Journal of Molecular and Cellular Cardiology 138 (2020) 110–114

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Short communication

# Plasma signature of apoptotic microvesicles is associated with endothelial dysfunction and plaque rupture in acute coronary syndromes

Effimia Zacharia[a,1], Alexios S. Antonopoulos[a,b,*,1], Evangelos Oikonomou[a], Nikolaos Papageorgiou[a], Zoi Pallantza[a], Antigoni Miliou[a], Vasiliki Chara Mystakidi[a], Spyridon Simantiris[a], Anastasios Kriebardis[a], Nikolaos Orologas[a], Eftychia Valasiadi[a], Spyridon Papaioannou[a], Nikolaos Galiatsatos[a], Charalambos Antoniades[a,b], Dimitris Tousoulis[a]

[a] 1st Department of Cardiology, National and Kapodistrian University of Athens Medical School, Hippokration Hospital, Athens, Greece
[b] RDM Division of Cardiovascular Medicine, University of Oxford, Oxford, UK

ARTICLE INFO

*Keywords:*
Microvesicles
Acute coronary syndromes
Apoptotic
Endothelial dysfunction
Plaque rupture

ABSTRACT

*Objective:* Circulating microvesicles (MV) are surrogate biomarkers of atherosclerosis. However, their role in acute coronary syndromes (ACS) has not been fully elucidated yet. We sought to examine the association of circulating apoptotic MVs with ACS pathophysiology.

*Approach and results:* One hundred and fifty-three patients ($n = 153$) were included in the study; 49 patients with ST-elevation myocardial infarction (STEMI), 35 with non-STEMI (NSTEMI), 38 with unstable angina, 15 with stable coronary artery disease and 16 control individuals. Flow cytometry analysis was used to quantify circulating apoptotic/non-apoptotic (phospatidyloserine$^+$/phospatidyloserine$^-$) endothelial cell (EMV), red blood cell (RMV) and platelet (PMV) derived MV. Flow-mediated dilatation (FMD) of the brachial artery was assessed by ultrasound to estimate endothelial function. The inflammatory profile was assessed by serum C-reactive protein (hsCRP) levels. Apoptotic only (but not non-apoptotic) MV were increased in patients with ACS (EMV, $P = 2.32 \times 10^{-9}$; RMV, $P = .0019$; PMV, $P = .01$). Hierarchical clustering of the total population of ACS patients ($n = 122$) by circulating levels of phospatidyloserine$^+$ EMV, RMV and PMV identified two discreet clusters of patients without any differences in traditional risk factors, but significant differences in brachial FMD (5.2% (2.5) vs. 4.1% (2.3), $P < .05$) that remained significant after adjustment for co-variates. The prevalence of STEMI, a surrogate for plaque rupture and vessel thrombotic occlusion, was significantly higher in the patient cluster with impaired endothelial function (60% vs 32%, $P = .016$, adjusted odds ratio for STEMI, 3.041, 95%CI, 1.160 to 7.968, $p = .024$).

*Conclusion:* Our findings indicate that the circulating levels of apoptotic MV are increased in ACS patients and their plasma profiles associate with endothelial dysfunction and thrombotic complications in ACS patients.

## 1. Introduction

Microvesicles (MV) are defined as cell-derived vesicles that are under 1 μm in diameter, do not carry a nucleus or nuclear remnants or present synthetic capacity, but contain cytoskeletal proteins and expose a certain amount of phosphatidylserine on their surface [1]. Phosphatidylserine is a membrane phospholipid that is actively held toward the cytosolic side of the cell membrane by the enzyme flippase. During apoptosis, phosphatidylserine is no longer restricted by flippase, whereas scramblase permits the rapid flip-flop of phosphatidylserine between the two sides of the membrane. The presence of phosphatidylserine on a MV surface acts as a signal for macrophage phagocytosis [2]; at the same time, phosphatidylserine permits the binding of annexin. Thus, annexin-binding indicates that MV blebbing has taken place due to cell apoptosis.

