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Circulating CD31$^+$/Annexin V$^+$ microparticles correlate with cardiovascular outcomes

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Aims CD31$^+$/Annexin V$^+$ microparticles (MPs) are increased in patients with cardiovascular risk factors and impaired coronary endothelial function. We evaluated whether MPs are an independent marker for cardiovascular events in patients with stable coronary artery disease (CAD).

Methods and results The number of CD31$^+$/Annexin V$^+$ MP was determined by flow cytometry in 200 patients (age 66.1 ± 10.4 years) and correlated with cardiovascular outcomes. The median follow-up time for major adverse cardiovascular and cerebral event (MACCE)-free survival was 6.1 (6.0/6.4) years. Four patients were lost to follow-up. A first MACCE occurred in 72 patients (37%). Microparticle levels were significantly higher in patients with MACCE compared with patients without event ($P = 0.004$). The prevalence of diabetes ($P = 0.02$) and male gender ($P = 0.05$) was significantly related to the MP level. In multivariate analysis (cardiovascular risk factors, number of diseased vessels, use of angiotensin-converting enzyme-inhibitors and statins), high MP levels were associated with a higher risk for cardiovascular death [Hazard ratio (HR) 4.0, 95% confidence interval (CI) 1.1–14.6; $P = 0.04$], the need for revascularization (HR 2.4, 95% CI 1.3–4.4; $P = 0.005$), and the occurrence of a first MACCE (HR 2.3, 95% CI 1.4–3.8; $P = 0.001$). Inclusion of the MP level into a classical risk factor model substantially increased *c*-statistics from 0.637 (95% CI: 0.557–0.717) to 0.702 (95% CI: 0.625–0.780) ($P = 0.03$).

Conclusion The level of circulating CD31$^+$/Annexin V$^+$ MPs is an independent predictor of cardiovascular events in stable CAD patients and may be useful for risk stratification.

Keywords Microparticles • Endothelial function • Prognosis • Risk factor • Coronary artery disease • Endothelium

Introduction

The vascular endothelium plays a pivotal role in the pathogenesis of atherosclerosis and its clinical manifestations coronary artery disease (CAD), myocardial infarction, heart failure, stroke, and peripheral artery disease. On the cellular level, endothelial dysfunction and consecutive atherosclerosis are based on a progressive loss of endothelial cells (ECs). Extended EC damage and apoptosis due to classical cardiovascular risk factors result in the loss of the endothelium's integrity. The consequences are an increased vascular permeability of the endothelium followed by facilitated migration of inflammatory cells, and proliferation of vascular smooth muscle cells, resulting in the manifestation of an atherosclerotic lesion.[1]

Cell apoptosis is commonly associated with conformational changes of the plasma membrane, condensation of the nucleus, followed by DNA fragmentation, and the release of small (submicroscopic) membranous particles, the so-called microparticles (MPs).[2] Microparticles are directly shed from circulating cells (erythrocytes, leucocytes, platelets) and ECs upon stimulation by activating agents or apoptosis-inducing factors.[3,4] Microparticles bear phospholipids (e.g. the negatively charged phosphatidylserine which binds to annexin V) and membrane proteins of their mother cell (e.g. CD144 and CD146 for ECs, CD31 for ECs and a proportion of platelets, CD42 and CD61 for platelets, CD45 for leucocytes) allowing partial differentiation between MPs.[5,6] Recent evidence suggests that MPs are abundantly released in patients with cardiovascular diseases. In case of atherosclerosis—the predominant

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pathology in cardiovascular disease—MPs derived from endothelial and inflammatory cells as well as platelets are increased.[4,5,7–19] Experimental and clinical studies suggest that endothelial dysfunction as an independent predictor of adverse events in CAD patients[20,21] can be assessed quantitatively by measurement of CD31$^+$/annexin V$^+$ MP plasma levels.[22,23]

We hypothesized that CD31$^+$/annexin V$^+$ MPs may contribute to risk stratification in CAD patients by adding information about cardiovascular outcomes beyond that obtained from classical cardiovascular risk factors.

