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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 ≥ 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 ≤ 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 (≤ 186 × 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*).

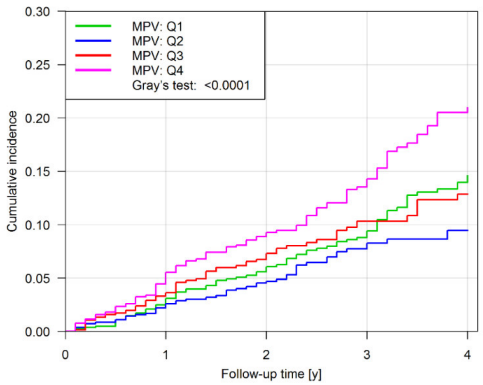
Cox regression analysis confirmed the worse outcome in subjects within the highest quartile of MPV in a model adjusted for age and sex (HR = 1.60, [95% CI: 1.26; 2.03]) and also after further adjustment for the cardiovascular risk profile and cancer (HR = 1.47, [1.16; 1.87]), as depicted in *Figure 2*. 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

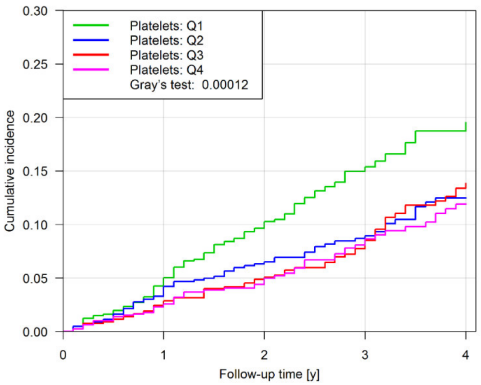
Looking into HF phenotypes, a higher incidence for worsening of HF was found among HFrEF individuals compared with HFpEF (*P* < 0.0001, *Figure S4*). However, the effect of MPV > 75th percentile was stronger in HFpEF (HR = 1.99 [1.22; 3.24]) than in HFrEF individuals (HR = 1.03 [0.69; 1.54]) independent 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).

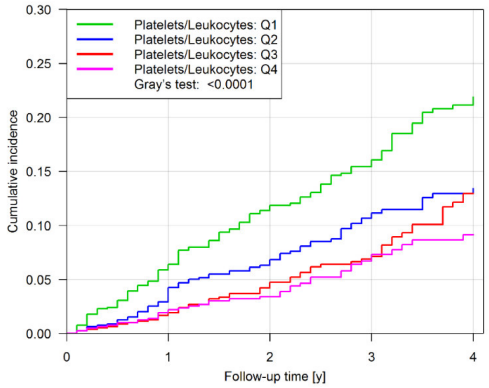
A. Worsening of HF according to MPV quartiles



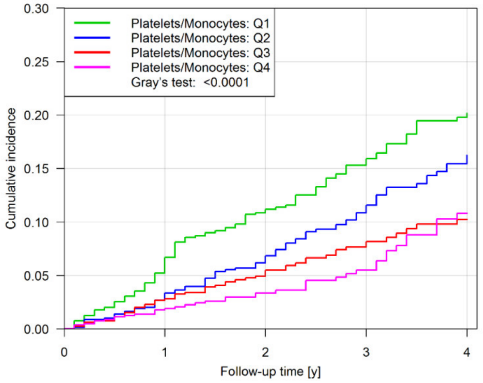
B. Worsening of HF according to platelet count quartiles



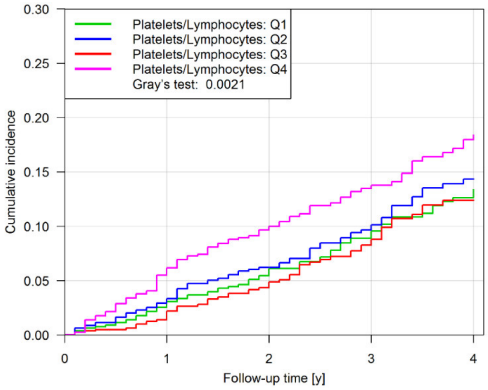
C. Worsening of HF according to platelet-to-leukocyte ratio quartiles