Over the last two decades, several studies have provided evidence on the role of MV as biomarkers of atherosclerosis. Circulating apoptotic MV have been associated with endothelial function impairment in patients with stable coronary artery disease (SCAD) [3], as they may cause premature endothelial senescence and dysfunction [4,5]. Apoptotic MV may also participate in inflammatory mechanisms; for example the levels of apoptotic MV positively correlate with the levels of

* Corresponding author at: Vasilissis Sofias 114, PO 11528, Hippokration Hospital, Athens, Greece.
*E-mail address:* alexios.antonopoulos@cardiov.ox.ac.uk (A.S. Antonopoulos).
[1] Joint first authors

https://doi.org/10.1016/j.yjmcc.2019.11.153
Received 2 October 2019; Received in revised form 4 November 2019; Accepted 21 November 2019
Available online 27 November 2019

high-sensitivity C-reactive protein (hs-CRP) [6,7]. Although there are reports that circulating apoptotic MV are elevated in small cohorts of patients with acute coronary syndromes (ACS) [8] and sudden cardiac death, their exact role in the pathophysiology of ACS remains unlcear.

In the present study we aimed to examine the role of both apoptotic (phosphatidylserine$^+$) and non-apoptotic (phosphatidylserine$^-$) MV in patients with ACS as well as their role in the pathophysiology of plaque rupture. Our aim was to a) investigate whether plasma MVs are increased in patients with ACS and b) explore associations between circulating plasma MVs and patient-related risk profile in ACS.

## 2. Material & methods

### 2.1. Study population

The present study included a total of 153 patients. A consecutive sample of 122 ACS patients was included: 49 patients with ST elevation myocardial infarction (STEMI), 35 patients with non-STEMI (NSTEMI) and 38 patients with unstable angina (UA) that presented to the emergency department of a Hippokration General Hospital of Athens between February 2016 and July 2016. The control groups included 15 patients with documented SCAD and 16 individuals undergoing elective diagnostic coronary angiography with normal coronary arteries (control group). Subjects with history of malignant or inflammatory disease, deep vein thrombosis/pulmonary embolism, renal failure (creatinine > 2 mg/dL) or age of > 75 years were excluded from the study. The initiation of clinical symptoms was reported by the patients to be at ≤2 h prior to hospital admission. Informed consent was obtained from all patients. The study protocol was approved by the Local Research Ethics committee of Hippokration General Hospital of Athens and conformed to the principles outlined in the Declaration of Helsinki.

### 2.2. Blood sampling

Citrated blood samples were obtained from an antecubital vein using a 21 Gauge catheter (BD Vacutainer) at 12–24 h after hospital admission. This specific time point was selected based on the results of previous studies that have shown that the levels of circulating MV in the plasma of patients with ACS peak at 12–24 h after the clinical event. The blood samples were processed within 30 min of venipuncture with two successive centrifugations at 2.500 RCF for 15 min at 20 °C, as previously described. The final platelet–free plasma (PFP) supernatant was immediately frozen and stored at −80 °C.

Plasma hs-cardiac troponin I and high-sensitivity C-reactive protein (CRP) levels were measured as representative of the extent of myocardial infarction and inflammation levels.

### 2.3. Flow cytometry

Flow cytometry analysis of circulating MV was performed within 15 min from thawing. Blood samples were strictly subjected to one freeze-thaw cycle. MV were identified by their size (< 1 μm), their annexin-binding capacity and/or the exposure of cell-specific surface antigens, as follows: Megamix beads (BioCytex) of 0.5 μm, 0.9 μm and 3 μm were used to standardize the set-up of MV analysis region. To distinguish apoptotic from non-apoptotic MV, the samples were stained with AnnexinV (PE Annexin V, 556422, BD). Annexin V binds to PS surface; thus, apoptotic MVP that express PS on their surface are stained positive for annexin V (phosphatidylserine$^+$), whereas non-apoptotic MV stained negative for annexin V (phosphatidylserine$^-$).