Methods

Patients

Between March and November 2003, 200 patients with angiographically proven stable CAD were included in this study. Patients with malignant and inflammatory diseases or severe acute ischaemia other than myocardial ischaemia were excluded from the study. Informed consent was obtained from all patients. The study protocol was approved by the ethics committee of the University of the Saarland.

The classification of previous events and follow-up data was made on the basis of medical records and personal interviews. Causes of death were determined by examination of hospital records, autopsy reports, and medical files of the patients' general practitioners. Deaths due to cardiovascular causes included sudden cardiac death and deaths from myocardial infarction, CAD, stroke, or congestive heart failure.

Coronary angiography

Cardiac catheterization was performed according to the guidelines for coronary angiography of the American College of Cardiology and the American Heart Association.[24] Biplane ventriculography was performed in standard projections. The ejection fraction was calculated by dividing the end-diastolic and end-systolic left ventricular areas with the use of an automated computer system (Digital Cardiac Imaging Software, Philips). The extent of CAD was scored, by at least two independent interventional cardiologists, as 0 (stenosis $<50\%$), 1 (stenosis of any main coronary artery $\geq 50\%$), 2 (stenoses of two main coronary arteries $\geq 50\%$), and 3 (stenoses of three main coronary arteries $\geq 50\%$).

Laboratory methods

Arterial blood was drawn under sterile conditions from the femoral artery before cardiac catheterization and was buffered using sodium citrate. Additional blood samples for routine analyses were obtained.

Plasma derived from 10-mL citrate-buffered blood was immediately centrifuged at 13 000 *g* for 2 min to generate platelet-poor plasma. Fifty microlitre of platelet-poor plasma was incubated with 4 μL of phycoerythrin (PE)-conjugated monoclonal antibody against CD31 (PE Mouse Anti-Human CD31, clone: WM59, BD Pharmingen^{TM}) followed by the incubation with fluorescein isothiocyanate (FITC)-conjugated annexin V (FITC Annexin V, material no. 556419, BD Pharmingen^{TM}) according to the manufacturer's instructions (negative control: PE Mouse IgG1 κ Isotype Control, clone: MOPC-21, BD Pharmingen^{TM}). Fluorescence-activated cell sorting (FACS) analysis was performed immediately after staining using a FACSCalibur flow cytometer (Becton Dickinson Biosciences, San Jose, CA, USA). CD31$^+$/annexin V$^+$ MPs were defined as particles positively labelled for CD31 and annexin V. To reduce the number of MP derived from non-EC which may occasionally show low expression of CD31, only CD31^{bright} MP were selected. This approach does not allow exclusive measurement of EC-derived MPs but may imply that platelet-derived MPs are additionally measured. However, a recent study showed an acceptable correlation between levels of CD31$^+$/annexin V$^+$ and CD31$^+$/CD42$^-$ MPs in 104 patients ($r = 0.89$, $P < 0.001$).[25] Data were analysed using Cellquest software (Becton Dickinson).

Statistical analysis

The association between baseline levels of CD31$^+$/annexin V$^+$ MPs and the occurrence of a first major adverse cardiovascular and cerebral event (MACCE) including non-fatal myocardial infarction, revascularization by percutaneous coronary intervention (PCI) or coronary artery bypass graft (CABG), stroke, and death from cardiac causes was evaluated. Levels of CD31$^+$/annexin V$^+$ MPs were analysed after log-transformation (on a base 10 scale) to normalize distribution. In categorical analyses, we used the median of MPs (2373 events/μL) at the time of enrolment.

Data are presented as mean ± standard deviation if normally distributed and as medians with inter-quartile range if not normally distributed. Continuous variables were tested for normal distribution with the use of the Kolmogorov–Smirnov test. Means between two categories were compared with the use of a two-tailed, unpaired Student's *t*-test. The one-way analysis-of-variance test was used for comparisons of categorical variables. For *post hoc* analysis, the Bonferroni correction was applied. Multiple linear regression analysis was applied to identify factors that were independently associated with the level of CD31$^+$/annexin V$^+$ MPs. We performed multivariable Cox proportional hazard models to examine the association between MPs and incident events. In the Cox model, the level of CD31$^+$/annexin V$^+$ MPs was included as categorical covariate (median MP level). All models were adjusted for gender, hyperlipoproteinaemia, diabetes, number of diseased vessels, chronic kidney disease, concomitant use of angiotensin-converting enzyme-inhibitors (ACE-inhibitors), and statins. We confirmed that the proportionality of hazards assumption was met. Survival was determined with the use of the Kaplan–Meier method. The log-rank test was used to determine statistical differences in terms of survival. To compare the prognostic information provided by a classical risk factor model alone and in combination with the level of CD31$^+$/annexin V$^+$ MPs, associated receiver operating characteristic (ROC) curves for predicted probabilities were generated, and the corresponding areas under the curve along with their 95% CIs were calculated and compared.

