH were described in individuals with primary hyperparathyroidism and end-stage renal failure patients (17, 18). In addition, an experimental study showed an important enhancing effect of the PTH-related protein, a protein initially isolated from hypercalcemia-associated tumors, on agonist-induced platelet activation and aggregation (19). Individuals with coronary artery disease presenting with higher PTH concentrations showed increased ADP-mediated platelet aggregation and suboptimal response to clopidogrel, despite receiving a dual antiplatelet therapy (7).

The relation between platelet function and PTH plasma concentration has been poorly explored in individuals with HF. This analysis aimed to investigate sex-specifically the associations between PTH concentrations and the platelet indices, platelet count, and MPV, across phenotypes of HF in individuals enrolled in the MyoVasc study.

METHODS

Analysis Sample

As a large prospective cohort study at the University Medical Center of the Johannes Gutenberg-University Mainz in Germany, the MyoVasc Study was primarily conceptualized to investigate the development and progression of HF and its interaction with vascular disease (20). The study included 3,289 participants aged from 35 to 84 years. All subjects underwent an extensive, standardized clinical and laboratory investigation including sampling of biomaterials for biobanking at the MyoVasc study center. Platelet count, MPV, and PTH were available in the first 2,000 participants enrolled in the MyoVasc study at their baseline examination between January 2013 and January 2016. The assessment of CVRFs, comorbidities, and medication as well as echocardiography of cardiac structure and function are described in the **Supplementary Material (Part A)**. Written informed consent was obtained from all study participants prior to entering the study. The study complies with the principles outlined in the Declaration of Helsinki, Good Clinical Practice and Good Epidemiological Practice. An approval from the responsible ethics committee [reference number 837.319.12 (8420-F)] and data safety commissioner was obtained in 2012, before study initiation. The MyoVasc study was registered at http://clinicaltrials.gov (identifier: NCT04064450).

Definition of HF Phenotypes

Based on measurement of LVEF following a standardized echocardiographic assessment (**Supplementary Material Part A**), subjects with LVEF $\geq$ 50% were defined as having preserved ejection fraction (EF) and those with LVEF $<$ 50% as having reduced EF, independent of presence of HF symptoms. Individuals with symptomatic HF (i.e., HF, stage C or D according to AHA) were further categorized according to LVEF into (i) HF with preserved ejection fraction (HFpEF) with LVEF $\geq$ 50%, (ii) HF with reduced ejection fraction (HFrEF) with LVEF $\leq$ 40%, and (iii) HFpEF borderline with LVEF in the range of 41–49% according to the ACCF/AHA Guideline for the Management of Heart Failure (21).

Laboratory Assessment

Venous blood sampling was performed for laboratory markers of the present analysis by using tripotassium ethylenediaminetetraacetic acid (K3-EDTA) tubes. Platelet count (10^9/L) and MPV (femtoliter, fl) were automatically determined on an ADVIA 120 Hematology System (Siemens, Erlangen, Germany) within 30 to 90 min after blood withdrawal in the Central laboratory of the Institute for Clinical Chemistry and Laboratory Medicine, University Medical Center Mainz,

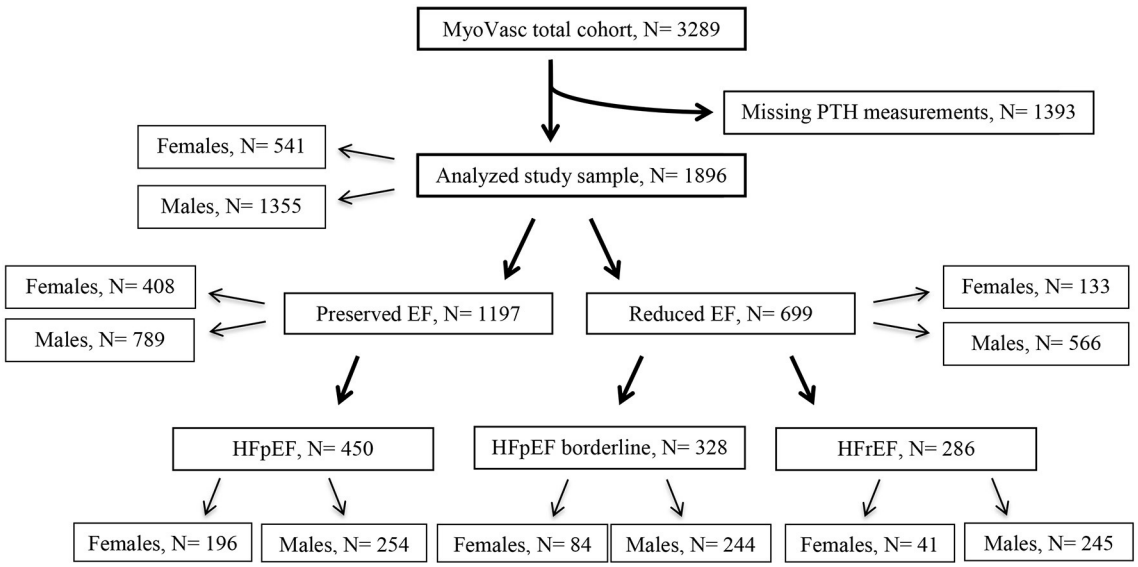

FIGURE 1 | Derivation of the analysis sample. Flow chart presenting the derivation of the analysis sample based on measurements of PTH and ejection fraction. *N*, number of individuals; PTH, parathyroid hormone; EF, ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFpEF borderline, heart failure with LVEF 41 to 49%; HFrEF, heart failure with reduced ejection fraction.

Germany. PTH was measured in pg/ml by an immunoassay with an automated chemiluminescence analyzer (Liaison XL, DiaSorin, Saluggia, Italy) in the Biomolecular Laboratory of the Clinical Epidemiology and Systems Medicine, Center for Thrombosis and Hemostasis, University Medical Center Mainz, Germany.

Data Management and Statistical Analysis

Statistical analysis was performed after data quality control including a review for correctness, completeness, representativeness, accuracy, and plausibility performed by the data management unit. Baseline characteristics of the analysis sample were presented according to phenotype of cardiac function. Normally distributed values were described by mean and standard deviation (SD), non-normally distributed variables were described by median and interquartile range. Associations between platelet indices (i.e., MPV and platelet count) and PTH were presented per phenotype of cardiac function by linear regression models, adjusted for the following variables in stepwise extended models: (i) age, sex, season, and vitamin D status; (ii) plus additionally with CVRFs (diabetes mellitus, arterial hypertension, smoking, dyslipidemia, obesity, and family history of myocardial infarction and stroke) and estimated glomerular filtration rate (eGFR); (iii) plus comorbidities subsuming CVD, venous thromboembolism (VTE), chronic obstructive pulmonary disease (COPD), cancer, and arthritis; and (iv) plus additionally medication (vitamin D supplements, calcium supplements, diuretics, beta-blockers, calcium channel blockers, RAAS antagonists, antiplatelet agents, antilipemic drugs, anti-inflammatory and rheumatic drugs, glucocorticoids, corticosteroids, antibacterial drugs, and immunosuppressant drugs). The subgroup analysis in males was conducted with adjustment for the same covariates as the whole analysis sample, whereas in females, it was additionally adjusted for oral contraceptives, hormone replacement therapy, and menstrual bleeding in the full model.

Because of the explorative character of the analysis, a significance threshold for *p*-values was not defined and *p*-values were interpreted as a continuous measure of statistical evidence. All statistical analyses were performed using R, version 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org).

RESULTS

Baseline Characteristics of the Analysis Sample

After exclusion of individuals with missing data on PTH, 1,896 subjects were available for analysis (**Figure 1**). Baseline characteristics of the individuals in the analysis sample are reported in **Table 1** according to phenotype of cardiac function. Based on EF and irrespective of presence of symptoms, 1,197 individuals were characterized with preserved EF and 699 individuals with reduced EF. Symptomatic HF was present in 1,064 (56.1%) individuals, of whom 42.3% (450) had HFpEF, 30.8% (328) HFpEF borderline, and 26.9% (286) HFrEF. More than 80% of individuals with reduced EF and HFrEF were males with a higher frequency of smokers, dyslipidemia, coronary artery disease, and history of myocardial infarction compared to individuals with preserved EF and HFpEF, respectively. In the subgroup with preserved EF and HFpEF, there were more females comparatively to the other phenotypes, but overall still more males. Individuals with preserved EF and HFpEF had more often arterial hypertension and a history of VTE compared to

TABLE 1 | Baseline characteristics according to phenotype of cardiac function (*N*= 1,896).

	Phenotype of cardiac function				
	Preserved EF (*N* = 1,197)	**Reduced EF (*N* = 699)**	**HFpEF (*N* = 450)**	**HFpEF borderline (*N* = 328)**	**HFrEF (*N* = 286)**
Age [years]	67.2 ± 9.4	65.6 ± 10.6	70.7 ± 8.2	66.2 ± 10.6	65.6 ± 10.6
Sex (women)	34.1% (408)	19.0% (133)	43.6% (196)	25.6% (84)	14.3% (41)
CVRFs					
Arterial hypertension	84.0% (1,006)	74.8% (523)	86.2% (388)	78.7% (258)	75.9% (217)
Diabetes mellitus	25.1% (300)	30.3% (212)	32.7% (147)	28.7% (94)	33.9% (97)
Smoking	10.5% (126)	17.3% (121)	9.1% (41)	17.1% (56)	18.9% (54)
Obesity	34.4% (412)	35.2% (246)	38.7% (174)	38.4% (126)	36.0% (103)
Dyslipidemia	79.1% (947)	84.4% (590)	78.2% (352)	84.5% (277)	86.0% (246)
FH of MI/stroke	24.3% (290)	27.0% (189)	23.3% (105)	27.7% (91)	29.0% (83)
Comorbidities					
History of MI	30.7% (368)	39.3% (275)	28.4% (128)	37.5% (123)	43.7% (125)
History of Stroke	10.3% (123)	10.4% (73)	10.4% (47)	11.9% (39)	10.8% (31)
CAD	50.9% (609)	56.2% (393)	49.8% (224)	57.6% (189)	57.3% (164)
AF	26.9% (322)	37.6% (263)	36.9% (166)	38.7% (127)	40.2% (115)
History of VTE	11.2% (134)	9.3% (65)	14.2% (64)	10.1% (33)	8.7% (25)
History of Cancer	16.8% (201)	17.6% (123)	19.6% (88)	18.3% (60)	16.8% (48)
Echocardiographic parameters					
EF [%]	58.3 ± 5.2	39.5 ± 8.1	58.1 ± 5.4	45.2 ± 2.8	31.5 ± 5.9
E/E'	8.57 (6.65/11.30)	10.17 (7.26/14.47)	11.16 (8.76/14.62)	9.22 (7.05/12.74)	12.40 (8.34/18.02)
Lab parameters					
MPV [fl]	8.22 ± 0.86	8.36 ± 0.93	8.25 ± 0.84	8.31 ± 0.89	8.43 ± 0.99
Platelet count [10^9/L]	219 (182/260)	208 (173/251)	218 (179/261)	213 (177/260)	206 (170/244)
PTH [pg/ml]	30.0 (23.0/38.4)	34.0 (26.3/46.7)	32.1 (23.6/42.3)	32.7 (24.8/45.3)	38.6 (29.1/52.1)
eGFR [ml/min/1.73 m^2]	76.44 ± 18.62	71.98 ± 21.50	69.80 ± 19.42	73.80 ± 20.54	66.36 ± 22.36
Medication					
Vitamin D supplements (A11CC)	8.7% (104)	5.9% (41)	8.7% (39)	5.5% (18)	6.6% (19)
Calcium supplements (A12A)	1.9% (23)	1.9% (13)	3.1% (14)	2.7% (9)	1.0% (3)
Antihypertensiva (C02)	4.4% (53)	2.1% (15)	5.8% (26)	2.7% (9)	1.4% (4)
Diuretics (C03)	28.5% (341)	66.1% (462)	43.6% (196)	60.1% (197)	86.4% (247)
Beta-blockers (C07)	69.0% (826)	79.4% (555)	75.8% (341)	79.6% (261)	84.6% (242)
Calcium channel blockers (C08)	25.4% (304)	14.2% (99)	32.2% (145)	19.8% (65)	8.4% (24)
Renin–Angiotensin–Aldosterone system antagonsists (C09)	78.6% (941)	81.3% (568)	80.2% (361)	85.4% (280)	86.0% (246)
Lipid-modifying agents (C10)	60.4% (723)	60.9% (426)	58.9% (265)	64.3.% (211)	60.1% (172)
Antithrombotic agents (B01A)	80.6% (965)	85.6% (598)	85.1% (383)	88.4% (290)	86.0% (246)

Presented are baseline clinical characteristics, echocardiographic and laboratory parameters, including intake of medications according to cardiac function phenotype in 1,896 subjects. EF, ejection fraction; HFpEF, heart failure with preserved ejection fraction (EF ≥ 50%); HFpEF borderline, heart failure with ejection fraction of 41%–49%; HFrEF, heart failure with reduced ejection fraction (EF ≤ 40%); CVRFs, cardiovascular risk factors; FH, family history; MI, myocardial infarction; CAD, coronary artery disease; AF, atrial fibrillation, PAD, peripheral artery disease, COPD, chronic obstructive pulmonary disease; VTE, venous thromboembolism; CKD, chronic kidney disease; EF, ejection fraction; MPV, mean platelet volume; PTH, parathyroid hormone.

other phenotypes. Subjects with HFpEF borderline had the lowest proportion of diabetes mellitus (28.7 vs. 32.7% in HFpEF and 33.9% in HFrEF).

Similarly to the clinical profile, differences between HF phenotypes were also evident in laboratory parameters: individuals with reduced EF presented with higher MPV and PTH concentrations, but lower platelet count as well as worse renal function (determined by eGFR), compared to individuals with preserved EF. Within the subsample with symptomatic HF, highest MPV and PTH and lowest platelet count and worst renal function were observed in individuals with HFrEF.

Individuals with preserved EF and particularly subjects with HFpEF were more frequently taking vitamin D supplements, antihypertensives, and calcium channel blockers compared to those with reduced EF, HFpEF borderline, and HFrEF. Intake of diuretics, beta-blockers, and antithrombotic agents were more often reported for subjects with reduced EF, HFpEF borderline, and HFrEF.

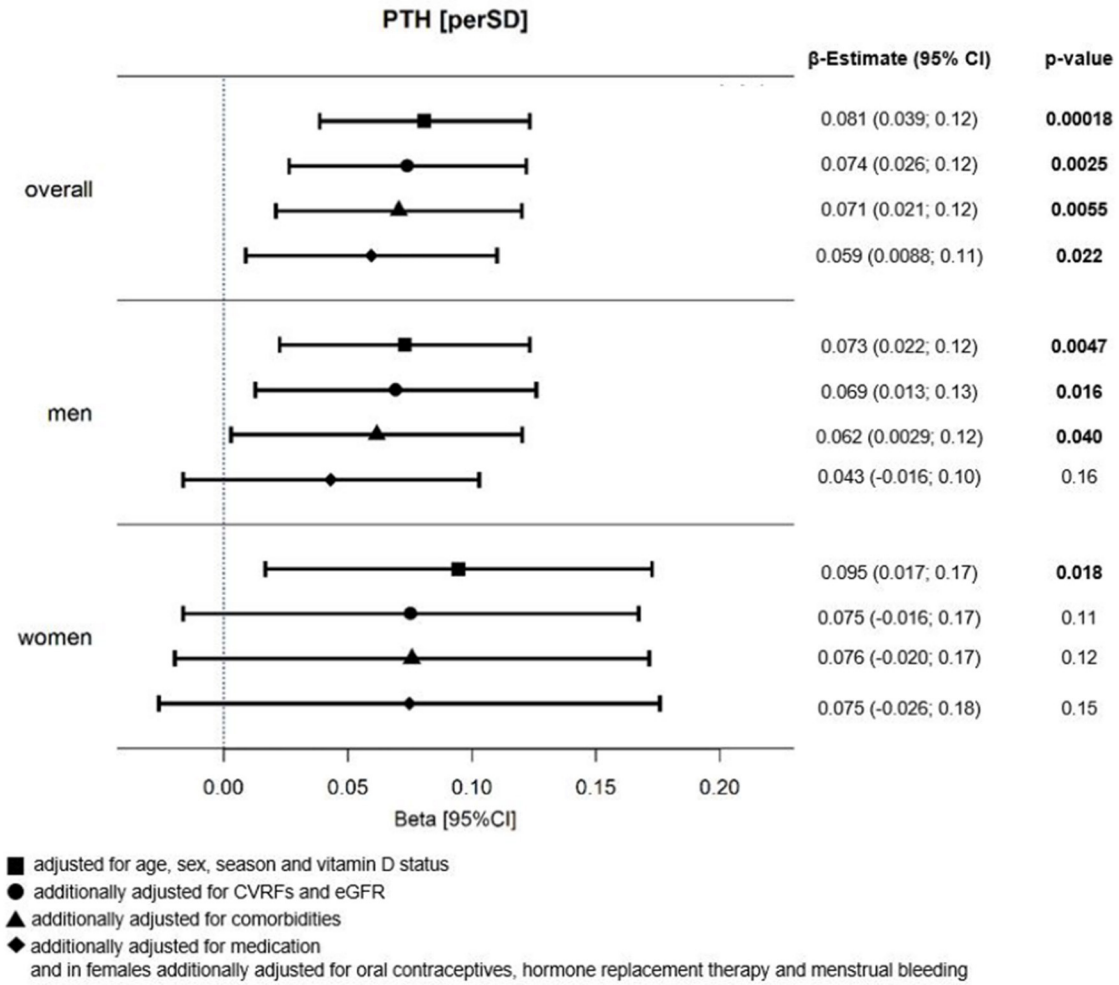

FIGURE 2 | Relation between MPV and PTH in HF individuals. Forest Plot of beta (β)-estimates with 95% confidence intervals (CIs) for the relation between MPV and PTH in all HF individuals and stratified by sex. $N = 1{,}861$; thereof $N = 532$ females and $N = 1{,}329$ males; adjustment for sex only in overall analysis sample; MPV, mean platelet volume; PTH, parathyroid hormone; CVRFs, cardiovascular risk factors; eGFR, estimated glomerular filtration rate.