MV were further stained with the following three monoclonal antibodies: vascular-endothelial (VE)-cadherin (CD144)–Alexa Fluor 647 (clone 55-7H1, 561,567, BD); Glycophorin-A (CD235a)–PE-Cy7 (clone GA-R2, HIR2, 563,666); and integrin-a2b (CD41a)–PE-Cy5 (clone HIP8, 559,768). MV that were stained positive for VE-cadherin were identified as endothelial- derived MV (EMV); MV that were stained positive for Glycophorin-A were identified as red blood cell-derived MV (RMV); and MV that were stained positive for integrin-a2b were identified as platelet-derived MV (PMV). Briefly, the staining protocol was as follows: 10 μL of the patients' PFP were re-suspended in 190 μL of diluted Annexin V binding buffer 10× concentrate (556,454, BD), which originally contained 25 mM of $CaCL_2$ solution. 5 μL of PE-AnnV, 5 μL of PE-Cy7 CD235a, 5 μL of Alexa Fluor-CD144 and 20 μL of PE-Cy5-CD41a were subsequently added. The samples were incubated for 15 min in a dark room at room temperature and the reaction was stopped by the addition of 400 μL of the diluted binding buffer. The samples were analyzed immediately in a FACSCanto flow cytometer (BD). Data were analyzed from 100,000 events with the aid of FACSDiva Software version 6.1.3 (BD) and with the aid of FlowJo version 10.4.1 for comparison and confirmation. TruCount beads (BD) were used to calculate the absolute number of circulating MV in plasma.

### 2.4. Assessment of endothelial function

Brachial artery flow-mediated dilatation (FMD) was determined by successive ultrasound measurements to assess endothelial function on the second day of hospitalization. In those patients that had undergone primary angioplasty from the radial artery, the contralateral brachial artery was studied, as recent radial catheterization may impair endothelial function [9]. FMD was reported as the percentage of maximum dilatation.

### 2.5. Statistical analysis

Data are presented as mean (standard deviation) if normally distributed and as median [interquartile range] if non-normally distributed. Shapiro-Wilk's test ($p > .05$) was used to determine whether the continuous variables were normally distributed. One-way analysis of variance, followed by Brown-Forsythe post-hoc test, and Kruskal-Wallis test were used for multiple comparisons, where appropriate. Differences in baseline characteristics were assessed with Chi Square. To perform hierarchical clustering of the population by plasma MV levels, the optimal number of clusters was first tested using *factoextra* R package. Hierarchical clustering of the population of patients with ACS based on levels of apoptotic MV was performed by *hclust* R package using Ward's method. The variation of apoptotic MV across the observation of the study population was represented on a relevant heat map with a row dendrogram indicating the clustering of patients. The differences in the presence of risk factors and variables of interest between clusters was tested by using chi-square or *t*-test as appropriate. The results of the chi-square or (prevalence of STEMI) and t-test (FMD) were internally validated by bootstrapping based on 2000 repetitions. A two-tailed *p*-value of < 0.05 was considered to be statistically significant. Statistical analysis was performed with the use of IBM SPSS Statistics Version 20 and R statistical package (v3.5.1).

## 3. Results

### 3.1. Levels of circulating MVs in acute coronary syndromes

The study population of ACS patients consisted of a typical cohort of high-risk patients, 84.4% males, mean age 60.2 (10.4) years) and with presence of multiple risk factors. The demographic characteristics of the study population are summarized in Table 1.

There were significant differences in the number of circulating MV across patient groups, i.e. controls, SCAD, UA and STEMI/NSTEMI (for EMV Kruskal Wallis $P = .0001$; for RMV Kruskal Wallis $P = .034$; for PMV Kruskal Wallis $P = .076$). These differences were driven by the differences in the number of apoptotic MV (Fig. 1a-c). There was a stepwise increase in the levels of circulating apoptotic MV across patient subgroups from controls to SCAD, UA and STEMI/NSTEMI patients. Conversely, there were no significant differences in the number