Statistical significance was assumed when the null hypothesis could be rejected at $P < 0.05$. Statistical analysis was performed with the use of SPSS Statistics version 17.0.0 (SPSS, Inc., Chicago, IL, USA) and MedCalc version 11.1.1.0 (MedCalc Software, Mariakerke, Belgium).

The investigators initiated the study, had full access to and analysed the data, and wrote the manuscript. All authors vouch for the data and analysis.

Results

Baseline characteristics

A total of 200 patients with stable CAD as diagnosed on coronary angiography were enrolled. Of these, four patients (2%) were lost to follow-up. The average age of the patients was 66.1 ± 10.4 years with a high prevalence of cardiovascular risk factors: hypertension (84%), hyperlipoproteinaemia (84%), diabetes (28%), smoking (21%). The number of CD31$^+$/annexin V$^+$ MPs ranged

from 136 to 11795 events per microlitre platelet-poor plasma (median 2373 events/μL). Levels of CD31$^+$/annexin V$^+$ MPs were analysed after log-transformation (on a base 10 scale) to normalize distribution.

The prevalence of diabetes was higher in patients with CD31$^+$/annexin V$^+$ MP levels above the median (37 vs. 19%; $P = 0.006$). Concerning other cardiovascular risk factors and clinical variables, no significant differences in baseline characteristics were found according to CD31$^+$/annexin V$^+$ MP levels (*Table 1*).

CD31$^+$/Annexin V$^+$ microparticle level in relation to baseline characteristics

By multiple regression analysis that used the log-transformed CD31$^+$/annexin V$^+$ MP level as the dependent variable, the MP level was independently associated with male gender ($P = 0.05$) and the prevalence of diabetes ($P = 0.02$) (*Table 2*). The r^2 value of this multiple regression model was 0.14.

CD31$^+$/Annexin V$^+$ microparticle levels and clinical outcomes

The median follow-up time for MACCE-free survival was 6.1 (6.0/6.4) years. A total of 29 patients died (15%), 14 from cardiovascular causes (7%); other causes included sepsis (6/29), pneumonia (4/29), and cancer (5/29). A first MACCE occurred in 72 (37%) patients after a mean follow-up of 2.1 ± 2.0 years. Patients with CD31$^+$/annexin V$^+$ MP levels above the median (2373 events/μL) had significantly more cardiovascular events ($P = 0.001$) than patients with lower levels (*Table 3*, *Figure 1*). In particular, cardiovascular mortality (10 vs. 4%; $P = 0.05$) and the need for revascularization (35 vs. 18%; $P = 0.006$) were higher in patients with MP levels above the median. The prevalence of diabetes was not associated with an increased risk for cardiovascular events ($P = 0.23$).

Multivariate Cox proportional hazards analysis identified the level of circulating CD31$^+$/annexin V$^+$ MPs (HR 2.3, 95% CI: 1.4–3.8; $P = 0.001$) as an independent predictor of future MACCE. Furthermore, increased MP levels were associated with a higher risk of cardiovascular mortality (HR 4.0, 95% CI: 1.1–14.6; $P = 0.04$) and the need for revascularization (HR 2.4, 95% CI: 1.3–4.4; $P = 0.005$) in multivariate analysis (*Table 4*). No significant association was detected between CD31$^+$/annexin V$^+$ MPs and non-fatal myocardial infarction, stroke, and all-cause mortality.