Pearson's correlation sex-specific analysis between PTH levels and age and according to HF phenotype showed a weak correlation in both males and females across different HF phenotypes as presented in **Supplementary Table 1**.

Association Between MPV and PTH

In the whole sample, a positive association between MPV and SD change of PTH with beta estimate (β) = 0.081 (95% confidence interval: 0.039; 0.12) was observed after adjustment for age, sex, season, and vitamin D status, which corresponded in males to $\beta = 0.073$ (0.022; 0.12) and in females to $\beta = 0.095$ (0.017; 0.17). Results from a linear regression model for MPV are presented in **Figure 2**. Further adjustment for CVRFs plus eGFR, comorbidities, and medication did not significantly change this association in the whole sample. A sex-specific analysis showed a mildly stronger association between MPV and PTH in females compared to males. The analysis stratified for cardiac function showed important sex-specific differences between phenotypes (**Table 2**): there was a positive association between MPV and PTH independent of age, season, and vitamin D status in individuals with preserved EF [$\beta = 0.078$ (0.020; 0.14)], which was only present in male participants [$\beta = 0.11$ (0.034; 0.18)], whereas in reduced EF and HFrEF, MPV and PTH were associated in females only [$\beta_{\text{reducedEF}} = 0.21$ (0.043; 0.37); $\beta_{\text{HFrEF}} = 0.36$ (0.063; 0.67)] after the same adjustment. For HFpEF borderline, a weak association was only found in women. Interestingly, the strongest and most robust association was found in females in HFrEF, where it remained relevant even after adjustment for CVRFs and comorbidities.

Association Between Platelet Count and PTH

Results of the multivariable analysis for platelet count showed a strong inverse association per SD of PTH independent of

TABLE 2 | Relation between MPV and PTH according to cardiac function in a sex-specific analysis.

		MPV							
		Adjusted for age, sex[a], season, vitamin D status[b]		Additionally adjusted for CVRFs and eGFR		Additionally adjusted for comorbidities		Additionally adjusted for medication[c]	
	N	*β*-estimate (95% CI)	*P*-value	*β*-estimate (95% CI)	*P*-value	*β*-estimate (95% CI)	*P*-value	*β*-estimate (95% CI)	*P*-value
Preserved EF	1,174	0.078 (0.020; 0.14)	**0.0086**	0.077 (0.013; 0.14)	**0.019**	0.060 (−0.0066; 0.13)	0.078	0.043 (−0.025; 0.11)	0.21
Females	401	0.014 (−0.078; 0.11)	0.77	−0.023 (−0.13; 0.084)	0.67	−0.039 (−0.15; 0.073)	0.50	−0.046 (−0.17; 0.073)	0.45
Males	773	0.11 (0.034; 0.18)	**0.0044**	0.12 (0.037; 0.20)	**0.0045**	0.10 (0.016; 0.18)	**0.020**	0.074 (−0.011; 0.16)	0.090
Reduced EF	687	0.067 (0.0012; 0.13)	**0.046**	0.064 (−0.011; 0.14)	0.093	0.073 (−0.0051; 0.15)	0.067	0.071 (−0.0089;0.15)	0.082
Females	131	0.21 (0.043; 0.37)	**0.015**	0.26 (0.050; 0.46)	**0.016**	0.27 (0.055; 0.49)	**0.016**	0.25 (0.029; 0.47)	**0.029**
Males	556	0.030 (−0.041; 0.10)	0.41	0.021 (−0.061; 0.10)	0.62	0.025 (−0.061; 0.11)	0.57	0.023 (−0.065; 0.11)	0.61
HFpEF	442	0.075 (−0.0046; 0.15)	0.065	0.049 (−0.040; 0.14)	0.28	0.031 (−0.063; 0.12)	0.52	0.019 (−0.076; 0.11)	0.69
Females	191	0.033 (−0.086; 0.15)	0.59	−0.0011 (−0.15; 0.14)	0.99	−0.018 (−0.17; 0.13)	0.81	n.a.	n.a.
Males	251	0.10 (−0.0087; 0.21)	0.073	0.076 (−0.045; 0.20)	0.22	0.056 (−0.073; 0.18)	0.40	0.028 (−0.10; 0.16)	0.68
HFpEF borderline	324	0.046 (−0.051; 0.14)	0.35	0.040 (−0.070; 0.15)	0.48	0.038 (−0.076; 0.15)	0.51	0.029 (−0.090; 0.15)	0.63
Females	82	0.22 (0.0076; 0.44)	**0.046**	0.22 (−0.066; 0.50)	0.14	0.21 (−0.095; 0.51)	0.18	0.10 (−0.30; 0.50)	0.62
Males	242	−0.028 (−0.14; 0.080)	0.61	−0.036 (−0.16; 0.087)	0.57	−0.044 (−0.17; 0.084)	0.50	−0.051 (−0.19; 0.087)	0.47
HFrEF	279	0.11 (0.0062; 0.21)	**0.038**	0.11 (−0.0033; 0.23)	0.058	0.14 (0.016; 0.26)	**0.028**	0.12 (−0.0055; 0.24)	0.062
Females	41	0.36 (0.063; 0.67)	**0.024**	0.59 (0.19; 0.99)	**0.0071**	0.60 (0.19; 1.0)	**0.0089**	n.a.	n.a.
Males	238	0.072 (−0.036; 0.18)	0.19	0.059 (−0.065; 0.18)	0.35	0.083 (−0.049; 0.22)	0.22	0.079 (−0.056; 0.22)	0.25

Multivariable linear regression analysis with MPV as dependent variable and PTH as independent variable in phenotypes of cardiac function and sex-specific. Results are presented as beta (β)-estimates for change per 1 standard deviation in PTH. MPV, mean platelet volume; PTH, parathyroid hormone; N, number of individuals; eGFR, estimated glomerular filtration rate; EF, ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFpEF borderline, heart failure with ejection fraction of 41–49%; HFrEF, heart failure with reduced ejection fraction; n.a., not available due to low sample size [a]Sex-adjustment only in overall analysis sample; [b]Vitamin D status was determined by concentrations of Calcifediol and Calcitriol; [c]In females additionally adjusted for oral contraceptives, hormone replacement therapy and menstrual bleeding. P-value < 0.05 were highlighted in bold.

age, sex, season, and vitamin D status with $\beta = -6.42$ (−9.21; −3.63), which remained after further adjustment for CVRFs and eGFR [$\beta = -6.79$ (−9.94; −3.63)], comorbidities [$\beta = -6.52$ (−9.78; −3.27)], and medication [$\beta = -6.21$ (−9.53; −2.88)] in the whole analysis sample (**Figure 3**). This reciprocal association was observed in males and females independent of all potential confounders, but with higher estimates in females then in men [$\beta_{females} = -8.36$ (−15.44; −1.27) vs. $\beta_{males} = -4.50$ (−8.32; −0.67)].

The analysis according to cardiac phenotypes, as presented in **Table 3**, showed relevant associations between platelet count and PTH in both individuals with preserved [$\beta = -6.7$ (−11; −2.0)] and reduced ejection fraction [$\beta = -5.6$ (−11; −0.77)]. In HFpEF, the largest effect estimates for an inverse association between PTH and platelet count were found, and these remained robust after adjustment for age, sex, season, and vitamin D status [$\beta = -9.5$ (−15; −3.6)], but also further adjustment for CVRFs and eGFR [$\beta = -9.9$ (−17; −3.2)], comorbidities [$\beta = -9.4$ (−16; −2.4)], and medication [$\beta = -8.9$ (−16; −1.7)]. The sex-specific analysis in this phenotype showed stronger associations in females than in males. Differently, in HFrEF, the inverse association between PTH and platelet count was only found in male individuals and present independent of all considered confounders [$\beta = -7.6$ (−15; −0.30)]. No associations were observed between platelet count and PTH in individuals with HFpEF borderline.

DISCUSSION

PTH and platelet activation have been independently implicated in the pathogenesis of the HF syndrome (15, 22). However, the sex-specific interplay of these factors, as well as their specific relationship in phenotypes of HF, is currently largely unknown. This study demonstrated an important relation between platelet indices and PTH, which varied in phenotypes of cardiac function and particularly in individuals with symptomatic HF. In addition, the present analysis reports on distinct sex-specific differences in HF phenotypes.

Previous studies in individuals with primary hyperparathyroidism and end-stage renal failure patients have shown positive associations between MPV and PTH; however, sex-specific aspects were not addressed (17, 18). Other research has already demonstrated sex-specific differences for MPV in the general population that was also differentially associated with total mortality (14).

In contrast to the findings for MPV and PTH, an inverse association between PTH and platelet count was found in the total sample, which was present in both men and women. The inverse direction of the association between platelet count and PTH compared to MPV is explained by the fact that platelet count and MPV are physiologically inversely related to keep the overall platelet mass stable (23). Similarly, as for the MPV and PTH relation, sex-specific associations observed between platelet count and PTH were distinct for phenotypes of symptomatic

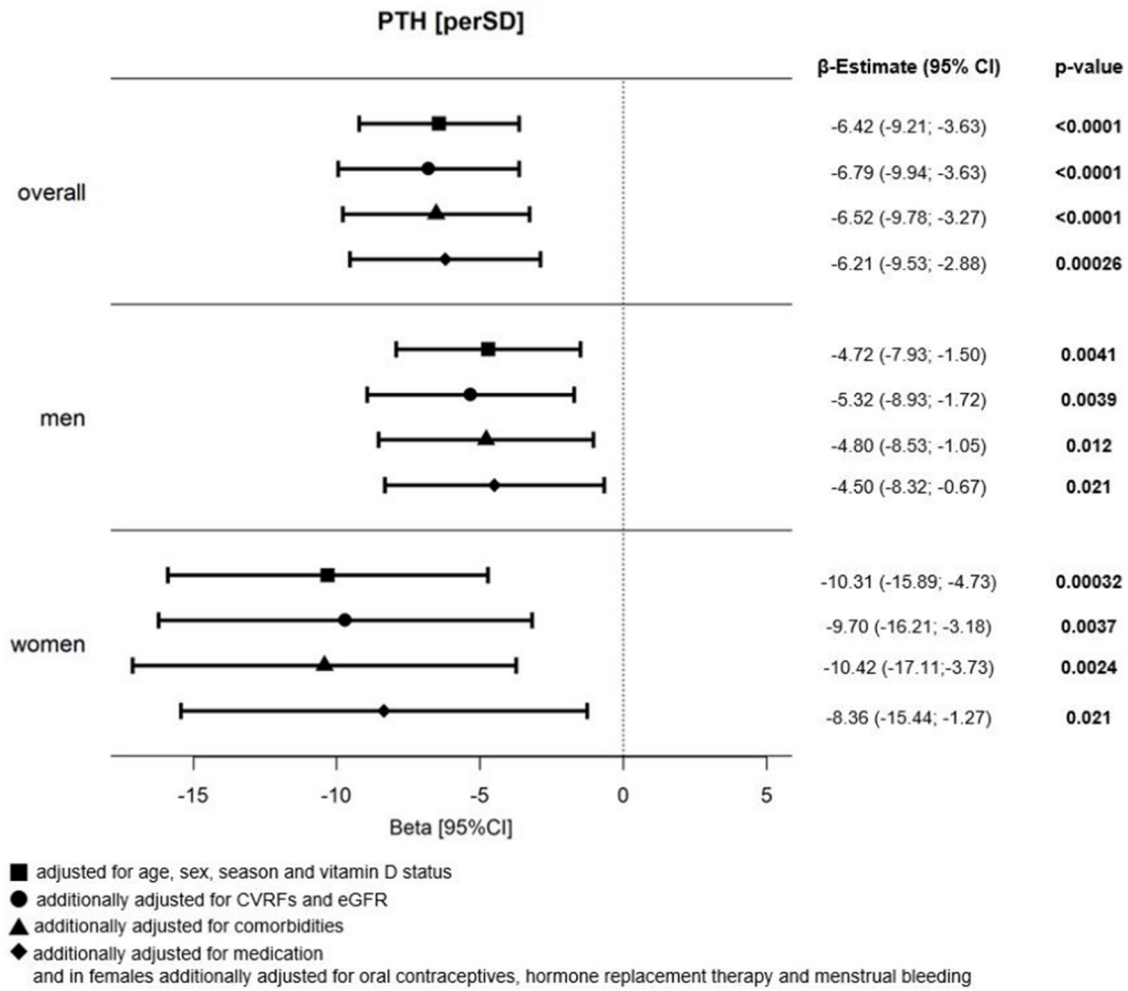

FIGURE 3 | Relation between platelet count and PTH in HF individuals. Forest Plot of beta (β)-estimates with 95% confidence intervals (CIs) for the relation between platelet count and PTH in all HF individuals and stratified by sex. *N* (total analysis sample) = 1,860, thereof *N* = 532 females and *N* = 1,328 males; adjustment for sex only in overall analysis sample; PTH, parathyroid hormone; CVRFs, cardiovascular risk factors; eGFR, estimated glomerular filtration rate.

HF: within HFpEF individuals, the inverse association was observed more consistent in females, whereas in HFrEF, the inverse association between PTH and platelet count was found in males only.

The etiology of HF differs between males and females regarding prevalence, risk factors, and comorbidities (2), and in part these differences could be explained by the sex-specific hormones, pregnancy, or preeclampsia (24). Also the pathophysiology differs between both sexes, as females tend to suffer more from a "microvascular" disease with vascular stiffness and systemic inflammation, whereas males tend to present with a more "macrovascular" pattern due to comorbidities such as MI or CAD (3, 25). Indeed, the results in the current analysis also differ between both sexes. The associations between MPV or platelet count, and PTH, if found, were with higher effect sizes in females compared to males. Notably, the association was also independent of known female factors influencing the platelet size, such as menstrual bleeding, hormone replacement therapy, and intake of oral contraceptives. Whether endogenous hormone levels influence the association between platelet indices and PTH in the HF syndrome requires further investigation. Genetically determined testosterone levels have been linked with development of HF, predominantly in men, as shown in a recent Mendelian randomization study (26). Post-menopause in women has been associated with an exponential increase in the incidence of HFpEF compared with men of the same age. Estrogen deprivation in post-menopause has been recognized as an important determinant of diastolic dysfunction as estrogen is shown to modulate many regulatory molecular pathways of cardiac diastolic function (27, 28). The present results further support the importance of hormones by showing an important effect of hormone-containing agents on the association between platelet count and PTH in female HF subjects.

TABLE 3 | Relation between platelet count and PTH according to cardiac function in a sex-specific analysis.