**Table 1**
Population demographics.

| | Acute coronary syndromes | | | SCAD | Controls | |
|---|---|---|---|---|---|---|
| | STEMI ($n$ = 49) | NSTEMI ($n$ = 35) | UA ($n$ = 38) | ($n$ = 15) | ($n$ = 16) | $P$-Value |
| Age, years | 56 (9.9) | 63.8(10.1) | 61.3 (9.9) | 62.4 (7.3) | 52 (11.9) | 0.001 |
| Males, n (%) | 45 (91.8) | 26 (74.3) | 32 (84.2) | 12 (80) | 11 (68.8) | 0.148 |
| Dyslipidemia, n (%) | 38 (77.6) | 25 (71.4) | 24 (63.2) | 6 (40) | 5 (31.3) | 0.387 |
| Diabetes mellitus, n (%) | 11 (22.4) | 11 (31.4) | 8 (21.1) | 5 (33.3) | 1 (6.3) | 0.289 |
| Hypertension, n (%) | 18 (36.7) | 12 (34.3) | 20 (52.6) | 9 (60) | 5 (31.3) | 0.190 |
| Current Smokers, n (%) | 36 (73.5) | 16 (45.7) | 11 (28.9) | 2 (13.3) | 2 (12.5) | 0.000 |
| BMI (kg/m$^2$) | 27.8 (6.3) | 29.8 (6.36) | 28.6(3.6) | 28.7(4.5) | 26.4 (3.8) | 0.224 |
| Family History of CAD | 15 (30.6) | 10 (28.6) | 11 (28.9) | 7 (46.7) | 4 (25) | 0.734 |
| History of AMI | 6 (12.2) | 11 (31.4) | 22 (57.9) | 5 (33.3) | 0 | 0.000 |
| History of PCI | 6 (12.2) | 7 (20) | 22 (57.9) | 3 (20) | 0 | 0.000 |
| History of CABG | 0 | 5 (14.3) | 7 (18.4) | 3 (20) | 0 | 0.015 |
| Peak troponin levels (pg/mL) | 50,000 (23,058–108,980) | 3849 (331–5,588) | 9.7 (4.4–18) | 4 (2 − 12) | 4 (1.4–7) | 0.000 |
| LVEF% | 43 [35–47] | 47 [40–55] | 50 [43–55] | 60 [41–60] | 60 [58–62] | 0.000 |
| FMD (%) | 4.6 (2.2) | 4.1 (2) | 4.9 (2.3) | 5.8 (2.6) | 6.2 (2.1) | 0.620 |

AMI: acute myocardial infarction; BMI: body mass index; CABG: coronary artery by-pass graft; CAD: coronary artery disease; FMD: flow-mediated dilatation; LVEF: left ventricular ejection fraction; NSTEMI: non ST-elevation myocardial infarction; PCI: Percutaneous coronary intervention; SCAD: stable coronary artery disease; STEMI: ST-elevation myocardial infarction; UA: unstable angina

of non-apoptotic MV between patient subgroups (for EMV Kruskal-Wallis $P$ = .075; for RMV Kruskal-Wallis $P$ = .413; for PMV Kruskal-Wallis $P$ = .326).

### 3.2. Signature of plasma microvesicles and plaque rupture

Having observed significant differences in the levels of apoptotic MV across groups, we then explored whether the signature of circulating apoptotic MV could be used to offer information on ACS phenotype. To this aim we used an unbiased approach performing unsupervised clustering of the study population with ACS based on the circulating levels of apoptotic MV. The resulting clustering of the population is graphically represented on a heat map with a row dendrogram indicating the patient clustering based on apoptotic MV levels (Fig. 1d). The association between the levels of apoptotic MV (EMV/RMV/PMV) in the ACS cohort is represented on a three-dimensional scatterplot (cloud plot, Fig. 1e). The two MV clusters had significant differences in the prevalence of STEMI (60% vs 32%, $P$ = .016). There were significant differences in the extent of myocardial necrosis as assessed by circulating cardiac troponin I levels (Fig. 1f), as expected due to a higher prevalence of STEMI. The difference in the prevalence of STEMI could not be explained by difference in traditional risk factors (p = NS for all, not shown) or differences in plasma CRP levels between clusters (Fig. 1h).

Notably though, the MV cluster with the higher MV levels (MV cluster B) had also significantly lower brachial artery endothelial function (Fig. 1j). After internal validation by bootstrapping the difference in brachial FMD remained significant (mean FMD difference of −1.07%, 95%CI -1.92 to −0.26). In multivariate regression analysis the association of MV clusters with FMD remained significant after adjustment for age, sex, presence of diabetes mellitus, arterial hypertension, dyslipidaemia, smoking status, history of acute myocardial infarction or percutaneous coronary intervention as well as the extent of MI assessed by circulating cardiac troponin I levels (for MV cluster B vs A: b = −0.115, [95%CI: −0.230 to −0.001], $p$ = .048).