Incremental prognostic value of CD31$^+$/Annexin V$^+$ microparticle level for risk stratification

Inclusion of the CD31$^+$/annexin V$^+$ MP level into a multivariate risk factor model (age, gender, hypertension, hyperlipoproteinaemia, smoking, diabetes, number of diseased vessels, concomitant use of ACE-inhibitors, beta-blockers, statins, and aspirin) increased the area under the curve (AUC) for the prediction of future MACCE from 0.637 (95% CI: 0.557–0.717) to 0.702 (95% CI: 0.625–0.780) ($P = 0.03$; *Figure 2*).

Discussion

In this prospective study of 200 patients, we sought to evaluate the prognostic value of circulating MP levels for the occurrence of cardiovascular events. We demonstrated that circulating CD31$^+$/annexin V$^+$ MPs are associated with a higher risk for a future MACCE in patients with stable CAD. During the observational period of 6 years, a significantly higher incidence of death from cardiovascular causes and need for revascularization was observed in CAD patients with an MP level above the median. Furthermore, increased CD31$^+$/annexin V$^+$ MP levels improved risk stratification of a classical risk factor model by a substantial increase in *c*-statistics and were associated with outcomes.

In a recent study, Nozaki *et al.*[26] assessed the prognostic value of CD144$^+$ MP levels as part of a multimarker strategy in a heterogeneous high-risk population for CAD. They demonstrated that increased MP levels were independent predictors of cardiovascular death and acute coronary syndromes. Our study extends this finding as it shows that circulating CD31$^+$/annexin V$^+$ MPs are significantly associated with the risk for future cardiovascular death and the need for revascularization during long-term follow-up in a population with angiographically proven, stable CAD. CD31$^+$/annexin V$^+$ MPs might significantly contribute to risk stratification in a CAD population.

Several studies show that MPs are increased in conditions of systemic cell damage, such as in patients with thrombotic thrombocytopenic purpura, lupus anticoagulant, multiple sclerosis, end-stage renal failure, and cardiovascular diseases.[5,27,28] Elevated MP levels are also associated with many cardiovascular risk factors, such as obesity,[29] hyperlipoproteinaemia,[9,30] hypertension,[12] and diabetes.[13,31,32] In our study, baseline CD31$^+$/annexin V$^+$ MPs differed significantly between diabetics and non-diabetics ($P = 0.004$). Surprisingly, this fact did not translate into a significantly different MACCE rate and, thus, has to be regarded as a problem of sample size since we observed a non-significant trend towards higher event rates in diabetics (44 vs. 34% for non-diabetics, $P = 0.23$). One other explanation for this observation may be that the summation of cardiovascular risk factors—expressed by a higher MP level—provides better prediction of outcome than a single risk factor alone.

Microparticles are released from various circulating blood cells: leucocytes, erythrocytes, and platelets as well as from ECs.[5,6,10] Thus, they express several different cell surface markers. The origin of CD31$^+$/annexin V$^+$ MPs are platelets and the endothelium.[5] Own *in vitro* experiments demonstrate an expression of the CD31 antigen of ~5% on activated and 10% of non-activated platelets, respectively. In case of EC apoptosis—the predominant pathology in cardiovascular disease—the relative part of EC-derived MPs detected by the combination of CD31 and annexin V is higher than in EC activation.[3,5,10] Furthermore, platelet MPs are also increased in acute coronary syndrome, hypertension, and diabetes. Thus, we cannot rule out that a proportion of CD31$^+$/annexin V$^+$ MPs measured in our study are derived from platelets. However, Yun *et al.*[25] demonstrated an acceptable correlation between levels of CD31$^+$/annexin V$^+$ MPs and CD31$^+$/CD42$^-$ EMPs in 104 patients ($r = 0.89$, $P < 0.001$) suggesting that both combinations of surface marker measure a similar,