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



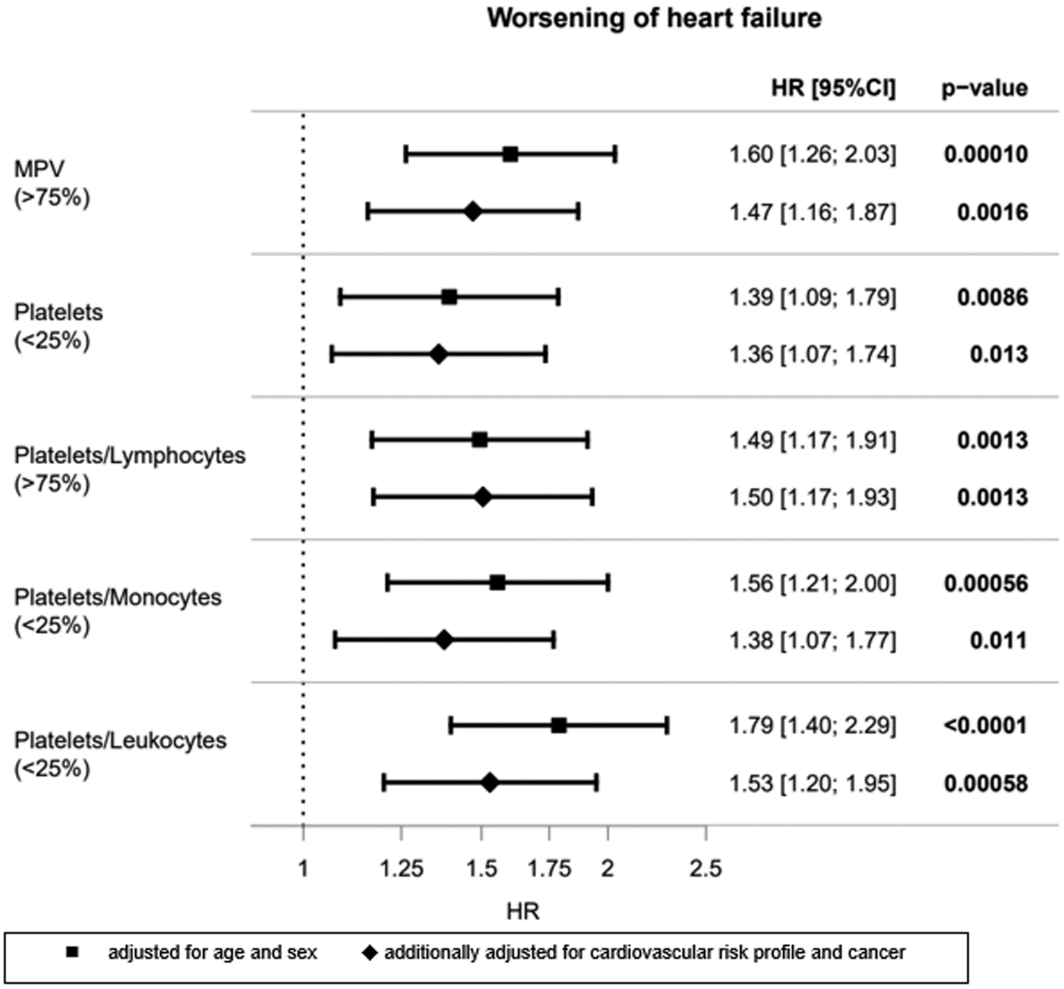
E. Worsening of HF according to platelet-to-lymphocyte ratio quartiles



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).

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.

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.

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.

Table S1. Characteristics of the study sample according to quartiles of platelet count (N = 3,250).
Table S2. Characteristics of study participants (N = 3,250) and according to HFpEF (N = 637) and HFrEF (N = 341).
Table S3. Quartiles of platelet indices.
Table S4. Relation between platelet indices and worsening of HF in HF phenotypes with additional adjustment for Antithrombotic medication (ATC code: B01).
Table S5. Relation between platelet indices and worsening of HF in total analysis sample and after excluding individuals with history of cancer.
Figure S1. Derivation of the analysis sample.
Figure S2. Boxplots for the distribution of cardiovascular diseases and comorbidities.
Figure S3. Scatter plots of LVEF and E/E' per increasing MPV (fL) or per increasing platelet count (10^9/L).
Figure S4. Cumulative incidence plot for worsening of HF in HFrEF and HFpEF.

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