The difference in the prevalence of STEMI between MV clusters remained statistically significant even after adjustment for age, sex, presence of diabetes mellitus, arterial hypertension, dyslipidaemia, smoking status, history of acute myocardial infarction or percutaneous coronary intervention and FMD (for MV cluster B vs. A: OR for STEMI 3.041, [95%CI: 1.160 to 7.968], $p$ = .24), suggesting a possible association between plasma MV signature, endothelial dysfunction, and thrombotic complications during ACS.

## 4. Discussion

In the present study we provide clinical evidence for the role of plasma MV in the pathophysiology of ACS. We demonstrate that circulating apoptotic EMV, RMV and PMV are significantly increased in patients with ACS. Moreover MV plasma profiling in ACS patients identifies a subgroup of ACS patients with lower endothelial function and a twofold increased prevalence of STEMI in the absence of differences in patient clinical profile. These observations suggest a possible mechanistic link between plasma MV, endothelial dysfunction and thrombotic complications in the setting of ACS.

### 4.1. Apoptotic vs. non-apoptotic microvesicles in acute coronary syndromes

The role of circulating MV as surrogate biomarkers of atherosclerosis has been a field of intensive research over the recent years. However, studies have so far focused on apoptotic MV. There is preliminary evidence that apoptotic MV are increased in the setting of ACS and can therefore be considered as biomarkers of atherothrombosis; however, the role of non-apoptotic MV in ACS is less-well studied. In the present study we investigated the role of both apoptotic and non-apoptotic circulating MV in the pathophysiology of ACS. The presence of MV that do not carry phosphatidylserine on their surface (non-apoptotic) in patients with ACS has been reported before; Skeppholm et al. [10] were the first to acknowledge the presence of phosphatidylserine$^-$ MV in ACS patients. In our study, the population of apoptotic MV was 15–20% compared to that of non-apoptotic MV for all MV subtypes. Currently, it remains unknown whether the ratio of apoptotic vs non-apoptotic EMV, RMV or PMV has any biological significance in coronary atherosclerosis. In our study we did not find a statistically significant difference in the levels of non-apoptotic circulating MV across the patient subgroups or association with ACS. However, there were significant differences in circulating apoptotic MV across patient subgroups, with the highest level observed in ACS patients. Thus, our findings support that only the population of apoptotic MV associates with CAD status and ACS pathogenesis.

### 4.2. Plasma signatures of apoptotic microvesicles, plaque rupture and thrombotic complication in acute coronary syndromes

Since we found that apoptotic MV only are increased in patients with ACS, we then examined whether variations of the apoptotic MV population could provide further information on ACS pathophysiology.