Table 1 Baseline characteristics of the study population

Characteristic	Total (n = 196)	Low MP level[a] (n = 98)	High MP level[a] (n = 98)	P-value
Age, years	66.1 ± 10.4	66.0 ± 10.8	66.1 ± 10.1	0.98
Gender, no. (%)				0.13
Female	59 (30.1)	34 (34.7)	25 (24.7)	
Male	137 (69.9)	64 (65.3)	73 (75.3)	
Cardiovascular risk factors, no. (%)				
Arterial hypertension	164 (83.7)	80 (81.6)	84 (85.7)	0.34
Hyperlipoproteinaemia	164 (83.7)	81 (82.7)	83 (84.7)	0.58
Diabetes	55 (28.1)	19 (19.4)	36 (36.7)	0.006
Family history of CAD	28 (14.3)	11 (11.2)	17 (17.3)	0.21
Smoking	41 (20.9)	22 (22.4)	19 (19.4)	0.62
Body-mass index, kg/m^2	28.0 ± 4.7	28.0 ± 5.1	28.0 ± 4.3	0.97
Laboratory parameters				
Cholesterol (mg/dL)	193 ± 43	192 ± 41	194 ± 46	0.78
LDL cholesterol (mg/dL)	116 ± 36	115 ± 38	118 ± 35	0.60
HDL cholesterol (mg/dL)	47 (42/45)	47 (42/56)	47 (42/55)	0.88
Triglycerides (mg/dL)	133 (95/176)	132 (98/177)	138 (90/176)	0.92
Serum creatinine (mg/dL)	1.0 (0.8/1.1)	0.9 (0.8/1.1)	1.0 (0.8/1.2)	0.38
Glomerular filtration rate (mL/min)	79.2 ± 24.8	79.8 ± 27.1	78.7 ± 22.4	0.76
Leucocytes (10^9/L)	7.2 ± 2.0	7.0 ± 1.9	7.4 ± 2.1	0.23
C-reactive protein (mg/L)	<3.0 (<3.0/10.4)	<3.0 (<3.0/10.3)	<3.0 (<3.0/11.5)	0.84
Medical history, no. (%)				
Myocardial infarction				
Acute (<24 h)	4 (2.0)	1 (1.0)	3 (3.1)	0.31
Subacute (24 h–7 days)	15 (7.7)	7 (7.1)	8 (8.2)	0.77
Previous MI (6 months ago)	60 (30.6)	30 (30.6)	30 (30.6)	0.96
Stroke	10 (5.1)	3 (3.1)	7 (7.1)	0.19
Chronic kidney disease	40 (20.4)	18 (18.4)	22 (22.4)	0.46
PCI				
Current	79 (40.3)	39 (39.8)	40 (40.8)	0.84
Previous	79 (40.3)	41 (41.8)	38 (38.8)	0.71
Coronary artery disease, no. (%)				0.97
1 Vessel	52 (26.5)	26 (26.5)	26 (26.5)	
2 Vessels	54 (27.6)	26 (26.5)	28 (28.6)	
3 Vessels	71 (36.2)	37 (37.8)	34 (34.7)	
Stenosis <50%	19 (9.7)	9 (9.2)	10 (10.2)	
Left ventricular ejection fraction, %	58.9 ± 15.7	59.9 ± 14.6	57.8 ± 16.9	0.38
Medication on admission, no. (%)				
ACE-inhibitors	113 (57.7)	52 (53.1)	61 (62.2)	0.17
Angiotensin receptor-blockers	19 (9.7)	9 (9.2)	10 (10.2)	0.79
Beta-blockers	128 (65.3)	64 (65.3)	64 (65.3)	0.92
Calcium-channel blockers	30 (15.3)	16 (16.3)	14 (14.3)	0.71
Diuretics	74 (37.8)	37 (37.8)	37 (37.8)	0.96
Statins	111 (56.6)	54 (55.1)	57 (58.2)	0.61
Nitrates	64 (32.7)	36 (36.7)	28 (28.6)	0.24
Aspirin	133 (67.9)	65 (66.3)	68 (69.4)	0.57
Clopidogrel	40 (20.4)	19 (19.4)	21 (21.4)	0.70

MP, microparticle; LDL, low-density lipoprotein; HDL, high-density lipoprotein; MI, myocardial infarction; PCI, percutaneous coronary intervention; and ACE, angiotensin-converting enzyme. Chronic kidney disease was defined as a glomerular filtration rate <60 mL/min.
[a]Cut-off value: 2373 events/μL (median).

however not identical, population of MPs. Interestingly, binding of platelet MPs with fibrinogen is also involved in thrombus formation *in vitro* suggesting an additional role in the process of atherosclerosis and, thus, might contribute to risk stratification in CAD patients by reflecting the procoagulatory potential of the individual.[11,12,17,18] Future studies will need to address the role of different MP subtypes on prognosis in patients with CAD.

