

Microparticles as Potential Biomarkers of Cardiovascular Disease

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Abstract

Primary prevention of cardiovascular disease is a choice of great relevance because of its impact on health. Some biomarkers, such as microparticles derived from different cell populations, have been considered useful in the assessment of cardiovascular disease. Microparticles are released by the membrane structures of different cell types upon activation or apoptosis, and are present in the plasma of healthy individuals (in levels considered physiological) and in patients with different pathologies. Many studies have suggested an association between microparticles and different pathological conditions, mainly the relationship with the development of cardiovascular diseases. Moreover, the effects of different lipid-lowering therapies have been described in regard to measurement of microparticles. The studies are still controversial regarding the levels of microparticles that can be considered pathological. In addition, the methodologies used still vary, suggesting the need for standardization of the different protocols applied, aiming at using microparticles as biomarkers in clinical practice.

Introduction

Microparticles (MP) are defined as a population of vesicles derived from different cell types (Table 1) after activation or apoptosis, measuring from 50 nm to 1000 nm, and containing cell material, such as proteins, mRNA and lipoproteins, which are fundamental to the identification of those vesicles by use of different techniques, such as flow cytometry¹. All blood cells produce MP, the greatest amount being released by platelets, platelet MP (PMP), corresponding to 70%-90% of the total amount of MP in the plasma of healthy individuals²⁻⁴.

Until the 1990s, no biological importance had been given to MP, which were considered inert particles resulting

from cell destruction or only markers of apoptosis. In 1996, however, Raposo et al.⁵ suggested that MP played an important role in adaptative immune response. Since then, several studies have shown the importance of MP as vectors of intracellular exchange of biological information, by use of identification, characterization and quantification of MP in several situations, such as obesity, diabetes mellitus, infarction, depression, cancer, HIV and renal failure.

As the atherosclerotic process develops, monocytes accumulate lipoproteins and change into cholesterol-rich macrophages, which undergo apoptosis, releasing a high amount of lipids to the extracellular medium, causing a vicious cycle of inflammation, oxidative stress, apoptosis of endothelial cells or endothelial erosion (Figure 1). This culminates in atherothrombotic outcomes, such as myocardial infarction or ischemic cerebral vascular accident, which result from the contact of inner plaque substances with blood, producing immediate coagulation and consequent total and sudden obstruction of the vessel⁶.

Healthy individuals and those with different diseases have MP in their plasma⁷. Inflammatory stimuli to the release of MP include Gram-negative bacterial lipopolysaccharides (LPS) and cytokines, such as tumor necrosis factor alpha (TNF- α), interleukine 6 (IL-6) and interleukine 1 (IL-1 β). After activation, the cells change their asymmetrical conformation, exposing phosphatidylserine, an aminophospholipid responsible for high procoagulant capacity⁸.

Microparticles derived from different cell populations

Platelet microparticles

The PMP are the most abundant in human plasma^{4,9}. Several studies have shown the relationship between those PMP and blood coagulation¹⁰, inflammatory processes¹¹, thrombosis and tumor progression¹², as well as the interaction between leukocytes and endothelial cells¹³.

However, some studies have suggested that PMP have an important role in tissue regeneration, because they are strongly associated with angiogenesis¹⁴. Some authors have shown that, *in vitro*, PMP promote budding of aortic rings by activating the signaling pathways of PI3-kinase and Extracellular Signal-Regulated Kinase (ERK)¹³, in addition to promoting post-ischemic revascularization¹⁵.

Endothelial microparticles

Endothelial MP (EMP) represent a smaller population of MP in plasma, but have been associated with cardiovascular

Keywords

Cardiovascular Diseases; Biomarkers, Pharmacological / analysis; Cell-Derived Microparticles; Atherosclerosis / prevention & control.

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Table 1 – Antigenes on the surface of microparticles derived from platelets, endothelium and monocytes

Microparticles	Surface antigens	References
PMP	CD31, CD41, CD42, CD61, CD62P, CD63	3, 8, 10, 39, 58, 62
EMP	CD31, CD51, CD54, CD62E, CD105, CD106, CD144, CD146, E-selectina, VE-caderina	8, 9, 23, 34, 36, 39, 42, 43, 58-61
MMP	CD14, CD54	28, 29, 31

PMP: platelet microparticles; EMP: endothelial microparticles; MMP: monocyte microparticles⁶²