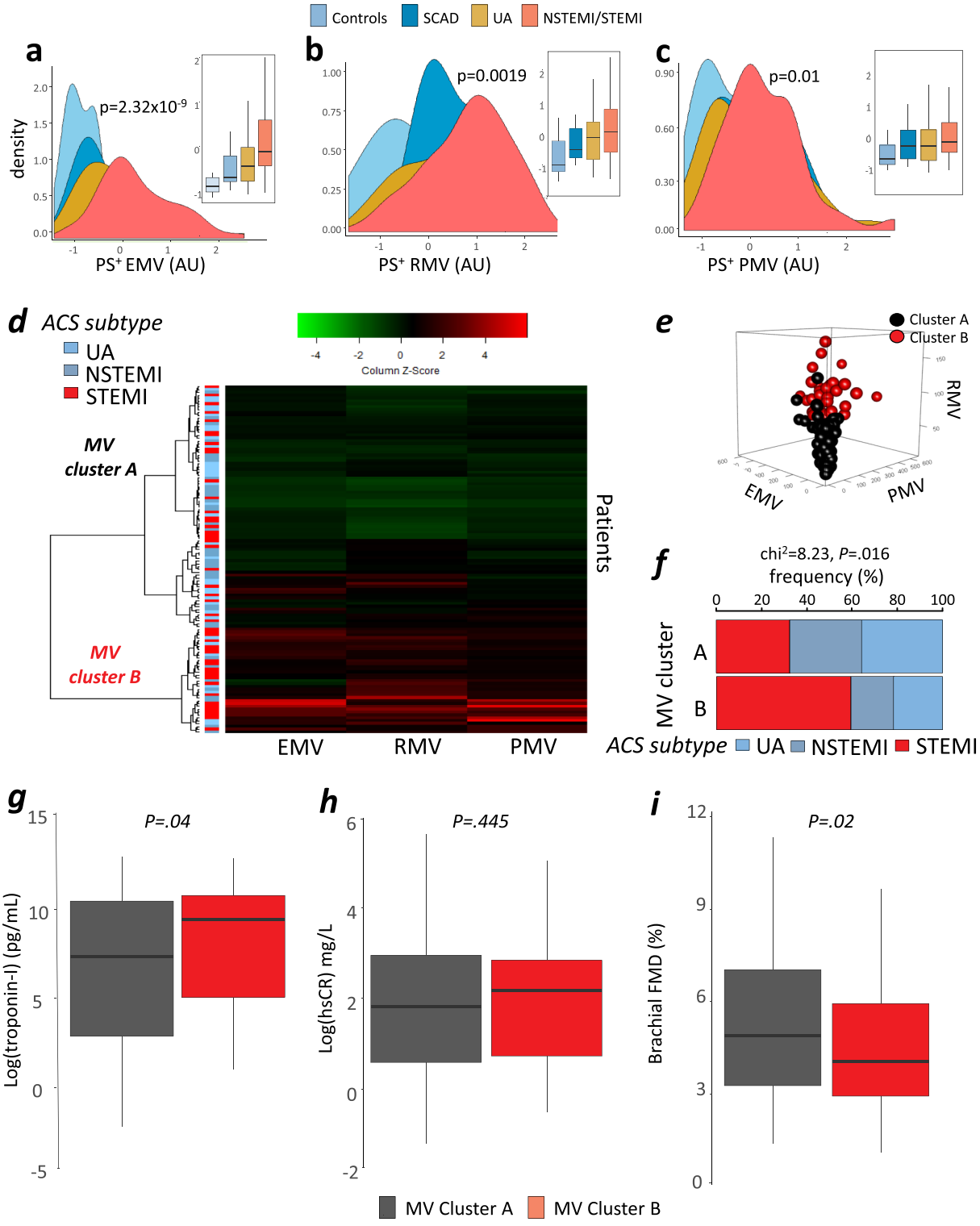


**Fig. 1.** Density plots representing the distribution of a. endothelial-derived (EMV) b. red blood cell-derived (RMV) and c. platelet-derived (PMV) apoptotic microvesicles (MV) in the different study groups ($n$ = 153). d. Hierarchical clustering of patients based on the plasma levels of apoptotic microparticles and row dendrogram indicating the patient clustering; a color legend indicates acute coronary syndrome (ACS) subtype; e. Cloud plot for the association between the different apoptotic MV subpopulations in the study; f. Prevalence of STEMI between MV clusters; g. Comparison of serum troponin-I, h. serum C-reactive protein, and i. endothelial function assessed by flow-mediated dilation (FMD) of brachial artery between MV clusters. EMV: endothelial-derived MV; RMV: red blood cell-derived MV; PMV: platelet-derived MV); PS: phosphatidylserine; SCAD: stable coronary artery disease; UA: unstable angina. a-c: $p$-values by Kruskal-Wallis. e. by chi-square and g-h. by unpaired $t$-test. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

It is already known that apoptotic MV are increased in the setting of ACS. However, the data are mostly derived from apoptotic circulating EMV, while the role of plasma PMV[7] and RMV is less clear. Evidence also suggests that MV are markers of coagulation. The majority of these results stems from studies that measured tissue-factor (TF)-bearing MVs. TF-bearing MV are increased in the culprit coronary artery of patients with STEMI [11] and are associated with fibrinolysis failure [12]. However, some studies have reported a depletion of circulating procoaculant MV in the setting of AMI, possibly as a result of MV adherence on the coronary thrombus [13,14]. The amount of evidence regarding the apoptotic MV is only preliminary. Wang et al. [15] have shown that apoptotic circulating MV decrease coagulation time in patients with NSTEMI following stent implantation. Apoptotic RMV, in particular, are released from thrombi of the coronary vasculature can thus be used as biomarkers of ongoing thrombosis. An association of EMV levels with endothelial dysfunction has been reported in stable patients [3]. In experimental studies, EMV and PMV can impair endothelium-dependent relaxation in rat aortas and procougulant MV cause premature endothelial senescence [4].