		Platelet count							
		Adjusted for age, sex[a], season, vitamin D status[b]		Additionally adjusted for CVRFs and eGFR		Additionally adjusted for comorbidities		Additionally adjusted for medication[c]	
	N	*β*-estimate (95% CI)	*P*-value	*β*-estimate (95% CI)	*P*-value	*β*-estimate (95% CI)	*P*-value	*β*-estimate (95% CI)	*P*-value
Preserved EF	1,173	−7.0 (−11; −3.0)	**0.00071**	−7.2 (−12; −2.8)	**0.0015**	−6.9 (−11; −2.3)	**0.0032**	−6.7 (−11;−2.0)	**0.0052**
Females	401	−11 (−18; −3.3)	**0.0046**	−10 (−19; −1.9)	**0.016**	−10 (−19; −1.7)	**0.019**	−8.0 (−17; 1.1)	0.084
Males	772	−5.0 (−9.8; −0.17)	**0.043**	−5.2 (−10; 0.060)	0.053	−4.8 (−10; 0.64)	0.084	−4.4 (−10; 1.1)	0.12
Reduced EF	687	−4.4 (−8.3; −0.41)	**0.031**	−5.7 (−10; −1.1)	**0.015**	−5.3 (−10; −0.52)	**0.030**	−5.6 (−11; −0.77)	**0.024**
Females	131	−5.9 (−14; 2.4)	0.17	−8.0 (−18; 2.4)	0.13	−9.1 (−20; 1.9)	0.11	−5.0 (−17; 6.8)	0.41
Males	556	−4.0 (−8.5; 0.41)	0.075	−5.1 (−10; 0.050)	0.053	−4.5 (−9.9; 0.94)	0.11	−4.7 (−10; 0.85)	0.098
HFpEF	442	−9.5 (−15; −3.6)	**0.0019**	−9.9 (−17; −3.2)	**0.0039**	−9.4 (−16; −2.4)	**0.0091**	−8.9 (−16; −1.7)	**0.015**
Females	191	−11 (−21; −0.96)	**0.033**	−12 (−23; −0.14)	**0.049**	−11 (−23; 1.4)	0.084	n.a.	n.a.
Males	251	−8.6 (−16; −1.2)	**0.024**	−8.3 (−17; 0.0084)	0.051	−7.7 (−17; 1.2)	0.090	−6.0 (−15; 3.2)	0.21
HFpEF borderline	324	−1.9 (−8.7; 4.8)	0.57	−3.0 (−11; 4.7)	0.44	−2.5 (−10; 5.4)	0.53	−3.9 (−12; 4.4)	0.35
Females	82	−11 (−23; 1.4)	0.086	−9.7 (−26; 6.5)	0.24	−12 (−29; 6.3)	0.21	−9.5 (−33; 14)	0.43
Males	242	0.71 (−7.2; 8.6)	0.86	−1.0 (−10; 8.1)	0.83	0.50 (−8.9; 9.9)	0.92	0.092 (−10; 10)	0.99
HFrEF	279	−5.4 (−11; −0.28)	**0.040**	−7.1 (−13; −1.2)	**0.019**	−7.0 (−13; −0.60)	**0.033**	−6.3 (−13; 0.18)	0.058
Females	41	−0.14 (−13; 13)	0.98	−10 (−27; 6.7)	0.25	−8.0 (−26; 10)	0.40	n.a.	n.a.
Males	238	−6.5 (−12; −0.79)	**0.027**	−7.4 (−14; −0.90)	**0.027**	−7.6 (−15; −0.49)	**0.037**	−7.6 (−15; −0.30)	**0.043**

Multivariable linear regression analysis with platelet count as dependent variable and PTH as independent variable in phenotypes of cardiac function and sex-specific. Results are presented as beta (β)-estimates for change per one standard deviation in PTH. PTH, parathyroid hormone; N, number of individuals; eGFR, estimated glomerular filtration rate; EF, ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFpEF borderline, heart failure with ejection fraction of 41–49%; HFrEF, heart failure with reduced ejection fraction; n.a., not available due to low sample size.

[a]Sex-adjustment only in overall analysis sample; [b]Vitamin D status was determined by concentrations of Calcifediol and Calcitriol; [c]In females additionally adjusted for oral contraceptives, hormone replacement therapy and menstrual bleeding. P-value < 0.05 were highlighted in bold.

The role of PTH according to HF severity has also been reported. A positive correlation between PTH and NYHA class and PTH and NT-proBNP levels as well as an inverse correlation between PTH and LVEF has been reported in different HF studies (8–11).

A positive relation between increasing age and PTH levels has been previously reported, primarily as a response to changes in serum calcium (29). The results from this study showed a weak positive correlation between age and PTH in males with predominantly HFpEF phenotype and in females with predominantly HFrEF phenotype.

In addition, patients with disorders of the parathyroid gland suffered more frequently from arterial hypertension, left ventricular hypertrophy, arrhythmia, and HF (13). Elevated PTH can stimulate cardiac myocyte hypertrophy, dysfunction of endothelium and vasculature, and hypercalcemia and activate aldosterone *via* RAAS (13). However, a community-based study in the Netherlands did not confirm PTH to be associated with a risk of developing HF or predicting new onset of HFpEF or HFrEF (30). Subjects with primary hyperparathyroidism and thus elevated concentrations of PTH presented with higher MPV compared to age- and sex-matched healthy controls (17). Higher MPV could suggest the presence of metabolically and enzymatically hyperactive platelets in HF individuals (17). Activated platelets release a plethora of different proinflammatory mediators that promote immune response, angiogenesis, and fibrosis (31, 32). Hypercalcemia can lead to oxidative stress and inflammation in the heart and finally contribute to cardiomyocyte necrosis (8). However, calcium is required as a cofactor in blood coagulation; a lack of calcium can also impair cardiac function and affect HF progression (33, 34). The presence of PTH-related protein and vitamin D receptors on platelets might lead to platelet activation after direct binding or after PTH-initiated increase of vitamin D or PTH-initiated increase of calcium (18, 19). The described pathways of platelet activation can result in a hypercoagulable state, an already recognized risk factor in HF syndrome (35). Vitamin D has been reported to have anti-inflammatory properties, and given the presence of vitamin D receptors in cardiac myocytes, vitamin D supplementation has been suggested as a possible supporting therapy in HF syndrome (36, 37). Indeed, the VINDICATE study showed the beneficial effects of Vitamin D supplementation on cardiac function and LV structure in patients with chronic HF and vitamin D deficiency for a duration of 1 year (38). On the other hand, suppressing PTH by vitamin D intake might present a potential therapeutic target to prevent PTH-driven endothelial dysfunction, atherosclerosis, and platelet activation as leading causes of cardiac ischemia and HF development (4, 39). In absence of robust experimental evidence for the direct interaction between PTH and platelets, it remains to understand if the observed relation depends on other PTH-dependent mechanisms such as plasma and platelet calcium level and vitamin D concentration and its association with platelet activation. Another hypothesis to be tested for the potential

improvement of the clinical outcome of individuals with HF syndrome, based on the present results on the interaction between PTH and platelets, could be the addition of antiplatelet agents in HF patients with higher PTH concentration. To increase the understanding of the interaction between PTH and platelet activation in HF phenotypes, a prospective investigation with specific platelet function tests depicting different aspects of platelet activation, that is, platelet aggregation and platelet procoagulant function, is needed. Furthermore, well-designed randomized controlled trials could importantly inform whether attenuating the levels of PTH intake and/or impeding platelet aggregation and procoagulant function by Vitamin D and antithrombotic agents, respectively, will decrease HF risk or mitigate its progression. Sex-related differences from biological mechanisms to treatment effects and prognosis have been already described in HF patients (40). Our findings for the sex differing association between PTH and platelet indices further support the recommendation to keep the sex-specific focus in future mechanistic, translational, and interventional studies.

CONCLUSION

The results of this analysis report important differences for the association between biomarkers of platelets and PTH that vary between sexes and with the phenotype of cardiac dysfunction. These differences are present independent of vitamin D status, CVRFs, and comorbidities. Particularly in phenotypes of symptomatic HF, distinct associations in males and females were observed, suggesting a sex-specific mechanism involved in the interaction between PTH and platelets. Further mechanistic studies are warranted to understand the effect of PTH at the molecular level of platelets, including the role of endogenous hormones in HF phenotypes.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics committee University Medical Centre Mainz, reference number 837.319.12 (8420-F). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BD, MP-N, and PW designed and performed research and wrote the manuscript. FM, S-OT, and MH contributed to discussion of results and to the critical review of the manuscript. AS performed the statistical analysis. NA, MH, SS-T, JP, TG, HC, KL, and TM contributed in critically reviewing the manuscript. All authors have read, critically reviewed, and approved the manuscript in its current form.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.682521/full#supplementary-material

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplement Material: Sex-specific relationship between parathyroid hormone and platelet indices in phenotypes of heart failure – Results from the MyoVasc study

Link to supporting information of the original publication:

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Part A. Supplemental Methods

Assessment of cardiovascular risk factors (CVRFs) and comorbidities

Cardiovascular risk factors are determined as:

- Diabetes mellitus was defined as HbA1c ≥ 6.5% or blood glucose level ≥ 126 mg/dL at baseline examination after an overnight fast or at least 8 hours or a blood glucose level of ≥ 200 mg/dL at baseline examination after a fasting period > 5 hours or diagnosed diabetes mellitus by a physician or intake of antidiabetic medication;

- Arterial hypertension was defined as blood pressure >140/90 mmHg or diagnose by a physician or intake of antihypertensive medication;

- Smoking was interrogated due to active and passive smoking; regular smoking was defined as smoking one cigarette per day or at least seven cigarettes per week or one package per month or one cigarillo per day or at least seven cigarillos per week or two pipes per day

- Dyslipidemia was defined as low density lipoprotein/ high density lipoprotein > 3.5 and/ or triglycerides level > 150 mg/dL or diagnose by a physician intake of lipid modifying medication;

- Obesity was defined as body-mass index (BMI) ≥ 30.0kg/m² or waist-to-hip-ratio > 0,85 in women and >1 in men;

- Family history of myocardial infarction and stroke was defined as myocardial infarction and/or stroke of male first-degree relatives until the age of 60 years or female first-degree relatives until the age of 65 years.

Comorbidities are self-reported and include cardiovascular diseases (CVD), venous thromboembolism (VTE), chronic obstructive pulmonary disease (COPD), cancer, and arthritis.

Assessment of medical treatment

Calcium and Vitamin D levels are each affected by supplementation and both effect PTH levels; therefore this analysis checked the following medication of the study individuals according to the Anatomical Therapeutic Chemical (ATC) Classification System: Vitamin D supplements (A11CC), calcium supplements (A12A), diuretics (C03), beta-blockers (C07), calcium channel blockers (C08), renin-angiotensin-aldosterone-system antagonists (C09), antiplatelet agents (B01), antilipemic drugs (C10a), anti-inflammatory, and rheumatic drugs (M01A), glucocorticoids (R03BA), corticosteroids (H02), antibacterial drugs (J01), and immunosuppressant drugs (I04A).

In addition, in females the analysis was adjusted for intake of oral contraceptives, hormone replacement therapy, and menstrual bleeding.

Assessment of cardiac structure and function

Resting two-dimensional transthoracic echocardiograms were performed according to recommendations by the American and European Societies of Echocardiography using an iE33 echocardiography system (Philips Medical Systems, Amsterdam, The Netherlands) to provide information on chamber dimensions, wall thickness, and measures of systolic and diastolic function. The mitral inflow velocity pattern was recorded from the apical four-chamber view with the pulsed waved Doppler sample volume positioned at the tips of the mitral valve leaflets during diastole in expiration. Peak early (E-wave) and late (A-wave) diastolic filling velocities were measured and their ratio (E/A) calculated. The lateral mitral annular early diastolic velocity (E') was measured by spectral tissue Doppler imaging and the E/E' ratio determined. Left ventricular ejection fraction (LVEF) was calculated by measurement according to Simpson from the apical four-chamber view. Preserved ejection fraction (PEF) was defined as LVEF≥ 50% and diastolic dysfunction according to one of the following criteria: (E/A< 0.75 and E/E'< 10), (E/A≥ 0.75 and E/E'≥ 10 and DTE ≥ 140ms), or (E/A> 2 and E/E'≥ 10 and DTE< 140ms); reduced ejection fraction (REF) was defined as LVEF≤ 40%. The definition of HF phenotypes was further based on a history of HF within the last 12 months and structural or functional heart disease according to ACCF/AHA guideline for the management of heart failure.2 Patients with a history of HF < 12 months ago or signs and symptoms of HF were classified as ACC/AHA Stage C/D and further categorized by LVEF into HFpEF (LVEF ≥ 50% and diastolic dysfunction), HFpEF borderline (LVEF: 41% to 49%) or HFrEF (LVEF ≤ 40%). Individuals with LVEF ≥ 50% without diastolic dysfunction were categorized as "Stage C/D not classifiable" and excluded for this analysis.

Part B. Supplemental Tables

Table S1. Correlation between PTH and age according to cardiac function in a sex-specific analysis

	PTH [pg/mL] * age [y] Correlation coefficient r
Preserved EF	0.084
- Females	0.052
- Males	0.102
Reduced EF	0.078
- Females	0.054
- Males	0.086
HFpEF	0.088
- Females	-0.032
- Males	0.159
HFpEF borderline	0.109
- Females	0.051
- Males	0.133
HFrEF	0.057
- Females	0.112
- Males	0.054

Pearson's product-moment correlation analysis presenting the correlation between PTH [pg/mL] and age [y] in males and females according to HF phenotypes. Abbreviations: PTH: parathyroid hormone; y: years; EF: ejection fraction; HFpEF: heart failure with preserved ejection fraction; HFpEF borderline: heart failure with ejection fraction of 41-49%; HFrEF: heart failure with reduced ejection fraction.

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Project: Platelet-related protein signature differs in heart failure phenotypes – results from the MyoVasc study

Results have not been published yet.

Platelet-related protein signature differs in heart failure phenotypes– results from the MyoVasc study

Abstract

Aims: This study aims to characterize platelet-related protein signatures in heart failure (HF) phenotypes (HF with preserved ejection fraction [HFpEF], HF with borderline ejection fraction [HFpEF borderline], and HF with reduced ejection fraction [HFrEF]). Furthermore, this study aims to assess HF phenotype-specific platelet-related protein scores with the clinical outcome worsening of HF, a composite of cardiac death and HF hospitalization.

Methods and results: From 178 unique proteins, analyzed with the proximity extension assay (PEA) technology in 3,289 individuals from the MyoVasc study, 39 were identified as platelet-related by literature research. In multivariable logistic regression models, adjusted for age, sex, antithrombotic agents, cardiovascular risk factors (CVRFs), and comorbidities, a different set of the 39 platelet-related proteins were associated with each HF phenotype.
Cox-regressions were used to analyze the identified platelet-related protein signatures from each HF phenotype for prediction of worsening of HF, independent of age, sex, and antithrombotic medication. Platelet-related protein scores for worsening of HF resulted with a hazard ratio (HR) of 1.94 (95% confidence intervals [CI]: 1.27; 1.78) in HFpEF, HR: 1.28 (95% CI: 1.04; 1.54) in HFpEF borderline; and in HFrEF a HR of 1.51 (95% CI: 1.27; 1.78) was assessed. Further adjustment for CVRFs and comorbidities did not substantially change the hazard ratios, but lowered the association between the HFpEF borderline protein score and outcome (HR: 1.24 [95% CI: 0.97; 1.57]).

Conclusion: HF phenotypes presented with distinct platelet-related protein signatures. Platelet-related protein scores predicted worsening of HF for each HF phenotype, independent of potential confounders. Circulating platelet-related proteins are promising biomarkers for an individualized risk profiling of patients suffering from HF syndrome.

Keywords: Heart failure, Platelets, Platelet activation, Proteins, Worsening of HF

One-sentence summary: This work showed for the first time distinct platelet-related protein signatures for HF phenotypes that were additionally related to worse clinical outcome.

Introduction

Activated platelets play an important role in the pathogenesis of cardiovascular diseases (CVDs).[27] Higher levels of mean platelet volume (MPV), a potential marker of platelet activation, and increased platelet aggregation have been reported in several CVDs, such as heart failure (HF), myocardial infarction (MI), and coronary artery disease (CAD).[27, 28] Activated platelets release plethora of proinflammatory factors implicated from atheromatosis, leukocyte adhesion, and accumulation to endothelial cell activation.[29] HF is a common CVD, associated with platelet activation and endothelial dysfunction, predisposing to a hypercoagulable state.[16]

Increased inflammation and immune cell activation further characterize HF syndrome.[6, 30] Higher levels of different inflammatory cytokines and chemokines have been correlated to incident HF and several of these proteins were associated with worsening of HF echocardiographic parameters such as lower left ventricular ejection fraction (LVEF) and worsened diastolic function.[31]

It has been well established that HF with preserved ejection fraction (HFpEF) and HF with reduced ejection fraction (HFrEF) presented with different characteristics beyond the ejection fraction distinction.[32] HFpEF is more prevalent in females and in individuals with cardiovascular risk factors (CVRFs) such as arterial hypertension and atrial fibrillation (AF), whereas HFrEF affects predominantly males with a history of ischemic heart disease, such as MI, stroke or CAD.[32, 33] Amongst others, these observations led to the assumption that different molecular mechanisms underlie the HF phenotypes. Distinctions in the inflammation protein profiles between HF phenotypes have not been extensively investigated yet. Moreover, platelets store a range of different molecules in the α-granules, dense granules, and lysosomes, released upon their activation.[34] The large diversity of these molecules contribute to the multifaceted role of platelets in physiological and pathophysiological conditions that until now have not been comprehensively addressed in HF syndrome. Targeted high-throughput proteomics assay, an emerging biomarker discovery technology, was used to characterize platelet-related protein signatures in HF phenotypes and to assess the link to worsening of HF.