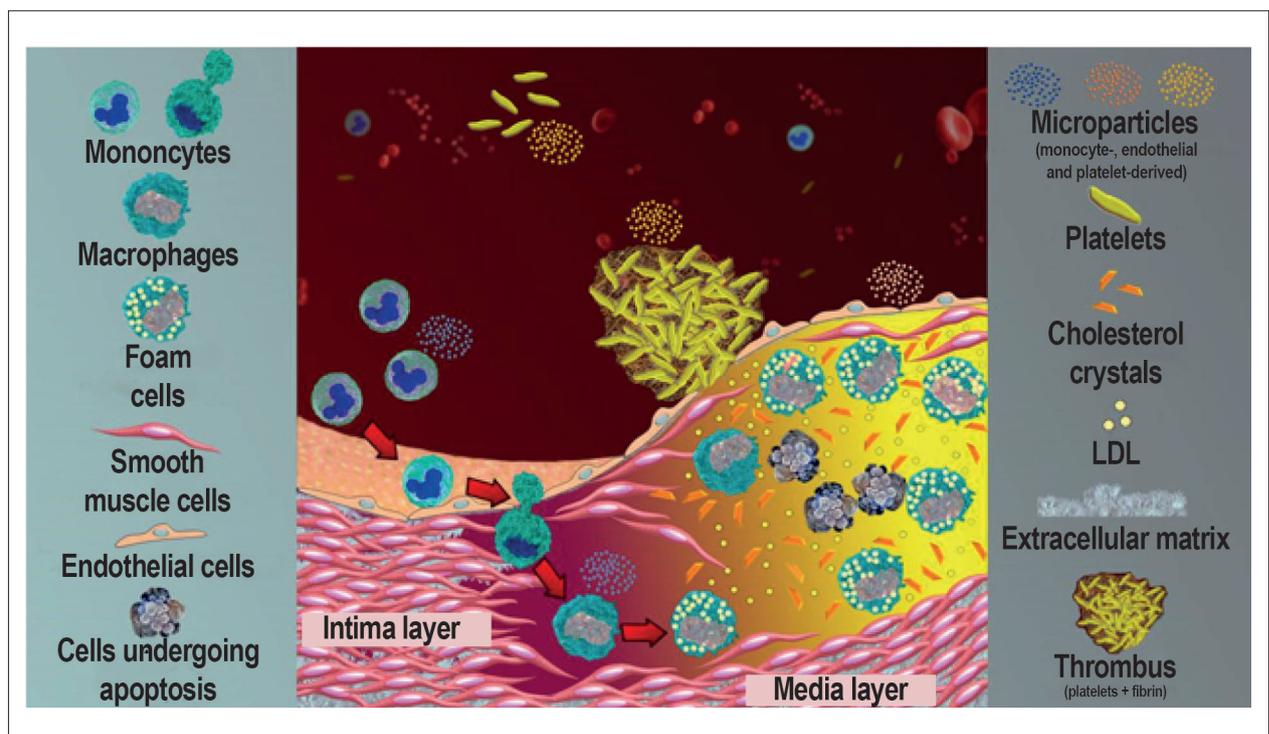


Figure 1 – Illustration of cell interactions during atheroma formation.

disease, mainly endothelial dysfunction⁸. Similarly to PMP, some studies have suggested a relationship between EMP and angiogenesis¹⁶, tumor growth¹⁷ and increased oxidative stress¹⁸.

Some studies have shown the importance of EMP in the proliferation and differentiation of endothelial progenitor cells, which are essential for vascular regeneration¹⁹, indicating a possible protective function related to vascular regeneration, repair and protection²⁰.

Mezentsev et al.²¹ have assessed *in vitro* different patterns of angiogenesis (cell division rate, capillary formation and apoptosis of endothelial cells), comparing physiological levels of EMP present in healthy individuals (between 10³ and 10⁴ EMP/mL)^{22,23} and pathological concentrations (present in individuals with cardiovascular disease, 10⁵ EMP/mL)²⁴⁻²⁶. Those authors have reported that pathological levels of EMP affected all parameters associated with angiogenesis in a directly proportional

manner to the concentration of EMP. Those same authors had previously shown that 10⁵ EMP/mL impaired endothelium-dependent relaxation, which was not seen with 10⁴ EMP/mL²⁷.

Monocyte microparticles

Similarly to PMP, the MP originated from monocytes, monocyte MP (MPM), can contain procoagulant substances and be related to endothelial dysfunction²⁸ and sepsis²⁹. The study by Wang et al.³⁰ has shown that MPM can activate endothelial cells, because MPM contain IL-1β, which enhances the inflammatory process.

Hoyer et al.³¹ have assessed the role of MPM in vascular inflammation and reported that the treatment of ApoE -/- mice with MPM promoted the formation of atherosclerotic plaque in the mice and increased the accumulation of macrophages in the vascular wall. Those authors have suggested an important interaction

between MPM and inflammatory cells in the atherosclerotic disease of ApoE $-/-$ mice.