Recent data support the hypothesis that MPs contribute to vascular homoeostasis and the pathogenesis of cardiovascular diseases, including inflammation and vascular dysfunction, in addition to their well-known action on the coagulation process.[5,33] Such data have led to the concept that MPs are key factors at the crossroads between inflammation, coagulation, and vascular repair.[4,34] The mechanisms depend on the cellular origin of MPs and may trigger differential consequences on vascular remodeling. The most important impact is probably on alteration of the shear stress-induced response, which is important in the processes leading to atherosclerosis.[34]

Increasing evidence suggests that the balance of EC apoptosis and EC regeneration may determine the degree and progression of atherosclerosis. The influence of MPs on endothelial progenitor cell (EPC) migration *in vitro* suggests a close interaction between EPC and EC apoptosis at the vascular wall.[35] Pirro *et al.*[28,30] recently demonstrated that MP release is caused by injured EPC, which are exposed to a pro-apoptotic milieu in the presence of several cardiovascular risk factors. Recently, Zernecke *et al.*[14] showed that EC-derived apoptotic bodies as paracrine alarm signals in response to tissue damage trigger the microRNA-126 (miR-126)-mediated production of the CXC chemokine CXCL12 and its receptor CXCR4, which counteract apoptosis and recruit progenitor cells. In this study, administration of apoptotic bodies or miR-126 limited atherosclerosis, promoted the incorporation of Sca-1$^+$ progenitor cells, and conferred features of plaque stability in different mouse models of atherosclerosis. Simultaneous assessment of endothelial progenitors and MP subtypes in patients with CAD should unravel potential additive effects on cardiovascular outcome prediction.[36]

In conclusion, measurement of circulating MP may serve as a useful tool, allowing us to mirror the actual detrimental effects of cardiovascular risk factors with one integrative marker. Larger studies are needed to further validate this promising cellular biomarker and to elucidate the value of MP measurement for risk stratification in patients with stable CAD.

Table 2 Independent association of the level of circulating CD31$^+$/Annexin V$^+$ microparticles with baseline characteristics

	B (95% CI)	*P*-value
Age	−0.05 (−0.11 to 0.01)	0.57
Male gender	−0.16 (−0.34 to 0.00)	0.05
BMI	−0.02 (−0.03 to 0.01)	0.70
Arterial hypertension	0.02 (−0.20 to 0.24)	0.84
Hyperlipoproteinaemia	0.07 (−0.12 to 0.31)	0.39
Diabetes	0.20 (0.04 to −0.40)	0.02
Family history of CAD	0.05 (−0.16 to 0.29)	0.55
Smoking	−0.04 (−0.26 to 0.16)	0.66
History of myocardial infarction	0.03 (−0.13 to 0.20)	0.67
History of stroke	0.09 (−0.16 to 0.57)	0.26
Chronic kidney disease	0.13 (−0.03 to 0.33)	0.10
No. of diseased vessels	−0.14 (−0.24 to −0.01)	0.08
Left ventricular ejection fraction	0.00 (−0.01 to 0.01)	0.98
ACE-inhibitors	0.07 (−0.10 to 0.23)	0.42
Beta-blockers	0.02 (−0.17 to 0.20)	0.87
Statins	−0.07 (−0.24 to 0.11)	0.48
Aspirin	0.05 (−0.12 to 0.23)	0.54
C-reactive protein (mg/L)	0.06 (−0.08 to 0.20)	0.57

Multiple linear regression analysis. CI indicates confidence interval; B, standardized B coefficient; BMI, body-mass index; and ACE, angiotensin converting enzyme.