Methods

Study participants

The MyoVasc study, a prospective cohort study at the University Medical Center Mainz (UMCM), Germany, included 3,289 participants with cardiac disorders, age range 35 to 84 years, enrolled from January 2013 to April 2018. The main aim of the MyoVasc study is to assess the development and progression of HF.[12] All participants underwent a standardized and thorough clinical and laboratory examination of 5 hours, including echocardiographic HF phenotyping and assessment of CVRFs, comorbidities, and treatment history as described in the **Supplemental Material Part A**. Standard laboratory parameters were assessed and samples of biomaterial, e.g. blood and urine, were collected for biobanking at the study visit for future analysis.

Prospective data on total and cause-specific mortality and incident cardiovascular events during follow-up were also collected and available for analysis.

Written informed consent was obtained from all study participants prior to entering the study. The study complies with the principles outlined in the Declaration of Helsinki, Good Clinical Practice and Good Epidemiological Practice. An approval from the responsible ethics committee (reference number 837.319.12 (8420-F)) and data safety commissioner was obtained in 2012, before study initiation. The MyoVasc study is registered at http://clinicaltrials.gov (identifier: NCT04064450).

Definition of HF phenotypes

The definition of HF phenotypes was based on a history of HF within the last 12 months and presence of structural or functional heart disease as defined by the American College of Cardiology Foundation/ American Heart Association (ACCF/AHA) guideline for the management of HF.[2] Individuals with neither a history of HF, HF treatment nor at risk for HF were grouped as stage 0. Individuals at risk for HF, who suffer from at least one CVRF, described in the **Supplemental Material Part A**, were clustered as stage A. Stage B was defined as having structural or functional heart disease but no signs or symptoms of HF (detailed description in **Supplemental Material Part A**). Patients with a diagnosed HF and treatment within the past 12 months or signs and symptoms of HF were classified as stage C/D and further categorized by LVEF into HFpEF (LVEF ≥ 50% and diastolic dysfunction), HFpEF borderline (LVEF: 41% to 49%) or HFrEF (LVEF ≤ 40%). In addition, individuals with LVEF ≥ 50% without diastolic dysfunction were categorized as “stage C/D not classifiable”.

Individuals with stage B (N= 759) and stage C/D not classifiable (N= 351) were excluded for further analyses (**Supplemental Figure S1**).

The primary outcome for individuals with confirmed HF is worsening of HF defined as the composite of cardiac death and hospitalization due to HF.

Blood withdrawal and laboratory assessment

Blood was taken from study participants after an overnight fasting period of at least eight hours. Fresh ethylenediaminetetraacetic acid (EDTA)-treated blood was used to measure routine parameters including MPV (femtoliter, fL) and platelet count (10^9/L) on an ADVIA 120 Hematology System (Siemens, Erlangen, Germany) within 30 to 90 minutes after blood withdrawal in the central laboratory of UMCM. Additional sample of EDTA blood was processed according to standard operating procedures (SOPs) and the isolated plasma stored at -80°C in the Biomaterial Bank Mainz, UMCM, until further measurements. Protein analysis was performed in plasma samples from 3,289 individuals at risk of or with confirmed HF from the MyoVasc study on the targeted proteomics platform of the Biomolecular laboratory of the Clinical Epidemiology and Systems Medicine department at the UMCM.

Relative protein quantification of 178 unique plasma proteins from the inflammation and cardiovascular (CVD III) panels have been determined by the proximity extension assay (PEA) technology (Olink Proteomics, Uppsala, Sweden). The 96-plex immunoassay ran a real-time quantitative polymerase-chain-reaction (PCR)-based signal amplification to detect the antigen molecules. Results were $\log_2$-transformed to normalized protein expression (NPX) units using the Olink NPX manager software supplied by the manufacturer.

Preselection of platelet-related proteins

Platelet-related proteins were identified from a total of 178 unique proteins assessed in the MyoVasc cohort as depicted in **supplemental Figure S2**. Firstly, the Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology (GO), and Reactome databases were searched using the search term “platelet activation”. The search matched nine proteins as follows: glycoprotein VI (GP VI), von Willebrand factor (vWF), CD40, hepatocyte growth factor (HGF), plasminogen activator inhibitor 1 (PAI-1), platelet endothelial cell adhesion molecule (PECAM-1), P-Selectin, transforming growth factor beta-1 (TGF-β1), and vascular endothelial growth factor A (VEGF-A). Secondly, each of the 178 proteins was individually searched in PubMed (https://pubmed.ncbi.nlm.nih.gov/) in conjunction with the search term “platelet activation”, resulting in additional 30 unique proteins. Proteins, such as tyrosine-protein kinase UFO

(AXL)[35] or C-X-C motif chemokine 16 (CXCL16)[36], were not considered for the analysis as they are only engaged in platelet activation, but were not released by platelets. In addition, proteins without supporting literature for the relation to human platelets were excluded. Both search steps identified a total of 39 proteins (**Supplemental Figure S2**) referred further in the text as "platelet-related proteins". The respective references for each of the selected platelet-related proteins were reported in the **Supplemental Table S1. Figure 1** summarizes the selected proteins storage and expression upon platelet activation.

Data management and statistical analysis

Central data management unit was responsible for collection, plausibility checks for completeness and correctness of collected data of all participants. Baseline characteristics were presented according to HF phenotypes including a control group of individuals with stages 0 and A. Normally distributed values were described by using mean ± standard deviation. Non-normally distributed variables were presented with median and interquartile range. Associations between platelet-related proteins and HF phenotypes were assessed by multivariable logistic regression models, adjusted for age, sex, and antithrombotic agents (anatomical therapeutical chemical [ATC] code: B01) and additionally adjusted for CVRFs and comorbidities. CVRFs and comorbidities were further described in **Supplemental Material Part A**.

STRING database (https://string-db.org/) was used to assess known and predicted protein-protein interactions (PPIs) including analysis on underlying protein pathways of the proteins associated with the HF phenotypes. An interaction score of 0.7 ("high confidence") for information on experiments, databases, co-expression, neighborhood, and gene fusion was applied in the network analysis.

Outcome analyses on worsening of HF were performed for platelet-related protein scores within each HF phenotype with Cox regression models and cumulative incidence plots according to score tertiles depicting the cumulative incidence for worsening of HF. Protein scores were calculated by a linear combination of proteins, weighted for their association with HF phenotypes, for each HF phenotype. Weights equal the coefficients in projected models adjusted for each selected protein singularly.

For this explorative analysis, p-values should be interpreted as continuous measures of statistical evidence. Statistical analysis was performed using R, version 4.0.3 (2020-10-10), R Foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org.

Results

Baseline characteristics of the study participants

Baseline characteristics of the study participants are presented in **Table 1.** HF subjects' characteristics are reported according to HF phenotype (HFpEF, HFpEF borderline and HFrEF). Control subjects (N= 789; 58.5 ± 11.5 years; 48.5% women) comprised individuals with ACCF/AHA stages 0 and A. Subjects with HFpEF (N= 646) were older (70.7 ± 8.2 years) and more often female (48.3%) compared to HFpEF borderline (N= 401; 66.5 ± 10.5 years; 25.2% women) and HFrEF individuals (N= 343; 66.3 ± 10.5 years; 14.9% women). HFpEF individuals presented with highest frequencies of arterial hypertension and obesity and lowest frequencies of smokers and subjects with dyslipidemia. CAD and AF were also less frequent in HFpEF compared to individuals with HFpEF borderline and HFrEF. Diabetes mellitus, MI, COPD, and CKD were more prevalent among HFrEF individuals compared to other HF phenotypes. Controls presented with a lower cardiovascular risk profile compared to HF subjects. However, the percentages of smokers and positive family history of MI/ stroke were similar to HF phenotypes.

MPV and N-terminal pro-B-type natriuretic peptide (NT-proBNP) increased gradually from controls, HFpEF to HFpEF borderline and presented with highest value in HFrEF individuals, whereas platelet count and estimated glomerular filtration rate (eGFR) were lowest among HFrEF individuals and highest among controls.

More than three quarters of HF subjects took an antithrombotic medication (B01, comprising antiplatelet and anticoagulant agents). The number of individuals on cardiovascular treatment with diuretics (C03), beta-blockers (C07), and renin-angiotensin-aldosterone system (RAAS) inhibitors (C09) increased from HFpEF to HFpEF borderline and HFrEF. Differently, the highest frequencies for intake of antihypertensives (C02) and calcium channel blockers (C08) as well as the lowest frequency of lipid modifying agents (C10) intake were among HFpEF individuals. Less than 40% of control subjects reported taking antithrombotic and cardiovascular medication.

Association between platelet-related proteins and HF phenotypes

A multivariable logistic regression analysis, accounting for large range of potential confounders, was applied to investigate the relationships between platelet-related proteins and HF phenotypes. **Table 2** presents the five proteins relevantly associated with the HFpEF phenotype after adjustment for age, sex, and antithrombotic agents compared to the control

group. Caspase-3 (CASP-3), HGF, and interleukin-8 (IL-8) were upregulated in HFpEF compared to controls, whereas C-X-C motif chemokine 5 (CXCL5) and GP VI were downregulated in HFpEF individuals. The associations remained after further adjustments for CVRFs and comorbidities. CASP-3, CXCL5, and GP VI were uniquely associated with HFpEF phenotype whereas HGF was additionally associated with HFrEF phenotype and IL-8 was additionally associated with HFpEF borderline phenotype.

Seven proteins were associated with HFpEF borderline phenotype independent of age, sex, CVRFs, and comorbidities (**Table 3**). IL-8, TIMP-4, vWF, and intercellular adhesion molecule 2 (ICAM-2) showed higher expression in HFpEF borderline, whereas cathepsin D (CTSD), matrix metalloproteinase 3 (MMP-3), and interleukin-18 binding protein (IL-18BP) were lower expressed in HFpEF borderline phenotype compared to control individuals. CTSD, ICAM-2, MMP-3, and vWF were associated with HFpEF borderline only whereas IL-18BP and TIMP-4 were additionally associated with HFrEF phenotype. From the seven proteins associated with the HFrEF phenotype in the fully adjusted model, six proteins (VEGF-A, CCL3, HGF, TIMP-4, MMP-9, and tissue factor pathway inhibitor [TFPI]) were upregulated, whereas only IL-18BP was downregulated when compared to control subjects (**Table 4**). CCL3, MMP-9, TFPI, and VEGF-A were uniquely associated with HFrEF phenotype. **Figure 2** further illustrates the shared proteins between HF phenotypes and proteins uniquely associated with each HF phenotype as Venn diagram.

Correlation and network analysis of platelet-related proteins

To consider PPIs and to rule out the omission of highly expressed proteins due to the preferential inclusion of other highly correlated proteins, a correlation heatmap of platelet-related proteins (N= 39) has been developed as depicted in **Supplemental Figure S3**. The highest positive protein-protein correlation was observed for CASP-3 and JAM-A (r= 0.86) whereas the strongest negative correlation was found between EGFR and HGF (r= -0.17). The high correlation between these proteins were not of statistical relevance as the variance inflation factor, a marker of the protein´s interaction potential, was below 10, the critical cut-off for relevant interactions (data not shown).

The STRING network analysis was implemented to elucidate known and predicted PPIs within the protein signatures, associated with each HF phenotype independent of potential confounders (**Figure S4**). Among the five platelet-related proteins associated with HFpEF phenotype a PPI was observed between IL-8 and CXCL5. The PPI analysis for proteins associated with HFpEF borderline phenotype showed no interaction. Differently, the proteins

associated with HFrEF phenotype resulted in two PPIs belonging to the cytokine-mediated signaling pathway: HGF - VEGF-A and VEGF-A – MMP-9.

Association of platelet-related protein scores and clinical outcome in HF phenotypes

The relationship of the identified platelet-related proteins and the clinical outcome was assessed by calculating an aggregate protein score specific for each HF phenotype. **Figures 3A-3C** present the cumulative incidence for worsening of HF for each HF phenotype according to score tertiles. The Cox regression models, adjusted for age, sex, and antithrombotic agents, confirmed that the calculated platelet-related protein scores predicted an increased risk for worsening of HF of more than 50% in both, HFpEF and HFrEF phenotypes. Based on the platelet-related protein score, the risk for worsening of HF was highest for the HFpEF phenotype particularly in the fully adjusted model with hazard ratio (HR): 2.19 (95% confidence interval: 1.76; 2.72), as presented in **Table 5**. For the HFrEF phenotype, the risk for worsening of HF slightly decreased after adjustment for all known confounders, e.g. $HR_{\text{(adjusted for age, sex, and antithrombotic medication)}}$: 1.51 (1.27; 1.78) vs $HR_{\text{(additionally adjusted for CVRFs, and comorbidities)}}$: 1.46 (1.21; 1.77). Differently, in HFpEF borderline the risk for worsening of HF attenuated after adjustments for CVRFs and comorbidities.

Discussion

The present study showed distinct platelet-related protein signatures in HF phenotypes. Platelet-related protein signatures were identified independent of clinical characteristics known to associate with platelet activation, such as age, sex, traditional CVRFs, comorbidities, and therapy. Moreover, a relation to clinical outcome was investigated, demonstrating highly relevant predictive power of the platelet-related protein signatures for worsening of HF.

Out of 178 unique proteins belonging to the inflammation and CVD III OLINK protein panels, 39 proteins were identified as platelet-related proteins, after performing a systematic analysis supported by literature. The platelet-related proteins were then tested for an association with each HF phenotype, controlling for potential confounders. The analysis resulted in three different unique protein signatures distinct for each HF phenotype. The findings indicated that platelet-related protein signatures differentiate between HF phenotypes, alluding potentially to different platelet-related mechanisms in the pathogenesis of HF phenotypes. HF phenotype-specific protein signatures remained consistent after adjustment for the subjects' clinical characteristics, demonstrating that the relations were not simply based on differences in the clinical profile between HF subgroups.

HFpEF phenotype has been characterized by higher expression of CASP-3, HGF, and IL-8 and lower expression of CXCL5 and GP VI. High levels of HGF have been found in HF individuals in response to cardiac damage and were correlated with severity of HF.[37] Animal studies demonstrated that HGF administration improves cardiac remodeling and dysfunction, presumably as a result of HGF angiogenic and anti-apoptotic mechanisms.[38] Interestingly, HGF was the only protein shared between HFpEF and HFrEF phenotype, indicating the presence of cardiac damage and activation of the cardiac protective mechanisms in both phenotypes. Conversely to HGF function, HFpEF was also characterized by higher expression of CASP-3, a recognized apoptotic protein implicated in cardiomyocyte progressive loss of contractile function in HF syndrome.[39, 40].

Higher expression of IL-8, as observed in HFpEF and HFpEF borderline phenotype, has been reported as one of the key chemokines increasing gradually with HF severity. Stimulation of mononuclear cells' release of IL-8 by activated platelets has been already demonstrated in patients with congestive HF.[30] Although proteins associated with HFpEF related to apoptosis and cardiac damage, platelet-related proteins associated with HFpEF borderline phenotype were rather involved in inflammatory activation, via increased expression of IL-8, vWF, and ICAM-2.[41-43]

Proteins related to extracellular matrix remodeling and atherosclerosis pathways were shared between HFpEF borderline and HFrEF.[44] TIMP-4 was upregulated, whereas IL-18BP was downregulated in both phenotypes.

Biomarkers of cardiac extracellular matrix turnover such as TIMP-4 and MMP-3 have been associated with fibrosis, diastolic dysfunction, and left ventricular hypertrophy.[45] The Bio-SHiFT study of chronic HF patients showed that higher levels of TIMP-4 were associated with the primary study end point, a composite of cardiac death, heart transplantation, left ventricular assist device implantation and hospitalization for the management of acute or worsened HF, independent of the cardiac biomarkers NT-proBNP and highly sensitive cardiac troponin T (hsTnT).[46]

Proteins positively regulating macrophage activation and angiogenesis mainly characterized HFrEF phenotype. Besides VEGF-A, CCL3 showed the strongest positive association with HFrEF phenotype independent of all potential confounders. CCL3 could augment MMP-9 expression in leukocytes and enhance HGF expression in fibroblasts, as recently reported in an animal model.[47] Indeed, another study demonstrated a role for fibroblasts in cardiac injury and cardiac remodeling of the infarcted heart.[48] Additionally, higher MMP-9 levels have been shown to also being related to worse outcome in chronic HF patients in the Bio-SHiFT study.[46] The network analysis of platelet-related proteins found a high confidence PPI-pathway along HGF - VEGF-A – MMP-9 in HFrEF phenotype. It has already been reported that these proteins were related to angiogenesis and have a role in fibrotic remodeling after MI, one of the main risk factors for HF development.[48] In patients with acute MI, several proteins were differentially regulated in coronary arterial endothelial cells compared to controls.[49] Only vWF and ICAM-2 matched our findings with an upregulation of vWF, but a downregulation of ICAM-2 in acute MI patients. However in our study, both proteins were found to be upregulated in HFpEF borderline independent of the cardiovascular risk profile. Nevertheless, a close link between MI and HF supports the conception that post MI subjects might exhibit a protein signature similar to HF subjects.