In the present study we used an unbiased hierarchical clustering approach and demonstrated that the signature of circulating EMV/RMV/PMV can be used to cluster ACS patients into two distinct groups, which are significantly different in the prevalence of STEMI subtype, a surrogate for definite plaque rupture and thrombotic lumen occlusion. Our findings, however, have a further implication. The difference in the prevalence of STEMI was not associated with differences in traditional cardiovascular risk factors, such as the presence of hypertension, dyslipidemia and diabetes mellitus. Notably, this distinct plasma EMV/RMV/PMV profile linked to higher (2fold) prevalence of STEMI was also associated with poorer endothelial function. This is the first, to the best of our knowledge, evidence that a plasma EMV/RMV/PMV signature may be related with endothelial dysfunction in the setting of ACS, an observation which could relate to the increased thrombotic complications and plaque rupture events in these patients.

## 5. Conclusion

Taken together, in this study we provide first, preliminary evidence that circulating apoptotic cell-derived MV are increased in patients with ACS. Profiling of circulating MV identifies a subgroup of ACS patients with a twofold increased prevalence of STEMI and impaired endothelial function independently of their clinical profile, which could predispose to plaque rupture and thrombotic complications in the setting of ACS. Although the temporal pattern or the causality of these associations remains unknown, our findings suggest that the use of plasma MV profile as a biomarker in ACS merits further investigation.

## Author contributions

Conception and design: DT, EO, EZ, CA, NP; acquisition of data: EZ, ZP, SS, AM, VM, AK, NO, EV, SP, NG; analysis and interpretation of data: AA, EO, DT.

## Funding

None.

## Disclosures

None for all authors.