Study limitations

At present, it remains unclear whether increased numbers of CD31$^+$/annexin V$^+$ MPs in CAD patients are increased because of a mechanical or (bio-)chemical injury or whether

Table 3 Number of events (percentage) during follow-up

Event	Total (n = 196)	Low MP level[a] (n = 98)	High MP level[a] (n = 98)	*P*-value
Cardiovascular mortality	14 (7.1)	4 (4.1)	10 (10.2)	0.05
All-cause mortality	29 (14.8)	13 (13.3)	16 (16.3)	0.29
Non-fatal myocardial infarction	8 (4.1)	3 (3.1)	5 (5.1)	0.34
Stroke	12 (6.1)	5 (5.1)	7 (7.1)	0.32
PCI	43 (21.9)	16 (16.3)	27 (27.6)	0.03
CABG	9 (4.6)	2 (2.0)	7 (7.1)	0.06
Revascularization	52 (26.5)	18 (18.4)	34 (34.7)	0.006
First MACE	66 (33.7)	22 (22.4)	44 (44.9)	0.001
First MACCE	72 (36.7)	25 (25.5)	47 (48.0)	0.001

MP, microparticle; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; and MAC(C)E, major adverse cardiovascular (and cerebral) event.
[a]Cut-off value: 2373 events/μL (median).

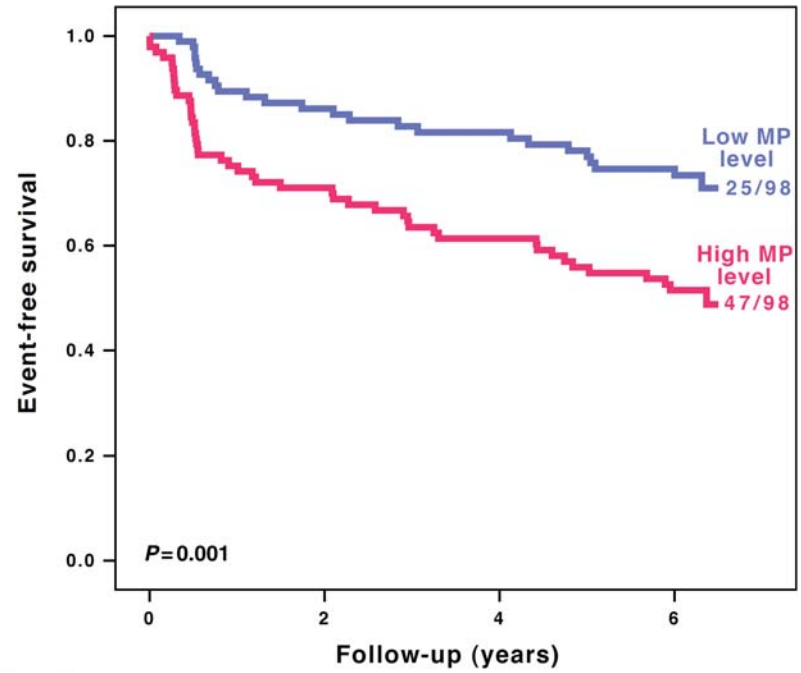


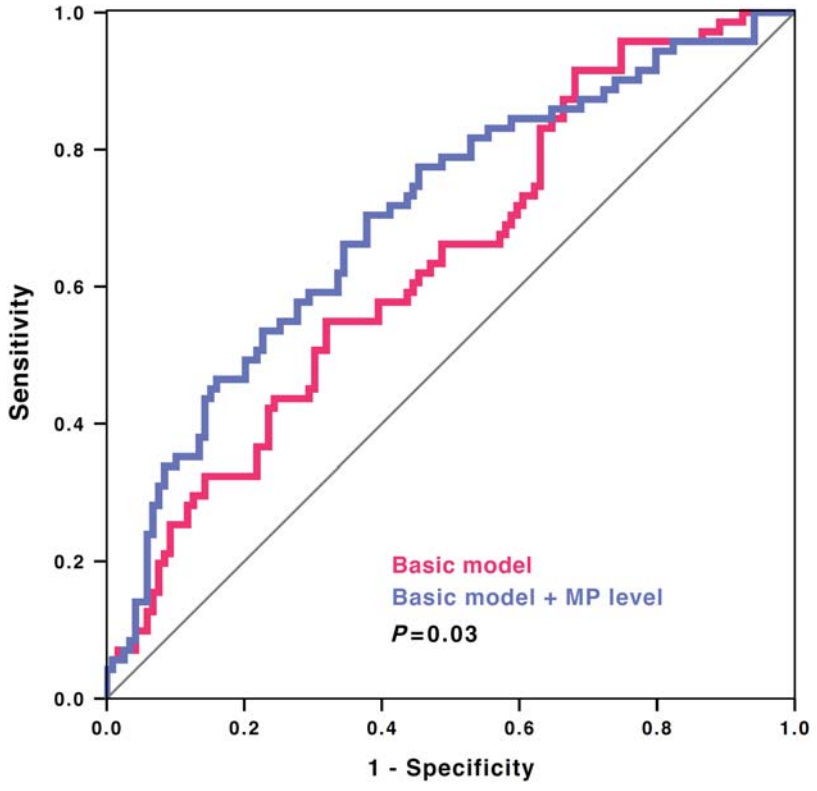
Figure 1 Major adverse cardiovascular and cerebral event rate according to level of CD31$^+$/Annexin V$^+$ microparticles. Survival rate free of a first major adverse cardiovascular and cerebral event according to the level of CD31$^+$/Annexin V$^+$ microparticles. Cut-off value: 2373 events/μL (median).