Additionally, this study comprised an important and potentially clinical relevant finding by demonstrating that platelet-related protein scores can predict worsening of HF. Platelet-related proteins provided additional relevant information about the severity and progression of HF. Inconsistent results were found regarding outcome incidence in HFpEF compared to HFrEF. HFpEF individuals were older and of higher risk for non-cardiovascular outcome compared to worse cardiovascular outcome in HFrEF individuals.[33] In accordance, platelet-related protein scores differed between HF phenotypes but predicted worse clinical outcome in each phenotype. An increased risk for worsening of HF of approximately 50% in HFrEF individuals and an even doubled risk in HFpEF characterized the impact of the platelet-related protein

scores. Just in HFpEF borderline the platelet-related protein score showed only a trend for worse outcome, predominantly driven by underlying CVRFs and comorbidities.

Strengths and limitation

Overall, the platelet-related protein signature may characterize each HF phenotype beyond LVEF. In addition to the clinical characteristics, distinct differences in the platelet-related protein signature were observed between HFpEF, HFpEF borderline, and HFrEF phenotypes, without proteins shared between all three phenotypes. Further, this work reported relationships between proteins that have not been previously reported in the literature in HF settings. For instance, this study found a high correlation between CASP-3 and JAM-A, not previously described in the context of platelets and HF. CASP-3 has a role in phosphatidylserine exposure and microparticle release from activated platelets[39], whereas JAM-A can be found on platelet surface and functions as a negative regulator of platelet activation.[50] This study showed that JAM-A was lower expressed in HF individuals compared to controls, indicating a role for promotion of activated platelets in HF.

In addition, the selected proteins, combined as a platelet-related protein score, predicted clinical outcome within each HF phenotype.

However, some limitations should be mentioned. By using only the inflammation and CVD III panels of PEA-based 96-plex immunoassays, all measured proteins were largely related to inflammation and less to platelets. Therefore, the results might be biased to an increased contribution of inflammation-related proteins to the HF phenotype characteristics. All 39 selected proteins were measured in EDTA plasma, so proteins that were not detectable in EDTA plasma could not be quantified. In addition, due to the freeze- and thaw-process in the sample preparation, various intact cells could have been destroyed. Therefore, the contribution of proteins and microparticles from other cells than platelets cannot be excluded. Monocytes, macrophages, and releasates from vascular endothelial cells, smooth muscle cells, and cardiomyocytes could also increase levels of circulating chemokines.[6, 30]

Further investigations on outcome are necessary in HFpEF borderline individuals as the platelet-related protein score showed only a trend for worsening of HF that, however, was more related to the underlying cardiovascular risk profile of the subjects.

Conclusion

This study demonstrated distinct platelet-related protein signatures associated with HF phenotypes. A network analysis of the three identified protein signatures did not exhibit shared PPI pathways, indicating that distinct mechanisms are involved in each HF phenotype.

Important relations of the platelet-related protein signatures with worsening of HF suggest that markers of platelet activation could be used for individual risk profiling. Finally, it remains to confirm these associations and to elucidate if a combination of platelet-related protein signatures with standard prognostic risk scores improves risk prediction for adverse outcome in HF syndrome.

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Tables

Table 1. Baseline characteristics according to cardiac function phenotype

	HFpEF	HFpEF borderline	HFrEF	Controls
Number of individuals	646	401	343	789
Age [y]	70.7 ± 8.2	66.5 ± 10.5	66.3 ± 10.5	58.5 ± 11.5
Sex (women)	48.3% (312)	25.2% (101)	14.9% (51)	48.5% (383)
CVRFs				
Arterial hypertension	86.8% (561)	78.8% (316)	75.5% (259)	51.1% (403)
Diabetes mellitus	30.3% (196)	29.4% (118)	34.7% (119)	9.1% (72)
Smoking	10.2% (66)	16.5% (66)	17.5% (60)	12.6% (99)
Obesity	40.2% (260)	38.2% (153)	34.1% (117)	22.1% (174)
Dyslipidemia	75.5% (488)	84.3% (338)	84.0% (288)	46.6% (368)
Familiy history of MI/ stroke	22.3% (144)	26.4% (106)	27.5% (94)	21.4% (169)
Comorbidities				
Coronary artery disease	44.0% (284)	57.6% (231)	58.0% (199)	11.3% (89)
History of MI	24.6% (159)	36.9% (148)	43.1% (148)	0% (0)
History of stroke	11.1% (72)	11.0% (44)	11.4% (39)	4.6% (36)

Atrial fibrilation	33.6% (217)	38.2% (153)	42.3% (145)	10.1% (80)
Peripheral artery disease	9.6% (62)	10.5% (42)	12.5% (43)	1.9% (15)
Chronic obstructive pulmonary disease	15.2% (98)	13.0% (52)	18.4% (63)	11.5% (91)
Deep vein thrombosis	11.0% (71)	9.0% (36)	7.3% (25)	5.3% (42)
Pulmonary embolism	5.9% (38)	3.7% (15)	2.9% (10)	3.0% (24)
History of cancer	21.8% (141)	20.2% (81)	16.9% (58)	12.9% (102)
Chronic kidney disease	22.9% (148)	18.5% (74)	30.9% (106)	11.7% (92)
Chronic liver disease	11.0% (71)	9.2% (37)	8.5% (29)	6.7% (53)
Echocardiographic parameter				
LVEF [%]	58.6 ± 5.6	45.2 ± 2.8	31.5 ± 6.1	62.2 ± 5.0
E/E´	11.13 (8.61/13.92)	9.11 (7.05/12.50)	12.39 (8.31/18.09)	6.78 (5.68/8.22)
Lab parameter				
MPV [fL]	8.28 ± 0.85	8.32± 0.87	8.44 ± 1.00	8.22 ± 0.84
Platelet count [10^9/L]	230.5 ± 70.4	218.2 ± 62.7	209.7 ± 57.1	234.9 ± 58.8
NT-proBNP [pg/mL]	290.5(150.4/688.3)	445.0 (200.0/1031.2)	1219.0 (542.2/2643.1)	74.5 (42.0/136.0)
eGFR [ml/min/1.73 m^2]	70.46 ± 19.06	74.26 ± 20.20	64.93 ± 23.23	86.92 ± 15.55
Medication				
Antithrombotic agents (B01)	77.4% (500)	85.5% (343)	86.3% (296)	28.1% (222)
Antihypertensives (C02)	6.2% (40)	2.5% (10)	1.5% (5)	1.9% (15)

Diuretics (C03)	39.8% (257)	58.1% (233)	85.7% (294)	8.1% (64)
Beta-blockers (C07)	70.1% (453)	77.3% (310)	84.0% (288)	23.2% (183)
Calcium channel blockers (C08)	30.3% (196)	18.7% (75)	8.5% (29)	9.0% (71)
RAAS inhibitors (C09)	79.1% (511)	83.0% (333)	83.7% (287)	36.4% (287)
Lipid modifying agents (C10)	54.2% (350)	63.6% (255)	59.2% (203)	20.9% (165)

Presented are baseline clinical characteristics, echocardiographic, and laboratory parameters, including intake of antithrombotic and cardiovascular medication by HF phenotypes in 646 HFpEF, 401 HFpEF borderline, and 343 HFrEF individuals as well as in 789 controls (stages 0 and A). Abbreviations: HFpEF: heart failure with preserved ejection fraction (EF≥ 50%); HFpEF borderline: heart failure withejection fraction of 41-49%; HFrEF: heart failure with reduced ejection fraction (EF≤ 40%); CVRFs: cardiovascular risk factors; MI: myocardial infarction; LVEF: left ventricular ejection fraction; MPV: mean platelet volume; NT-proBNP: N-terminal pro-B-type natriuretic peptide; eGFR: estimated glomerular filtration rate; RAAS: renin-angiotensin-aldosterone system

Table 2. Association between platelet-related proteins and HFpEF phenotype

Platelet-related proteins	adjusted for age, sex, and antithrombotic agents		additionally adjusted for CVRFs and comorbidities		
	OR (95% CI)	p-value	OR (95% CI)	p-value	
Caspase-3	1.595 (1.122; 2.267)	**0.0092**	1.629 (1.137; 2.334)	**0.0075**	upregulated in HFpEF
HGF	1.453 (1.137; 1.860)	**0.0029**	1.391 (1.075; 1.802)	**0.012**	
Interleukin-8	1.309 (1.074; 1.601)	**0.0081**	1.259 (1.021; 1.556)	**0.032**	
CXCL5	0.731 (0.573; 0.929)	**0.011**	0.733 (0.569; 0.942)	**0.015**	downregulated in HFpEF
GP VI	0.708 (0.534; 0.936)	**0.016**	0.655 (0.488; 0.876)	**0.0045**	

Multivariable logistic regression models for HFpEF phenotype (N= 621) vs. controls (N= 768) as dependent variable and associated platelet-related proteins as independent variables. Results are presented as odds ratios (OR) with 95% confidence intervals (CI) for change in NPX with standard deviation for each protein. CVRFs: Cardiovascular risk factors; HFpEF: heart failure with preserved ejection fraction; HGF: Hepatocyte growth factor; CXCL5: C-X-C motif chemokine 5; GP VI: Platelet glycoprotein VI

Table 3. Association between platelet-related proteins and HFpEF borderline phenotype

Platelet-related protein	adjusted for age, sex, and antithrombotic agents		additionally adjusted for CVRFs and comorbidities		
	OR (95% CI)	p-value	OR (95% CI)	p-value	
Interleukin-8	1.276 (1.010; 1.612)	**0.041**	1.303 (1.022; 1.664)	**0.033**	upregulated in HFpEF borderline
TIMP-4	1.240 (1.000; 1.544)	0.052	1.266 (1.012; 1.590)	**0.040**	
vWF	1.188 (0.983; 1.440)	0.69	1.219 (1.002; 1.489)	**0.049**	
ICAM-2	1.181 (0.962; 1.454)	0.11	1.292 (1.035; 1.617)	**0.024**	
CTSD	0.886 (0.716; 1.097)	0.27	0.801 (0.642; 0.997)	**0.048**	downregulated in HFpEF borderline
MMP-3	0.721 (0.580; 0.894)	**0.0030**	0.719 (0.570; 0.902)	**0.0048**	
IL-18BP	0.742 (0.560; 0.980)	**0.037**	0.685 (0.507; 0.922)	**0.013**	

Multivariable logistic regression models for HFpEF borderline phenotype (N= 388) vs. controls (N= 768) as dependent variable and associated platelet-related proteins as independent variables. Results are presented as odds ratios (OR) with 95% confidence intervals (CI) for change in NPX with standard deviation for each protein.

CVRFs: Cardiovascular risk factors; HFpEF borderline: heart failure with ejection fraction of 41% to 49%; vWF: von Willebrand factor; TIMP-4: Metalloproteinase inhibitor 4; ICAM-2: Intercellular adhesion molecule 2; CTSD: Cathepsin D; MMP-3: matrix metalloproteinase 3; IL-18BP: Interleukin-18 binding protein

Table 4. Association between platelet-related proteins and HFrEF phenotype

Platelet-related proteins	adjusted for age, sex, and antithrombotic agents		additionally adjusted for CVRFs and comorbidities		
	OR (95% CI)	p-value	OR (95% CI)	p-value	
VEGF-A	2.036 (1.398; 2.994)	0.00025	1.890 (1.275; 2.824)	0.0017	upregulated in HFrEF
CCL3	1.538 (1.188; 1.993)	0.0011	1.575 (1.195; 2.077)	0.0012	
HGF	1.465 (1.049; 2.057)	0.026	1.452 (1.021; 2.073)	0.039	
TIMP-4	1.371 (1.065; 1.774)	0.015	1.500 (1.152; 1.965)	0.0029	
MMP-9	1.349 (1.086; 1.684)	0.0073	1.460 (1.158; 1.852)	0.0016	
TFPI	1.307 (1.048; 1.634)	0.018	1.278 (1.007; 1.626)	0.045	
vWF	1.257 (1.011; 1.569)	0.041	1.222 (0.973; 1.540)	0.087	
MMP-3	0.757 (0.588; 0.972)	0.030	0.765 (0.582; 1.001)	0.053	downregulated in HFrEF
IL-18BP	0.567 (0.400; 0.799)	0.0013	0.554 (0.384; 0.796)	0.0015	

Multivariable logistic regression models for HFrEF phenotype (N= 331) vs. controls (N= 768) as dependent variable and associated platelet-related proteins as independent variables. Results are presented as odds ratios (OR) with 95% confidence intervals (CI) for change in NPX with standard deviation for each protein. CVRFs: Cardiovascular risk factors; HFrEF: heart failure with reduced ejection fraction; VEGF-A: Vascular endothelial growth factor A; CCL3: C-C motif chemokine 3; HGF: Hepatocyte growth factor; TIMP-4: Metalloproteinase inhibitor 4; MMP-9: Matrix metalloproteinase 9; TFPI: Tissue factor pathway inhibitor; vWF: von Willebrand factor; MMP-3: Matrix metalloproteinase 3; IL-18BP: Interleukin-18 binding protein

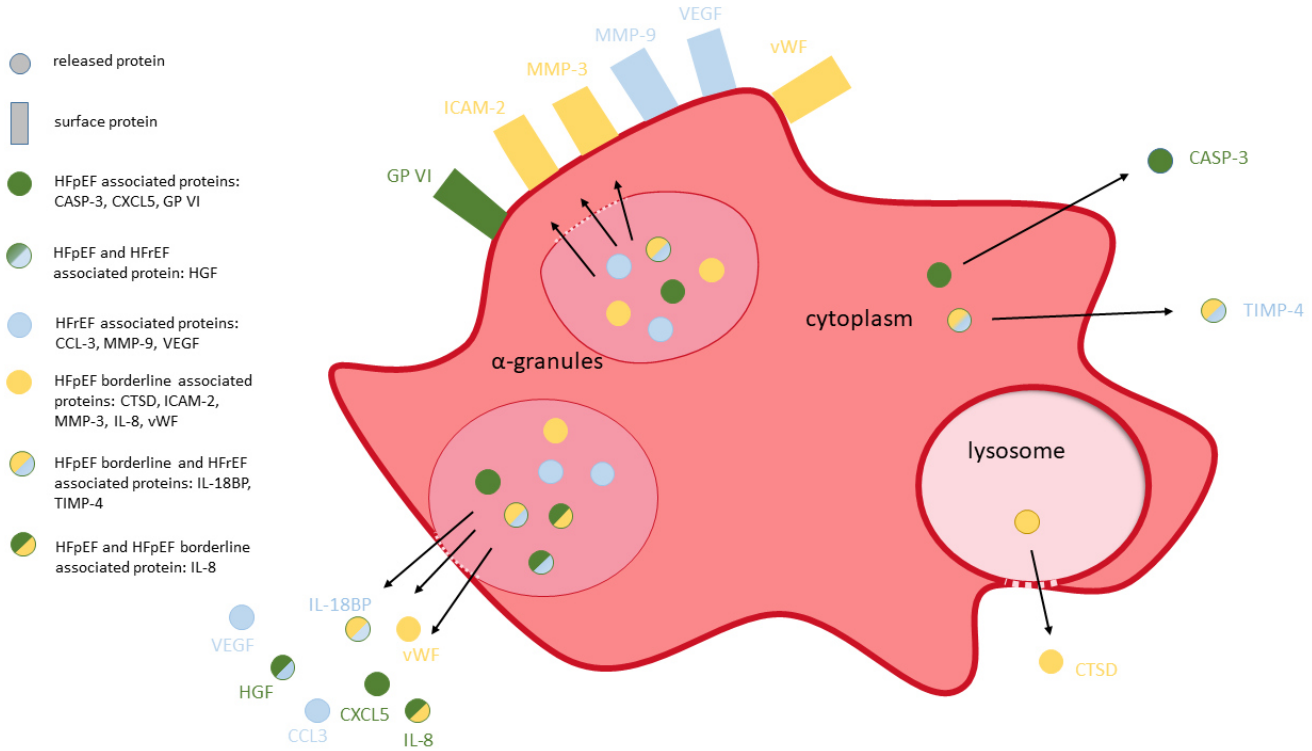
Table 5. Association between protein score and worsening of HF in HF phenotypes