## References

[1] L.A. Hargett, N.N. Bauer, On the origin of microparticles: from “platelet dust” to mediators of intercellular communication, Pulm. Circulat. 3 (2013) 329–340, https://doi.org/10.4103/2045-8932.114760.
[2] B. Verhoven, R.A. Schlegel, P. Williamson, Mechanisms of phosphatidylserine exposure, a phagocyte recognition signal on apoptotic t lymphocytes, J. Exp. Med. 182 (1995) 1597–1601, https://doi.org/10.1084/jem.182.5.1597.
[3] P. Horn, M.M. Cortese-Krott, N. Amabile, C. Hundsdorfer, K.D. Kroncke, M. Kelm, C. Heiss, Circulating microparticles carry a functional endothelial nitric oxide synthase that is decreased in patients with endothelial dysfunction, J. Am. Heart Assoc. 2 (2012) e003764, , https://doi.org/10.1161/JAHA.112.003764.
[4] M. Abbas, L. Jesel, C. Auger, L. Amoura, N. Messas, G. Manin, C. Rumig, A.J. Leon-Gonzalez, T.P. Ribeiro, G.C. Silva, R. Abou-Merhi, E. Hamade, M. Hecker, Y. Georg, N. Chakfe, P. Ohlmann, V.B. Schini-Kerth, F. Toti, O. Morel, Endothelial microparticles from acute coronary syndrome patients induce premature coronary artery endothelial cell aging and thrombogenicity: role of the ang ii/at1 receptor/nadph oxidase-mediated activation of mapks and pi3-kinase pathways, Circulation. 135 (2017) 280–296, https://doi.org/10.1161/CIRCULATIONAHA.116.017513.
[5] W.Q. Han, F.J. Chang, Q.R. Wang, J.Q. Pan, Microparticles from patients with the acute coronary syndrome impair vasodilatation by inhibiting the akt/enos-hsp90 signaling pathway, Cardiology. 132 (2015) 252–260, https://doi.org/10.1159/000438782.
[6] S. Bernard, R. Loffroy, A. Serusclat, L. Boussel, E. Bonnefoy, C. Thevenon, M. Rabilloud, D. Revel, P. Moulin, P. Douek, Increased levels of endothelial microparticles cd144 (ve-cadherin) positives in type 2 diabetic patients with coronary noncalcified plaques evaluated by multidetector computed tomography (mdct), Atherosclerosis. 203 (2009) 429–435, https://doi.org/10.1016/j.atherosclerosis.2008.07.039.
[7] L.M. Biasucci, I. Porto, L. Di Vito, G.L. De Maria, A.M. Leone, G. Tinelli, A. Tritarelli, G. Di Rocco, F. Snider, M.C. Capogrossi, F. Crea, Differences in microparticle release in patients with acute coronary syndrome and stable angina, Circ. J. 76 (2012) 2174–2182, https://doi.org/10.1253/circj.cj-12-0068.
[8] P.K. Min, J.Y. Kim, K.H. Chung, B.K. Lee, M. Cho, D.L. Lee, S.Y. Hong, E.Y. Choi, Y.W. Yoon, B.K. Hong, S.J. Rim, H.M. Kwon, Local increase in microparticles from the aspirate of culprit coronary arteries in patients with st-segment elevation myocardial infarction, Atherosclerosis. 227 (2013) 323–328, https://doi.org/10.1016/j.atherosclerosis.2013.01.032.
[9] A.S. Antonopoulos, G. Latsios, E. Oikonomou, K. Aznaouridis, A. Papanikolaou, D. Syrseloudis, G. Siasos, M. Vavuranakis, K. Toutouzas, D. Tousoulis, Long-term endothelial dysfunction after trans-radial catheterization: a meta-analytic approach, J. Card. Surg. 32 (2017) 464–473, https://doi.org/10.1111/jocs.13181.
[10] M. Skeppholm, F. Mobarrez, K. Malmqvist, H. Wallen, Platelet-derived microparticles during and after acute coronary syndrome, Thromb. Haemost. 107 (2012) 1122–1129, https://doi.org/10.1160/TH11-11-0779.
[11] O. Morel, B. Pereira, G. Averous, A. Faure, L. Jesel, P. Germain, L. Grunebaum, P. Ohlmann, J.M. Freyssinet, P. Bareiss, F. Toti, Increased levels of procoagulant tissue factor-bearing microparticles within the occluded coronary artery of patients with st-segment elevation myocardial infarction: role of endothelial damage and leukocyte activation, Atherosclerosis. 204 (2009) 636–641, https://doi.org/10.1016/j.atherosclerosis.2008.10.039.
[12] M.G. Huisse, N. Ajzenberg, L. Feldman, M.C. Guillin, P.G. Steg, Microparticle-linked tissue factor activity and increased thrombin activity play a potential role in fibrinolysis failure in st-segment elevation myocardial infarction, Thromb. Haemost. 101 (2009) 734–740.
[13] J.F. Keuren, K.S. Jie, M.P. Leers, Increased expression of tf on monocytes, but decreased numbers of tf bearing microparticles in blood from patients with acute myocardial infarction, Eur. J. Haematol. 83 (2009) 387–388, https://doi.org/10.1111/j.1600-0609.2009.01306.x.
[14] M. Maly, I. Hrachovinova, P. Tomasov, P. Salaj, P. Hajek, J. Veselka, Patients with acute coronary syndromes have low tissue factor activity and microparticle count, but normal concentration of tissue factor antigen in platelet free plasma: a pilot study, Eur. J. Haematol. 82 (2009) 148–153, https://doi.org/10.1111/j.1600-0609.2008.01175.x.
[15] L. Wang, Y. Bi, M. Cao, R. Ma, X. Wu, Y. Zhang, W. Ding, Y. Liu, Q. Yu, Y. Zhang, H. Jiang, Y. Sun, D. Tong, L. Guo, Z. Dong, Y. Tian, J. Kou, J. Shi, Microparticles and blood cells induce procoagulant activity via phosphatidylserine exposure in nstemi patients following stent implantation, Int. J. Cardiol. 223 (2016) 121–128, https://doi.org/10.1016/j.ijcard.2016.07.260.