Microparticles and coronary disease

Several studies have suggested a direct relationship between the increase in MP and development of coronary disease. Augustine et al.³², assessing patients undergoing dobutamine stress echocardiography, have reported an elevation in MP derived from different cell types (platelets, erythrocytes and endothelial cells) immediately after the test followed by a rapid MP clearance from the circulation during the next hour in response to cardiac stress. Those authors have suggested that the release of MP is a protective mechanism to clear cell stress in those patients.

Sarlon-Bartoli et al.³³ have measured the plasma levels of leukocyte-derived MP (LMP) in 42 individuals with carotid artery stenosis greater than 70%. They have shown that patients with unstable plaque had increased levels of the CD11bCD66b+ and CD15+ LMP, suggesting that even less frequently found subpopulations of MP in plasma, as compared to PMP, can provide important information regarding clinical studies on atherosclerotic plaque vulnerability in patients with high-grade carotid stenosis.

Morel et al.³⁴ have assessed the levels of LMP and EMP within occluded coronary arteries of ST-segment elevation myocardial infarction patients treated with primary angioplasty and have compared them with the levels of MP in peripheral blood. Those authors have reported an increase in MP within arteries, indicating the importance of those vesicles in the development of coronary atherothrombosis.

Failla et al.³⁵ have measured CD11b+ MP (monocyte marker) in patients with acute coronary syndrome with no ST-segment elevation on the electrocardiogram, aiming at assessing whether the quantification of those MP could contribute to the identification of patients at higher risk for a recurring cardiovascular event within one month after coronary stent implantation. A smaller amount of CD11b+ MP was found in individuals with recurring cardiovascular event as compared to that in patients with no complications, suggesting greater capture of those MP in sites of atherosclerotic lesions.

Jeanneteau et al.³⁶ have assessed in rats and humans the role of MP in the mechanism of remote ischemic conditioning (RIC), which has been described as an infarction-related cardioprotective strategy. No differences were found in the total number of MP in the group of animals undergoing RIC as compared to the control group. After phenotypic characterization of MP, elevations in the endothelial and Annexin V+ (apoptotic) subpopulations were observed in the RIC group. Similarly, elevations in EMP and Annexin V+ MP were found in the group of individuals submitted to RIC.

Porto et al.³⁷ have assessed the concentrations of MP in ST-segment elevation myocardial infarction patients undergoing primary percutaneous coronary intervention, and the relationship of those vesicles with microvascular obstruction (defined by multiple angiography and

electrocardiography). The main finding was that the MP subpopulations assessed (PMP and EMP) showed higher levels within the coronary arteries as compared to those in aortic blood. In addition, a greater release of both MP subpopulations was observed in the impaired coronary artery than in ascending aorta, indicating local MP production. Those authors have suggested that their findings can support the hypothesis that MP act as active elements in the embolization and pathophysiology of microvascular obstruction.

Kaabi et al.³⁸ have assessed the relationship between the levels of MP and treatment of stable coronary artery disease patients with external counterpulsation (ECP). That therapy has been considered effective and safe for patients with refractory angina pectoris. Those authors have found an increase in PMP after ECP therapy, and no difference in EMP and MPM levels.

Willians et al.¹¹ have assessed platelet activation and depression levels in coronary artery disease patients, because depression, even mild, is an independent predictor of increased mortality after myocardial infarction. Those authors have reported that patients with moderate depression and high levels of TNF- α , IL-6 and PCR also released more PMP, indicating that a pro-inflammatory component could change platelet function in those patients.

Bernal-Mizrachi et al.³⁹ have shown that, depending on the cell stimulus (activation or apoptosis), different surface proteins are expressed. Those authors have conducted a study analyzing two subpopulations of EMP (CD31+/CD42- and CD51+) in coronary artery disease patients and have reported that CD31+/CD42- EMP were more frequently expressed in acute events (myocardial infarction and unstable angina), and CD51+ EMP were released in similar amounts both in acute and chronic events (stable angina).

Microparticles and diabetes mellitus

Some studies have shown higher concentrations of PMP related to diabetes mellitus. Ogata et al.⁴⁰ have assessed the levels of PMP in 92 patients with diabetic retinopathy. Those authors have reported increased release of PMP in those patients as compared to that in healthy individuals, and the increase was higher the more severe the retinopathy. In atherothrombosis, MP are related to the release of cytokines by leukocytes and endothelial cells, monocyte recruitment to the atherosclerotic plaque, smooth muscle cell proliferation, angiogenesis and increased oxidative stress. In addition, MP can be signalers of cell homeostasis, promoting balance between cell stimulus, proliferation and apoptosis¹².

Lumsden et al.⁴¹ have assessed patients with type 2 diabetes mellitus after acute coronary syndrome (six months prior to event), who had reduced levels of EMP and no PMP changes. Those authors have suggested that those unexpected findings, which disagree with most studies in the literature, can result from concomitant medications used by patients⁴²⁻⁴⁴.