Worsening of HF	adjusted for age, sex, and antithrombotic agents		additionally adjusted for CVRFs and comorbidites	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Platelet-related protein score HFpEF	1.94 (1.61; 2.33)	<0.0001	2.19 (1.76; 2.72)	<0.0001
Platelet-related protein score HFpEF borderline	1.28 (1.04; 1.58)	0.022	1.24 (0.97; 1.57)	0.081
Platelet-related protein score HFrEF	1.51 (1.27; 1.78)	<0.0001	1.46 (1.21; 1.77)	<0.0001

Cox regression analyses for the assiciation between worsening of HF and platelet-related proteins in HFpEF phenotype (N= 621, 89 events), HFpEF borderline (N= 388, 87 events), and HFrEF (N= 331; 153 events) individuals. Results are hazard ratios (HR) with 95% confidence intervals (CI) for change. CVRFs: Cardiovascular risk factors; HFpEF: heart failure with preserved ejection fraction; HFpEF borderline: heart failure with ejection fraction of 41% to 49%; HFrEF: heart failure with reduced ejection fraction

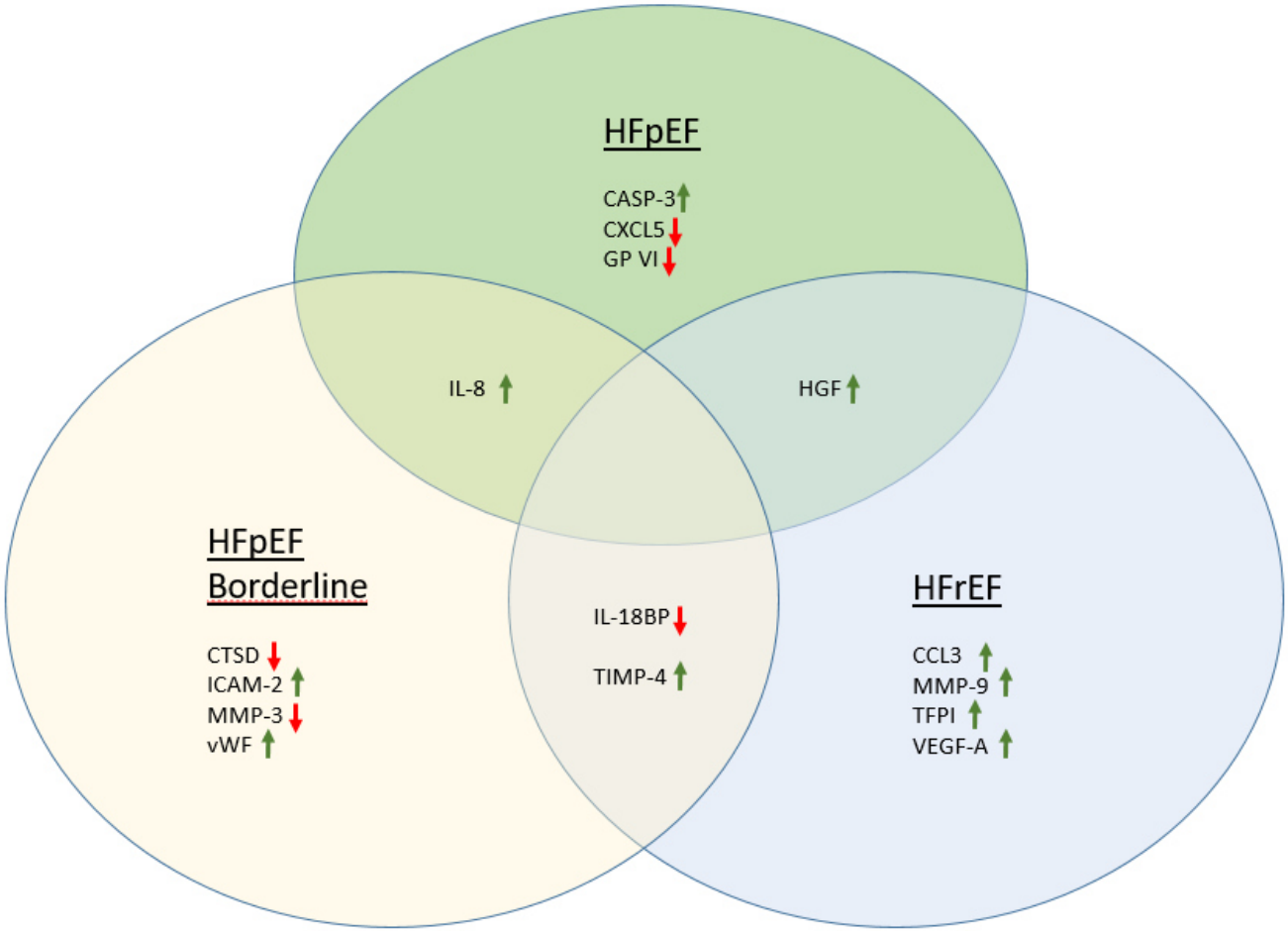
Figures

Figure 1.



Scheme of an activated platelet releasing platelet-related proteins that are associated with HF phenotypes, according to their colors. Surface proteins might be shed. Proteins are listed alphabetically.

Figure 2.



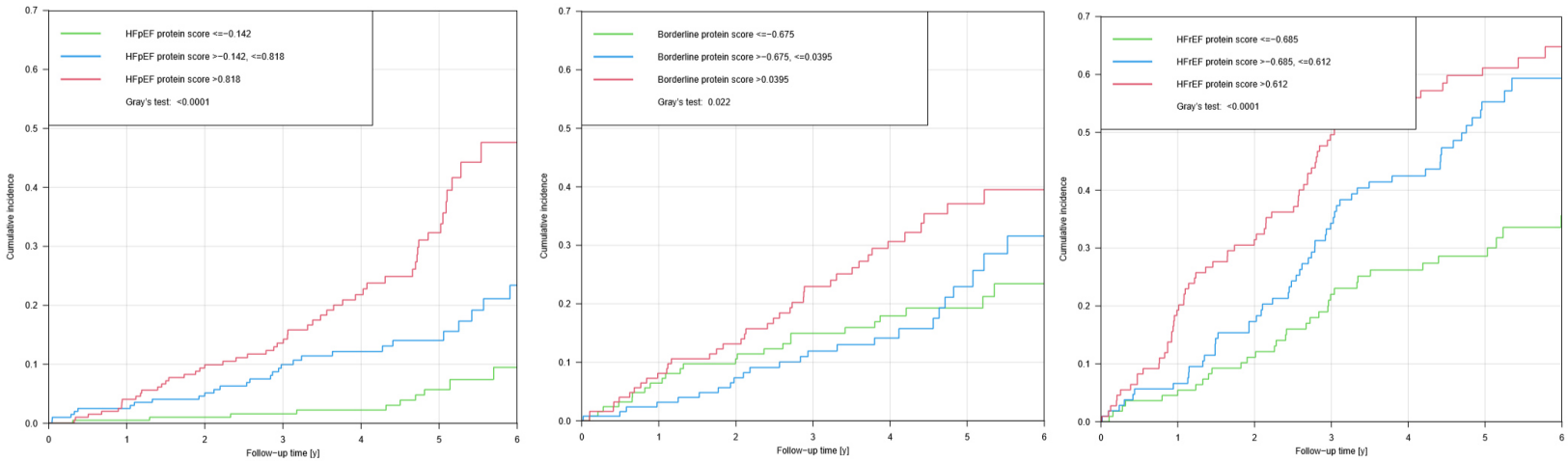
Venn diagram showing shared proteins between the HF phenotypes after adjusting for age, sex, antithrombotic agents, CVRFs, and comorbidities. Proteins are listed alphabetically. Arrows describe an up- (green) or downregulation (red) of proteins in HF phenotypes.

Figure 3. Cumulative incidence plots for worsening of HF

3A. Cumulative incidence of worsening of HF in HFpEF

3B. Cumulative incidence of worsening of HF in HFpEF borderline

3C. Cumulative incidence of worsening of HF in HFrEF



Plots of cumulative incidence for worsening of HF in HFpEF, HFpEF borderline, and HFrEF phenotypes according to tertiles of protein scores calculated for each HF phenotype. Median follow-up times are in HFpEF: 4.01 years (interquartile range [IQR]: 2.23-5.02 years); in HFpEF borderline: 3.97 years (IQR: 2.12-5.03 years); in HFrEF: 3.26 years (IQR: 1.75-5.04 years).

Supplement material: Platelet-related protein signature differs in heart failure phenotypes – results from the MyoVasc study

Part A. Supplemental Methods

Assessment of cardiovascular risk factors (CVRFs), comorbidities, and signs or symptoms of HF

Cardiovascular risk factors are determined as:

- Diabetes mellitus was defined as HbA1c ≥ 6.5% or blood glucose level ≥ 126 mg/dL at baseline examination after an overnight fast or at least 8 hours or a blood glucose level of ≥ 200 mg/dL at baseline examination after a fasting period > 5 hours or diagnosed diabetes mellitus by a physician or intake of antidiabetic medication (ATC A10);

- Arterial hypertension was defined as blood pressure RR_{sys} >140/90 mmHg or RR_{diast} >90mmHg in mean of 2nd/ 3rd measurement; or diagnose by a physician or intake of antihypertensive medication with the last 14 days;

- Smoking was interrogated due to active and passive smoking; regular smoking was defined as smoking one cigarette per day or at least seven cigarettes per week or one package per month or one cigarillo per day or at least seven cigarillos per week or two pipes per day;

- Dyslipidemia was defined as low density lipoprotein/ high density lipoprotein > 3.5 and/ or triglycerides level ≥ 150 mg/dL or HDL cholesterol in men ≤40mg/dL, in women ≤45mg/dL or diagnose by a physician or intake of lipid modifying medication;

- Obesity was defined as body-mass index (BMI) ≥ 30.0kg/m² or elevated waist circumference, i.e. men ≥94 cm or women ≥80cm;

- Family histories of myocardial infarction (MI) or stroke of male first-degree relatives until the age of 60 years or female first-degree relatives until the age of 65 years;

Comorbidities are self-reported and include coronary artery disease (CAD), myocardial infarction (MI), stroke, atrial fibrillation (AF), peripheral artery disease (PAD), chronic

obstructive pulmonary disease (COPD), deep vein thrombosis (DVT), pulmonary embolism (PE), cancer, chronic kidney disease (CKD), and chronic liver disease (CLD).

Signs and symptoms of HF are defined as at least one of the following:

- NYHA class ≥ II
- bilateral ankle swelling or rales or nocturia and NT-proBNP > 125pg/mL
- NYHA class = I and NT-proBNP > 125pg/mL and HF medication (ATC codes C09 and at least one of the following C01AA, C07, C03CA/C03CB or C03DA)

Assessment of cardiac structure and function

Resting two-dimensional transthoracic echocardiograms were performed using an iE33 echocardiography system (Philips Medical Systems, Amsterdam, The Netherlands) to provide information on chamber dimensions, wall thickness, and measures of systolic and diastolic function. Measurements were performed according to recommendations of the American and European Societies of Echocardiography. Mitral inflow velocity pattern was recorded from the apical four-chamber view with the pulsed waved Doppler sample volume positioned at the tips of the mitral valve leaflets during diastole in expiration. Peak early (E-wave) and late (A-wave) diastolic filling velocities were measured and their ratio (E/A) calculated. The lateral mitral annular early diastolic velocity (E') was measured by spectral tissue Doppler imaging and the E/E' ratio determined. Left ventricular ejection fraction (LVEF) was calculated by measurement according to Simpson from the apical four-chamber view.

Part B. Supplemental Tables

Table S1. Literature references of selected proteins

Protein	UniProt ID	Reference(s) of proteins for platelet activation
ADA (Adenosine Deaminase)	P00813	Franco, R., et al. (1990). J Histochem Cytochem 38(5): 653-658.
CASP-3 (Caspase-3)	P42574	Boing, A. N., et al. (2008). Platelets 19(2): 96-103. ; Shcherbina, A et al. Blood. 1999 Jun 15;93(12):4222-31
CCL3 (C-C motif chemokine 3)	P10147	Gear, A. R. and D. Camerini (2003). Microcirculation 10(3-4): 335-350.
CD40 (CD40L receptor)*	P25942	Inwald, D. P., et al. (2003). Circ Res 92(9): 1041-1048.
CTSD (Cathepsin D)	P07339	Garcia, B. A., et al. (2005). J Proteome Res 4(5): 1516-1521. ; Sixma, J. J., et al. (1985). Blood 65(5): 1287-1291.
CXCL1 (C-X-C motif chemokine 1)	P09341	Gear, A. R. and D. Camerini (2003). Microcirculation 10(3-4): 335-350.
CXCL5 (C-X-C motif chemokine 5)	P42830	Gear, A. R. and D. Camerini (2003). Microcirculation 10(3-4): 335-350.
EGFR (Epidermal growth factor receptor)	P00533	Chen, R., et al. (2018). J Immunol 201(7): 2154-2164.
GP VI (Platelet glycoprotein VI)*	Q9HCN6	Jung, S. M. and M. Moroi (2008). Adv Exp Med Biol 640: 53-63. ; Handtke, S., et al. (2019). Thromb Haemost 119(3): 407-420.
HGF (Hepatocyte growth factor)*	P14210	Boswell, S. G., et al. (2012). Arthroscopy **28**(3): 429-439. ; Taniguchi, Y., et al. (2019). J Exp Orthop 6(1): 4.
ICAM-2 (Intercellular adhesion molecule)	P13598	Diacovo, T. G., et al. (1994). J Clin Invest 94(3): 1243-1251.
IL-1 alpha (Interleukin-1 alpha)	P01583	Sedlmayr, P., et al. (1995). Scand J Immunol 42(2): 209-214.
IL-7 (Interleukin-7)	P13232	Damas, J. K., et al. (2003). Circulation 107(21): 2670-2676

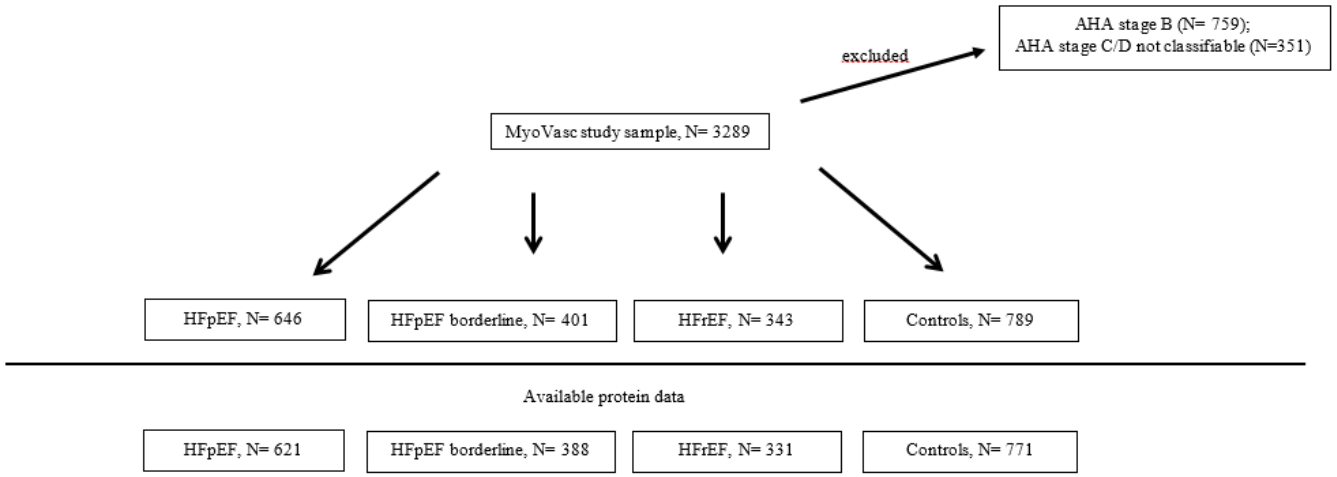
IL-8 (Interleukin-8)	P10145	Gear, A. R. and D. Camerini (2003). Microcirculation 10(3-4): 335-350.
IL-17RA (Interleukin-17 receptor A)	Q96F46	Maione, F., et al. (2011). Biochem Biophys Res Commun 408(4): 658-662.
IL-18 (Interleukin-18)	Q14116	Allam, O., et al. (2017). Cytokine 90: 144-154.
IL-18BP (Interleukin-18-binding protein)	O95998	Allam, O., et al. (2017). Cytokine 90: 144-154.
IL-33 (Interleukin-33)	O95760	Takeda, T., et al. (2016). J Allergy Clin Immunol 138(5): 1395-1403.e1396.
JAM-A (Junctional adhesion molecule A)	Q9Y624	Naik, M. U., et al. (2012). Blood 119(14): 3352-3360; Sobocka, M. B., et al. (2004). J Recept Signal Transduct Res 24(1-2): 85-105.
MCP-1/ CCL2 (Monocyte chemotactic protein 1)	P13500	Liu, D., et al. (2018). Biochim Biophys Acta Mol Basis Dis 1864(9 Pt B): 2901-2912.
MCP-3 (Monocyte chemotactic protein 3)	P80098	Gear, A. R. and D. Camerini (2003). Microcirculation 10(3-4): 335-350.
MMP-1 (Matrix metalloproteinase-1)	P03956	Mastenbroek, T. G., et al. (2015). Arterioscler Thromb Vasc Biol 35(12): 2554-2561. ; Trivedi, V., et al. (2009). Cell 137(2): 332-343.
MMP-2 (Matrix metalloproteinase-2)	P08253	Seizer, P. and A. E. May (2013). Thromb Haemost 110(5): 903-909. ; Radomski, A., et al. (2002). Br J Pharmacol 137(8): 1330-1338.
MMP-3 (Matrix metalloproteinase-3)	P08254	Trivedi, V., et al. (2009). Cell 137(2): 332-343.
MMP-9 (Matrix metalloproteinase 9)	P14780	Fernandez-Patron, C., et al. (1999). Thromb Haemost 82(6): 1730-1735. ; Sheu, J. R., et al. (2004). Br J Pharmacol 143(1): 193-201.
PAI-1/ SERPINE1 (Plasminogen activator inhibitor 1)*	P05121	Huebner, B. R., et al. (2018). Shock 50(6): 671-676. ; Morrow, G. B., et al. (2019). Haematologica.
PDGF subunit A (Platelet derived growth factor subunit A)	P04085	Harrison, P. and E. M. Cramer (1993). Blood Rev 7(1): 52-62.
PD-L1 (Programmed cell death 1 ligand 1)	Q9NZQ7	Rolfes, V., et al. (2018). Oncotarget 9(44): 27460-27470.
PECAM-1 (Platelet endothelial cell adhesion molecule 1)*	P16284	Dhanjal, T. S., et al. (2007). Platelets 18(1): 56-67; Feng, Y. M., et al. (2016). Eur Rev Med Pharmacol Sci 20(19): 4082-4088.
P-Selectin/ SELP*	P16109	Furie, B., et al. (2001). Thromb Haemost 86(1): 214-221.