Table 4 Cox regression analysis for cardiovascular outcomes according to the median level[a] of CD31$^+$/Annexin V$^+$ microparticles

	Univariate		Multivariate	
	HR (95% CI)	***P*-value**	**HR (95% CI)**	***P*-value**
Cardiovascular mortality	4.2 (1.1–16.3)	0.04	4.0 (1.1–14.6)	0.04
All-cause mortality	1.6 (0.7–3.7)	0.26	1.3 (0.6–2.7)	0.48
Non-fatal myocardial infarction	2.5 (0.5–12.3)	0.27	2.3 (0.5–10.8)	0.29
Stroke	2.3 (0.7–8.4)	0.18	2.2 (0.7–6.9)	0.19
PCI	2.3 (1.2–4.4)	0.02	2.0 (1.0–3.8)	0.04
CABG	4.3 (0.8–22.5)	0.09	4.1 (0.9–19.8)	0.08
Revascularization	2.5 (1.4–4.7)	0.003	2.4 (1.3–4.4)	0.005
First MACE	2.9 (1.6–5.0)	<0.001	2.3 (1.3–3.9)	0.002
First MACCE	2.7 (1.6–4.5)	<0.001	2.3 (1.4–3.8)	0.001

Multivariate analysis included gender, hyperlipoproteinaemia, diabetes, number of diseased vessels, chronic kidney disease, and concomitant use of angiotensin-converting enzyme-inhibitors, and statins. MP, microparticle; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; and MAC(C)E, major adverse cardiovascular (and cerebral) event.
[a]Cut-off value: 2373 events/μL (median).

ECs try to eliminate noxious agents. Owing to the dual expression of CD31 on ECs and platelets, platelet-derived MPs were additionally measured. It will be most intriguing to determine the role of MP subtypes derived from platelets, leucocytes, or monocytes as predictors for cardiovascular morbidity and mortality. One may speculate that, for example, monocytic MPs may have additional impact in patients with cardiovascular disease. Finally, it is necessary to standardize the MP assay for



	AUC (95% CI)	SE	*P*-value
Basic model	0.637 (0.557–0.717)	0.0409	
Basic model + MP level	0.702 (0.625–0.780)	0.0395	0.03

Figure 2 Incremental effect of the level of $CD31^+$/Annexin V^+ microparticles in addition to traditional risk factors. Data from a receiver operating characteristic curve analysis concerning a first major adverse cardiovascular and cerebral event. The basic model includes information on age, gender, hypertension, hyperlipoproteinaemia, smoking, diabetes, number of diseased vessels, chronic kidney disease, concomitant use of angiotensin-converting enzyme-inhibitors, beta-blockers, statins, and aspirin. The MP level was treated as log-transformed continuous variable. AUC, area under the curve; CI, confidence interval; and MP, microparticle.

the development and establishment of clinical routine tests in a larger cohort.

Conclusions

Our results suggest that circulating $CD31^+$/annexin V^+ MPs provide information about prognosis and the risk for a future MACCE beyond that obtained from classical cardiovascular risk factors and, thus, might contribute to risk stratification in stable CAD patients.

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