Other studies have reported that the increase in the expression of adhesion molecules is associated with the

activation of monocytes, which can bind to endothelial cells in vessel walls, leading to diabetic retinopathy progression. Those data suggest that measuring the levels of MPM can be a useful biomarker of diabetic retinopathy progression⁴⁵.

Microparticles in endothelial dysfunction and dyslipidemia

Leroyer et al.⁴⁶ have shown that MP originated from macrophages (CD40+) can promote angiogenesis within the plaque, suggesting that these MP can determine plaque vulnerability. However, studies assessing the relationship between MP and angiogenesis have proved controversial and inconclusive, because some MP have been reported to be able to stimulate angiogenesis (PMP, for example)^{13,47}, while other studies have reported that MP can both stimulate and inhibit angiogenesis, depending on cell origin⁴⁸⁻⁵⁰.

Some studies have shown that individuals with metabolic syndrome have increased levels of MP as compared to those of healthy individuals, and that MP are related to endothelial dysfunction, due to decreased eNOS expression and increased release of reactive oxygen species⁵¹.

The first study to show the direct effect of MP on vascular function has been developed by Boulanger et al.⁵² Those authors have assessed whether the MP present in peripheral blood of patients with non-ischemic syndrome and after acute myocardial infarction would influence the endothelium-dependent response in aortic rings of rats. The MP of post-infarction individuals have been reported to reduce acetylcholine-induced vascular relaxation (by influencing the nitric oxide pathway), suggesting that MP could contribute to the endothelial dysfunction observed after the acute event.

Diehl et al.⁵³ have analyzed different subpopulations of MP in individuals with pulmonary hypertension, and have reported increased levels of LMP, EMP and PMP, indicating higher inflammatory and procoagulant activity, which can be related to thromboembolic complications and endothelial dysfunction in those patients.

The improvement in endothelial function promoted by calcium channel blockers has been well described in the literature. Nomura et al.⁵⁴ have shown a reduction in EMP in patients with type 2 diabetes mellitus after treatment with the calcium channel blocker, nifedipine. Similar result has been reported by the same group⁵⁵ with patients with type 2 diabetes mellitus and hypertension after treatment with benidipine, belonging to the same drug class. The effect of the renin-angiotensin system blocker valsartan on the levels of MPM in individuals with type 2 diabetes mellitus has also been assessed by the same group⁵⁶, who has reported that the drug inhibited the release of MPM. Those results have suggested that the renin-angiotensin system blocker can contribute to the treatment of atherosclerosis.

Microparticles and lipid-lowering therapies

New strategies that can either inhibit MP functions or provide greater clearance have been searched. Several studies have assessed the effect of different lipid-lowering strategies on the amount of MP released by different cell types^{57,58}.

Pinheiro et al.⁵⁹ have assessed the effect of the antiplatelet drug clopidogrel in association or not with rosuvastatin (40 mg) on the levels of EMP and PMP in patients with stable coronary disease on statins for at least three months. Those authors have identified an increase in the levels of PMP after suspension of rosuvastatin and maintenance of only clopidogrel for four weeks and a tendency towards greater release of EMP in those patients. They have suggested that an increase in the apoptosis of platelets occurred, and that rosuvastatin might have a protective effect on the endothelium when associated with clopidogrel. In a similar study, França et al.⁶⁰ have assessed the influence of atorvastatin (80 mg) in association or not with clopidogrel in patients with stable coronary disease. Those authors have suggested higher vascular stability promoted by atorvastatin after identifying an inverse relationship between the plasma concentration of atorvastatin and the levels of PMP.

Another study⁶¹ has assessed the effect of the treatment with vitamin C for five days on the levels of MP in patients with diabetes, dyslipidemia, or at least two risk factors for post-infarction cardiovascular disease. A reduction in the amount of EMP and PMP was observed, which has been associated with the reduction in oxidative stress caused by vitamin C.

Thus, the studies have shown that MP can be useful markers not only to assess cardiovascular disease, but also cancer^{10,17}, sepsis²² and other illnesses. However, the MP levels considered physiological and pathological are still controversial. Although flow cytometry is considered a reference for the identification and phenotypic characterization of MP, the studies have used several methods (time of centrifugation and incubation with antibodies, different markers), which makes the comparison between publications in the literature difficult.

In conclusion, the search for new biomarkers that might be related to cardiovascular disease has increased the interest in MP derived from different cells, especially platelets. However, studies are still controversial, and further research on standardization of more sensitive techniques to obtain, characterize and quantify those vesicles is required, so that the findings can be applied to clinical practice.

Author contributions

Acquisition of data: França CN. Writing of the manuscript: França CN, Amaral JB, Tegani DM. Critical revision of the manuscript for intellectual content: Izar MCO, Fonseca FAH. Figure Confection: Amaral JB.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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