TFPI (Tissue factor pathway inhibitor)	P10646	Winckers, K., et al. (2017). PLoS ONE 12(2): e0168273. ; Maroney, S. A. and A. E. Mast (2008). Transfus Apher Sci **38**(1): 9-14.
TGF-β1 (Transforming growth factor beta-1)*	P01137	Grainger, D. J., et al. (1995). Nat Med 1(9): 932-937.
TIMP-4 (Metalloproteinase inhibitor 4)	Q99727	Radomski, A., et al. (2002). Br J Pharmacol 137(8): 1330-1338.
TNFSF14/ LIGHT (Tumor necrosis factor ligand superfamily member 14)	O43557	Otterdal, K., et al. (2006). Blood 108(3): 928-935. ; Celik, S., et al. (2007). Thromb Haemost 98(4): 798-805.
TRAIL (TNF-related apoptosis-inducing ligand)	P50591	Crist, S. A., et al. (2004). Exp Hematol 32(11): 1073-1081.
TWEAK (Tumor necrosis factor (Ligand) superfamily member 12)	O43508	Meyer, T., et al. (2010). Platelets 21(7): 571-574.
uPA (Urokinase-type plasminogen activator)	P00749	Camoin-Jau, L., et al. (2002). Thromb Haemost 88(3): 517-523.
VEGF-A (Vascular endothelial growth factor A)*	P15692	Salgado, R., et al. (2001). Angiogenesis 4(1): 37-43.
vWF (Von Willebrand Factor)*	P04275	Gralnick, H. R., et al. (1991). Mayo Clin Proc **66**(6): 634-640.

List of proteins, selected as platelet-related, with UniProt IDs and literature references. Proteins highlighted with * were also selected by at least one of the databases used for the selection.

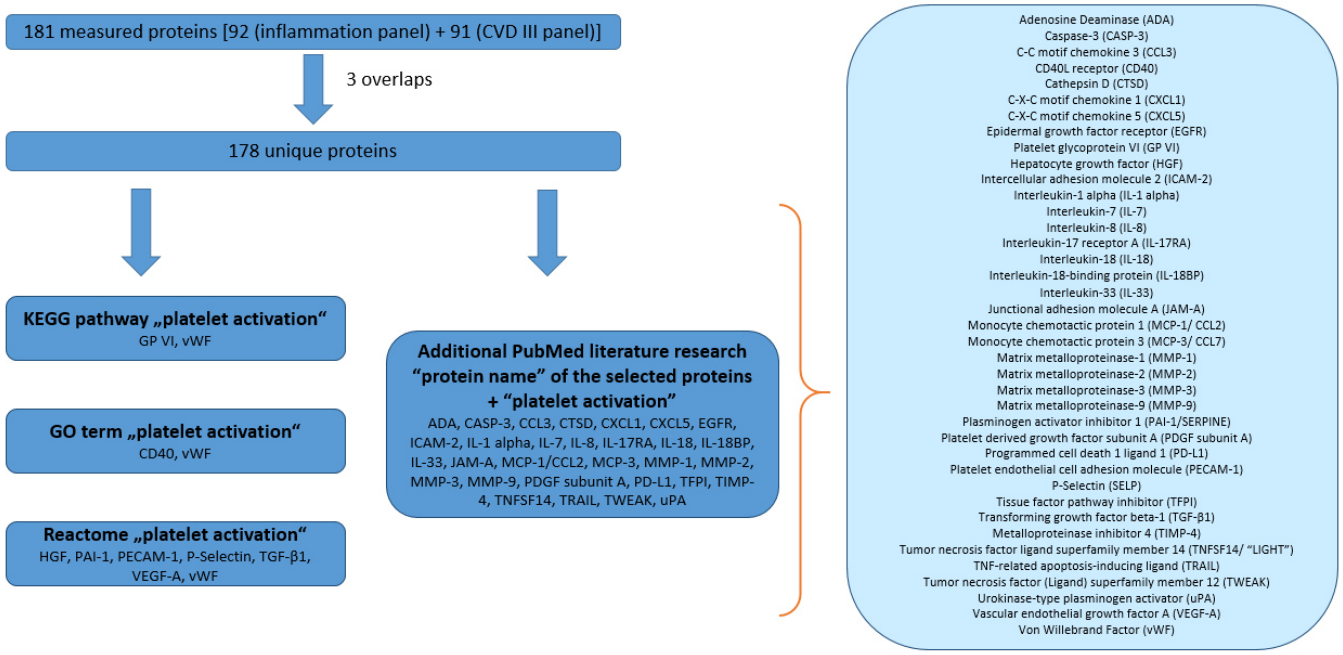
Part C. Supplemental Figures

Figure S1. Derivation of the analysis sample



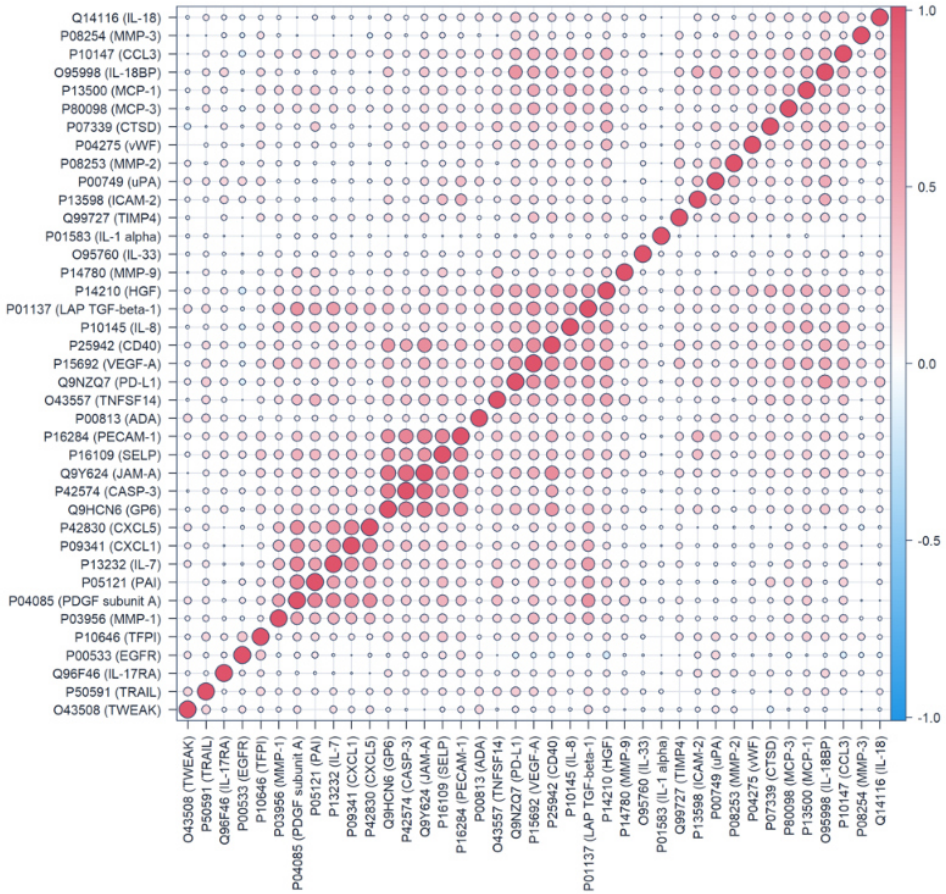
Flow chart presenting the derivation of the analysis sample based on baseline information and available data of protein measurements; control individuals comprise individuals with stage 0 and AHA stage A. N: number of individuals; HFpEF: heart failure with preserved ejection fraction; HFpEF borderline: heart failure with LVEF 41% to 49%; HFrEF: heart failure with reduced ejection fraction

Figure S2. Preselection of platelet-related proteins



Scheme of the selection of the 39 platelet-related proteins by searching the databases from Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology (GO), and Reactome using the search term “platelet activation” and an additional keyword search of each protein + “platelet activation” in PubMed (https://pubmed.ncbi.nlm.nih.gov/).

Figure S3. Correlation between platelet-related proteins



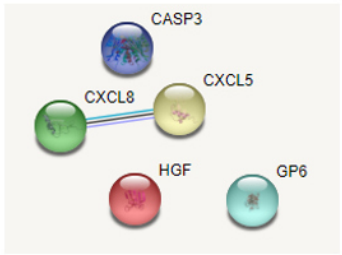
Heat map presenting the correlation between proteins, correlation between proteins ranges from r= -0.17 to r= 0.86.

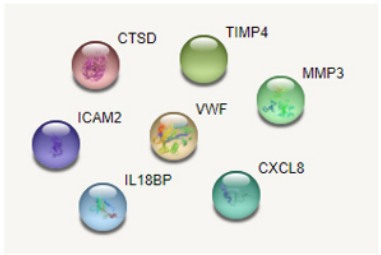
Figure S4. Network analyses of protein-protein interactions according to HF phenotype

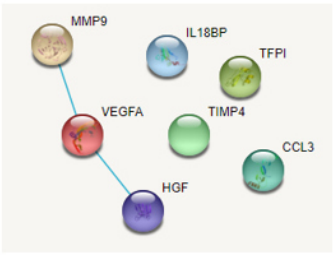
HFpEF
CASP-3 (P42574)
CXCL5 (P42830)
GP VI (Q9HCN6)
HGF (P14210)
IL-8 (CXCL8) (P10145)

HFpEF borderline
CTSD (P07339)
ICAM-2 (P13598)
IL-8 (CXCL8) (P10145)
IL-18BP (O95998)
MMP-3 (P08254)
TIMP-4 (Q99727)
vWF (P04275)

HFrEF
CCL3 (P10147)
HGF (P14210)
IL-18BP (O95998)
MMP-9 (P14780)
TIMP-4 (Q99727)
TFPI (P10646)
VEGF-A (P15692)







Cytokine signaling

For each HF phenotype a network analysis was conducted on string-db.org to visualize possible protein-protein interactions and pathophysiological pathways that were related to the selected platelet-related proteins after adjustments for age, sex, antithrombotic medication, CVRFs, and comorbidities. Cardiovascular risk factors (CVRFs) are arterial hypertension, diabetes mellitus, smoking, obesity, dyslipidemia, and family history of MI/stroke and comorbidities were defined as stroke, CAD, PAD, COPD, cancer, CKD, CLD, DVT, and PE. CXCL8= IL-8

General discussion

This doctoral thesis was the first to investigate platelet indices as surrogate for platelet activation in a large cohort of 3,289 well-characterized HF subjects from the MyoVasc study. The first aim was to investigate associations between platelet indices as potential markers of platelet activation and HF phenotypes, cardiac function parameters, and clinical outcome. Considering the important role of PTH and cardiac function, the second aim of this thesis was to assess the association between platelet indices and PTH in a sex-specific analysis in HF phenotypes. Furthermore, circulating plasma proteins, defined as platelet-related due to comprehensive literature research, were identified with specific signatures for HF phenotypes. Scores of the signatures were prognostic of the clinical outcome “worsening of HF”.

Higher MPV, a potential marker of platelet activation, was associated with worse systolic and diastolic cardiac function in HF subjects and worse clinical outcome. Individuals with higher MPV or lower platelet count presented with worse cardiovascular risk profile. This phenomenon can be explained by the physiologically inverse interplay of MPV and platelet count to keep the platelet mass stable.[51] Lower platelet count was associated with a lower LVEF, worse diastolic function, and worse clinical outcome independent of age and sex. Despite a higher incidence of worsening of HF among HFrEF individuals, the effects of increased MPV and/or lower platelet count were stronger in HFpEF individuals. With respect to a history of cancer, a worse outcome was found for HF patients with lower platelet count as also described in a Danish population study,[52] suggesting for a worse general health compared to the total study sample. Whereas for higher MPV, HF patients with a history of cancer presented with a better outcome. In addition to MPV and platelet count, platelet-to-leukocyte, platelet-to-monocyte, and platelet-to-lymphocyte ratios were analyzed. A high platelet-to-lymphocyte ratio depicted an increased risk for worsening of HF, especially among HFrEF compared to HFpEF phenotype. Increased levels of platelet-to-lymphocyte ratios have been found in CVRFs such as hypertension, venous thromboembolism, myocardial infarction (MI), and cancer.[53] However, the underlying cardiovascular risk profile did not alter the association between high platelet-to-lymphocyte ratio and worse clinical outcome. After excluding cancer patients, the risk for worsening of HF was higher compared with the total sample. Cancer patients were more often under medical supervision and a deterioration of the health status might be recognized earlier and faster. The platelet-to-lymphocyte ratio has been reported as a marker of systemic inflammation, atherosclerosis, and platelet activation that could help to improve patients´ risk profile.[53] However, this marker is still not of clinical relevance and it did not provide additional information about cardiac function in HF subjects. No association was found between platelet-to-lymphocyte ratio and LVEF or E/E’, measures of systolic and diastolic function, respectively. However, lower platelet-to-leukocyte and platelet-to-monocyte

ratios were related to worse cardiac function and worsening of HF in the total sample and particularly among individuals with HFpEF phenotype and in those with cancer history, assuming lower platelet counts and/or increased immune response by leukocytes and monocytes may play a role in HF pathophysiology in those individuals. The intake of antithrombotic agents did not change the associations between any of the platelet indices and clinical outcome.

In CVRFs and comorbidities, underlying inflammatory processes and activated cytokines were observed, that might trigger severity of HF: In diabetes mellitus and obesity, low-grade and microvascular inflammation caused endothelial dysfunction and atherosclerosis, drove diastolic stiffness, fibrosis, and HF progression; whereas in HFrEF, high-grade inflammation occurred in response to necrosis and trauma of ischemia, that directly caused cardiomyocyte damage.[54, 55] A better characterization of these inflammation types and adequate timely therapeutic management may help to attenuate worsening of HF. Individuals with symptomatic HF showed a worse cardiovascular risk profile and elevated markers of inflammation, such as fibrinogen and leukocytes, compared with the total study sample. Inflammation is a recognized factor of HF progression.[56] Inflammatory markers potentially interact with platelets and promote platelet activation and coagulation.

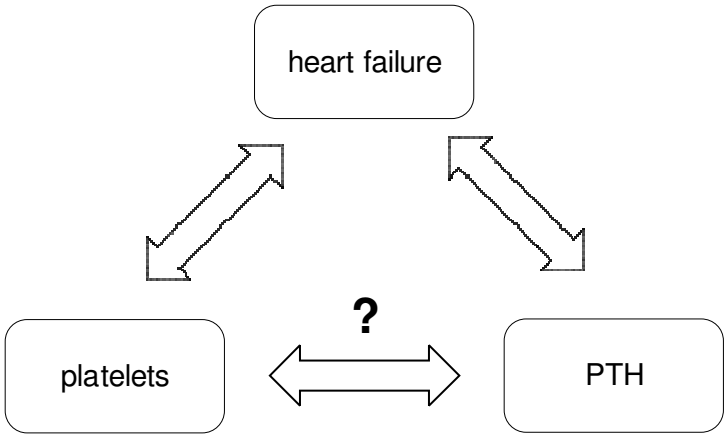
Nevertheless, the diagnosis of HFpEF is quite difficult as it is accompanied with unspecific symptoms, such as dyspnea and edema.[1] HFpEF was found to be more prevalent among females, the elderly, and individuals with hypertension.[33] In contrast, HFrEF is more common in males and individuals with ischemic heart diseases.[57] With increasing MPV, the proportion of individuals with diabetes mellitus, obesity, and atrial fibrillation increased. Indeed, activated platelets play an important role in the severity of HF, as expressed by lower LVEF and increased E/E' ratio, a parameter of diastolic dysfunction, and can be assumed to be an additional risk factor for HF. The incidence of worsening of HF was higher in HFrEF compared to HFpEF. However, higher MPV and lower platelet count had stronger effects on worse clinical outcome in HFpEF phenotype. Higher platelet-to-lymphocyte ratio and lower platelet-to-monocyte and platelet-to-leukocyte ratios were associated with worse clinical outcome in both HF phenotypes. Thus, specific platelet indices have the potential to provide additional information about the pathophysiology and severity of HF. This analysis demonstrated the important link between platelets and HF, as higher MPV, platelet-leukocyte ratios and scores of platelet-related proteins were associated with increased incidence of worse clinical outcome. Nevertheless, these results only outlined the role of platelets in HF, but further risk stratification according to platelet function should be elucidated.

In addition, platelet indices showed differential and sex-specific links to PTH within HF phenotypes, with stronger effects in women regardless of the underlying cardiovascular risk profile, medication, and vitamin D status. A positive association was found for PTH and MPV

in females with reduced ejection fraction and symptomatic HFrEF, whereas PTH was inversely associated with platelet count in male individuals with HFrEF as well as in HFpEF phenotype.

As already known from other studies, PTH can directly interact with the heart, stimulating hypertrophy, arrhythmia, and inflammation by promoting necrosis of cardiomoycytes.[23] PTH was found to be associated with all-cause mortality and cardiovascular mortality in 148 HF patients.[58] There was also an interaction between PTH and platelets.[23, 25] To date, the interaction of PTH and platelets in HF subjects has not been investigated and was therefore of interest for this doctoral thesis (**Figure 1**).

Figure 1. Interaction between heart failure, platelets, and parathyroid hormone (PTH)



An important role of PTH was found for platelet interaction in individuals with cardiac dysfunction independent of the cardiovascular risk profile. Associations were found between PTH and both platelet indices, namely MPV and platelet count, independent of the underlying cardiovascular risk profile, vitamin D status, and season. A positive association between PTH and MPV was found particularly in females with HFrEF, whereas an inverse association between PTH and platelet count was found only in male HFrEF subjects, but did exist in both males and females with HFpEF. Beside the interaction of PTH and platelets, this study additionally found sex-specific differences within the HF phenotypes with regard to the mechanism involved in the interaction between platelets and PTH. However, the findings were independent of common female hormonal factors such as menstrual bleeding, hormone replacement therapy, and intake of oral contraceptives. Endogenous female hormones have not been investigated in this study cohort, but may be of interest for further research on sex differences in HF patients, particularly for elaborating the platelet interaction of and/or the role of PTH in individuals with HF. Other studies in post-menopausal women with estrogen deprivation showed an increase in diastolic dysfunction.[59] The association between PTH and platelet count was stronger in women than in men, regardless of HF phenotype. However, in female HFrEF patients, the association between PTH and platelet count was related to CVRFs

and renal function, whereas these factors did not alter the association in males with HFrEF or females with HFpEF, suggesting a particular role in female HFrEF subjects. Overall, HFpEF is more common in women, whereas men are more likely to have HFrEF, reflecting characteristic risk factors and comorbidities for each phenotype.[60] A role for elevated PTH in individuals with HFpEF has already been described in several studies.[61] Among individuals from the MyoVasc study, the association between high PTH levels and lower platelet count was found in both sexes in HFpEF, whereas in HFrEF the association was found in males only. This might be due to the different cardiac characteristics of HF in individuals with preserved EF, having rather concentric remodeling, compared with those with reduced EF, presenting with eccentric remodeling of the heart.[4] Nevertheless, further sex-specific research is needed to understand the particular role of PTH in HFrEF individuals as associations with higher MPV were found in females only, and PTH and lower platelet count in males with HFrEF only. In addition, the cardiovascular risk profile in female HFrEF subjects should be analyzed to highlight the different clinical characteristic profile as they represent a minority in HFrEF phenotype. Moreover, mechanistic studies are warranted to understand the biochemical interaction potential between PTH and platelets in general. To date, no medical studies have targeted the role of PTH and the interaction with platelets in HF patients, as previously suggested by Gruson et al. in 2014.[23] Evidence from the literature demonstrated an association between PTH and all-cause mortality as well as cardiovascular mortality.[58, 62] The results may suggest a potential benefit of antiplatelet medication in HF patients with high PTH levels. Additionally, the subject´s vitamin D status should be routinely assessed as PTH increases in response to low vitamin D levels. In chronic HF subjects with hypovitaminosis D, a vitamin D supplementation should be discussed.[63] Those individuals exhibited impaired cardiac function and platelet activation.[62] Results from this work, which highlight important sex-specific mechanisms involved in the interaction between PTH and platelets in different cardiac function phenotypes, underline the value of a sex-specific design and analyses of clinical studies investigating HF. Further interventional studies are needed to investigate whether interfering the interaction of PTH with platelets may improve the clinical manifestations and outcome in HF patients.

In addition to the role in thrombosis and hemostasis, activated platelets release a plethora of inflammatory and immune-associated mediators.[26] Platelet-related protein signatures can help to distinguish between HF phenotypes and may provide new mechanistic insights into differences in the underlying pathophysiology of each phenotype. The investigation of circulating platelet-related proteins provided new insights into the differentiating role of inflammatory and immune processes in HF phenotypes, including an increased risk for worsening of HF. With a targeted protein biomarker discovery approach, 178 proteins have been quantified in a large sample of more than 3,200 HF patients from the MyoVasc study. After a systematic literature research, 39 of these proteins have been determined as “platelet-related” based on their expression at platelet activation and were further analyzed regarding

their association with HF phenotypes and clinical outcome within the phenotypes. Unique protein profiles were identified for each HF phenotype independent of the cardiovascular risk profile and potential confounders, including medication. The results demonstrated that signatures were related to distinct roles of platelets in HF phenotypes that were not based on the clinical profile of the individuals. HFpEF was found to be associated with a proinflammatory state due to related risk factors and comorbidities.[4] The platelet-related protein profile in HFpEF was characterized by five proteins involved in apoptosis and cardiac damage, whereas in HFrEF, four proteins related to extracellular matrix remodeling, macrophage activation, and atherosclerosis, were more highly expressed. The protein profile in HFrEF patients was similar to that in post-MI subjects. MI is one of the major causes leading to HF and especially to HFrEF.[64] In the heart of both HFrEF and post MI individuals, similar cardiac and fibrotic remodeling occurred in combination with angiogenesis.[48] In HFmrEF, increased expression of seven immune response-related proteins were found. Although HFmrEF represents a distinct HF phenotype with a separate platelet-related protein profile indicative of activation of inflammatory processes, some overlap with HFrEF phenotype was found. Metalloproteinase inhibitor 4 (TIMP-4) was higher expressed in both phenotypes. It is involved in extracellular matrix turnover and wound repair after MI.[65] The literature suggests TIMP-4 to be related to worse clinical outcome in HF patients.[46] The distribution of identified proteins showed shared and unique proteins in HF phenotypes, but there was no overlap between all three HF phenotypes. It should be assumed that there is no general “heart failure” protein profile related to platelets, as the protein expression profiles of HF phenotypes presented with large disparities. However, by measuring and selecting a wider spectrum of proteins or not only platelet-related ones, additional information on HF pathophysiology might be obtained. The results provided more detailed information about the role of platelet-related proteins in pathophysiology of HF phenotypes. Unique protein signatures provided new insights into the role of platelets in inflammatory and immune processes as well as into a role in HF characteristics beyond EF.

Biomarkers of inflammation were suggested to be useful determinants for HF severity and mortality.[6, 55] Platelet-related protein signatures were shown to be unique for each HF phenotype and were further scored by a linear combination of proteins weighted by their association with HF phenotypes, for each HF phenotype. The generated protein scores added important information on the clinical outcome “worsening of HF” for each HF phenotype independent of the cardiovascular risk profiles. The protein score in HFrEF phenotype predicted an approximately 1.5-fold risk of worsening of HF. In the HFpEF phenotype, the risk for worsening of HF was even twice as high, demonstrating the highest impact of platelet-related inflammation and immune response on outcome. These results complied with previous findings on MPV and platelet count as routine platelet markers.[52, 66] It has been demonstrated that platelet activation had an important role in increasing risk of worsening of HF, especially

in HFpEF patients and should therefore be of high interest in improving the clinical outcome in this HF phenotype.

This doctoral thesis highlights the diversified role of platelets in HF syndrome and HF phenotypes, including the interaction of platelets with immune mediators or endogenous hormones such as PTH. Moreover, this work underlines the necessity for sex-specific research to improve knowledge of HF pathophysiology and adequate therapy in males and females, as both sexes presented with different platelet characteristics beyond the clinical risk profile in HF phenotypes with reduced and preserved ejection fraction. HF is defined as a clinical syndrome with several clinical components. A potential role of activated platelets should be taken into consideration in specific HF phenotypes that would benefit from antiplatelet agents, similar to HF patients with AF, in whom antiplatelet agents are already recommended in guidelines.[5]

Strength and limitations

A large number of HF subjects with different stages of HF severity and from different HF phenotypes were included in this dissertation work. All participants from the MyoVasc study underwent a comprehensive clinical examination. Outcome recording was thoroughly obtained via annual computer-assisted telephone interviews and regular checks with the German registration offices.[12] The provided information was assessed by a clinical event committee. Further data was checked for completeness and correctness according to predefined procedures before data sheets were saved electronically and physically separated at central data management department. Cross-sectional, multivariable linear, and logistic regressions and prospective outcome analyses with Cox regression have been performed to address the research objectives of this dissertation. With these advanced statistical methods, strong effects of platelets were found in HF phenotypes as well as important links to clinical outcome. High-throughput analyses offered further detailed information on proteins that were related to platelet activation and part of the immune response in HF pathophysiology.

Nevertheless, there are some limitations to be mentioned. The association of platelet indices with cardiac function was determined in HFpEF and HFrEF only. No information was obtained for the HFmrEF phenotype. Regarding the role of platelets in patients with cancer history, there was no detailed information about cancer type or treatment, as both might be of potential interest for a deeper analysis in this subgroup. PTH measurements were only available in the first 2,000 individuals from the MyoVasc study, thus information was lacking for approximately one-third of all study participants. Next to important information about sex-specific associations between PTH and platelet indices, results on clinical outcome and mortality were missing. In addition to MPV and platelet count, surrogates for platelet activation, further mechanistic studies may provide information on platelet function, such as platelet aggregation or platelet

activation. For the analysis on platelet-related proteins, the total number of 178 unique proteins were provided only from panels related to inflammation and cardiovascular diseases, leading to an inflammation-related bias of the identified proteins. Further investigations on the role of proteins related to platelet activation in a wider cardiovascular range may be of interest to gain more detailed knowledge about platelet-related proteins in HF patients. By now, only the expression profile of platelet-related proteins was evaluated. It needs to be clarified, whether the expression of these proteins might have a causal relationship with HF or whether these proteins were up- or downregulated as compensatory mechanism due to HF to repair damages of heart and vasculature.

Conclusion

This doctoral thesis reported several aspects for a role of platelets in HF subjects. Beside the role platelet indices as surrogate for platelet activation, the associations of MPV and platelet count with PTH were of interest in HF pathophysiology. Additionally, the proteins related to activated platelets showed interesting mechanisms beyond MPV and platelet count. If platelet activation caused HF or resulted from HF needs to be evaluated in cell cultures and animal models. HF is a multifaceted syndrome with different signs and symptoms at initial identification and the present research reported important evidence for a role of platelets that triggers further novel research, e.g. investigating (sex-) specific platelet function in HF phenotypes. As protein research is still too expensive, it is not used for clinical routine measurements, but a clinical relevance of protein signatures for the outcome in HF patients should further be elaborated.

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Supplement

Original publications

First authorship

- The impact of platelet indices on clinical outcome in heart failure: results from the MyoVasc study.

  Bianca Dahlen, Andreas Schulz, Sebastian Göbel, Sven-Oliver Tröbs, Sören Schwuchow-Thonke, Henri M. Spronk, Jürgen H. Prochaska, Natalie Arnold, Karl J. Lackner, Tommaso Gori, Hugo ten Cate, Thomas Münzel, Philipp S. Wild, Marina Panova-Noeva

  ESC Heart Fail. 2021 Aug; 8(4):2991-3001. doi: 10.1002/ehf2.13390. Epub 2021 May 3

- Sex-specific relationship between parathyroid hormone and platelet indices in phenotypes of heart failure – Results from the MyoVasc study

  Bianca Dahlen, Felix Müller, Sven-Oliver Tröbs, Marc William Heidorn, Andreas Schulz, Natalie Arnold, M. Iris Hermanns, Sören Schwuchow-Thonke, Jürgen H. Prochaska, Tommaso Gori, Hugo ten Cate, Karl J. Lackner, Thomas Münzel, Philipp S. Wild, Marina Panova-Noeva

  Front Cardiovasc Med 2021 Jun 16;8:682521. doi: 10.3389/fcvm.2021.682521. eCollection 2021.

Co-authorship

- Relevance of polypharmacy for clinical outcome in patients receiving vitamin K antagonists

  Lisa Eggebrecht, Markus Nagler, Sebastian Göbel, Heidrun Lamparter, Karsten Keller, Bianca Wagner, Marina Panova-Noeva, Vincent ten Cate, Christoph Bickel, Michael Lauterbach, Christine Espinola-Klein, Roland Hardt, Thomas Münzel, Jürgen H. Prochaska, Philipp S. Wild

  J Am Geriatr Soc. 2019 Mar;67(3):463-470. doi: 10.1111/jgs.15712. Epub 2018 Dec 11.

- Relation between platelet coagulant and vascular function, sex-specific analysis in adult survivors of childhood cancer compared to a population-based sample

  Marina Panova-Noeva, Bianca Wagner, Markus Nagler, Natalie Arnold, Jürgen H. Prochaska, Susan Eckerle, Henri M. Spronk, Hiltrud Merzenich, Arthur Wingerter, Astrid Schneider, Sven Danckwardt, Hugo ten Cate, Jörg Faber, Philipp S. Wild

  Sci Rep. 2019 Dec 27; 9(1):20090. doi: 10.1038/s41598-019-56626-1.

- Comprehensive platelet phenotyping supports the role of platelets in the pathogenesis of acute venous thromboembolism – results from clinical observation studies

  Marina Panova-Noeva, Bianca Wagner, Markus Nagler, Thomas Koeck, Vincent ten Cate, Jürgen H. Prochaska, Stefan Heitmeier, Imke Meyer, Christoph Gerdes, Volker Laux, Stavros Konstantinides, Henri M. Spronk, Karl J. Lackner, Kirsten Leineweber, Hugo ten Cate, Philipp S. Wild

  EBioMedicine. 2020 Oct; 60:102978. doi: 10.1016/j.ebiom.2020.102978. Epub 2020 Sep 10.

- Characterization of thrombin generation curve shape in presence of platelets from acute venous thromboembolism patients

  Jeremy Lagrange, Bianca Wagner, Markus Nagler, Vincent ten Cate, Alejandro Pallares Robles, Thomas Koeck, Steffen Rapp, Jürgen H. Prochaska, Henri M. Spronk, Philip Wenzel, Wolfram Ruf, Hugo ten Cate, Philipp S. Wild, Marina Panova-Noeva

  J Clin Med. 2020 Sep 7; 9(9):2892. doi: 10.3390/jcm9092892.

- Variation of platelet function in clinical phenotypes of acute venous thromboembolism - Results from the GMP-VTE project.

  Panova-Noeva M, Wagner B, Nagler M, Koeck T, Ten Cate V, Eggebrecht L, Prochaska JH, Meyer I, Gerdes C, Spronk HM, Lackner KJ, Ten Cate H, Leineweber K, Heitmeier S, Konstantinides S, Wild PS.

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