

Impaired Phenotype of Circulating Endothelial-Derived Microparticles: Novel Marker of Cardiovascular Risk

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ABSTRACT

Circulating microparticles (MPs) delivered from the native cell membrane are suitable forms of cell-to-cell communication and transfer of biological active effectors. Circulating endothelial-derived MPs as a marker of endothelial dysfunction may discuss as integrated effectors interrelated target cells, endothelium, and conventional cardiovascular risk factors. Endothelial-derived MPs play a pivotal role in vascular damage, integrity and tissue repair. However, the role of MPs as biomarker of cardiovascular outcomes is required scrutinizes. The present review accumulates data regarding the role of circulating endothelial-derived MPs as novel marker of cardiovascular risk.

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Key words: Circulating microparticles, Endothelial-derived microparticles, Cardiovascular disease, Risk

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abbreviations

APCs: antigen-presenting cells;

CVD: cardiovascular disease

MPs: microparticles

miRNA: microRNA

RNA: ribonucleic acid

INTRODUCTION

Cardiovascular disease (CVD) progression is mediated by several pathogenetic mechanisms and demonstrates complex and multi factorial nature that frequently relates to genetics, ethnicity, age- and sex-related causes, life style particularities, culture and educational level, exiting comorbidities, etc.^[1-3]. Moreover, integrating CVD phenotype highly coordinates with complex risk factors (lipid abnormalities, elevated blood pressure and fasting blood glucose, insulin resistance, increased body mass index) that demonstrate good predictive discrimination for each component^[4]. The risk factor stratification is necessary to identify high-risk candidates for CVD who require preventive measures of risk profile and CVD phenotype determination^[5]. Although CVD-specific profiles of risk factors are satisfied, other diseases (heart failure, peripheral artery disease, stroke, thromboembolism) and comorbidities, which sometimes does not essentially relate to CVD, such as cancer, share similar profile of risk^[6-9]. Collectively, CVD phenotype requires more definition and probably it is attractive component of risk stratification of the patients.

Microparticles (MPs) are defined as secreted membrane-enclosed extracellular vesicles and they together microvesicles, microparticles, exosomes, ectosomes, apoptotic bodies, etc. are currently undergoing intense investigations^[10-12]. Their size appears to be a one of the key criteria of the selection in subsets, despite confusing nomenclature is existed and currently various names of vesicles have been emerged (Table 1). MPs are represented to be a

Table 1 The criteria of selection of secreted membrane-enclosed extracellular vesicles.

Name of vesicles	Origin	Key forming mechanism	Size range	Expression of membrane proteins
Microvesicles	Any cell types	membrane budding	50-1000 nm	CD34, CD45, band 3, glycoporins, or blood group antigens depending origin
Microparticles	haematopoietic cells, APCs, platelets, oligodendrocytes, endothelial cells, epithelial cells	membrane vesiculation	100-1000 nm	glycoprotein Ib, CD133+, phosphatidylserine and many other receptors (TF, P-selectin, E-selectin, etc.)
Exosomes	haematopoietic and nonhematopoietic cells (cytotoxic T cells, platelets, mast cells, neurons, oligodendrocytes, Schwann cells, and intestinal epithelial cells, APCs, multivesicular bodies)	exocytosis	50 - 100 nm	Rab, GTPases, annexins, flotillin, and ESCRT, MHC
Ectosomes	Any cell types (?)	haematopoietic cells, APCs, fibroblasts, tumor cells	0.1-1 µm	tetraspanin proteins
Multivesicular bodies (endosomes)	Any cell types	exocytosis of the exosomes	40-100 nm	flotillin
Apoptotic bodies	Any cell types	blebbing of plasma membrane due to karyorrhexis with further "budding"*	50 - 2000 nm	Annexin V
Oncosomes	Tumor cells	blebbing of plasma membrane	1 - 10 µm	caveolin-1, Myr Akt1, HB-EGF

APCs: antigen-presenting cells; ESCRT: endosomal sorting complex required for transport complex; tetraspanin proteins: CD9, CD37, CD63 and CD81; HC: antigen-major histocompatibility complex. Notes: * - separation of cell fragments into apoptotic bodies.

heterogeneous population of anucleoid phospholipid vesicles with diameter ranged 100-1000 nm^[13] and thereby they are distinguished from other subsets of extracellular vesicles, such as exosomes and apoptotic bodies^[14-17]. Microparticles are released (*in vivo* and *in vitro*) following apoptosis and cell activation by specific mechanisms named vesiculation and fission of the membrane enriched in phosphatidylserine of the various originated cells, i.e. mononuclears, platelets, endothelial cells, erythrocytes, oligodendrocytes, antigen-presenting cells, and even tumour cells^[18]. The formation of MPs is under controlled through several genomic mechanisms and is trigged specific (cytokines, thrombin, endotoxins) and none-specific (shear stress) stimuli^[19]. Biological role of MPs are not fully understood and depends on origin of the vesicles (Figure 1). However, MPs is considered disseminated storage pool of bioactive effectors required for cell-to-cell communication, which is mediated through delivering various peptides, lipids, active proteins and molecules, mRNA and microRNA (miRNA)^[20,21]. Therefore, it has suggested that MPs may mediate inter cells communications via arranging of expressed surface receptors^[22]. These signals are sufficient for CVD phenotype and may play a pivotal role in disease progression^[23]. In this context circulating endothelial-derived MPs as a marker of endothelial dysfunction may discuss as integrated effectors interrelated target cells, endothelium, and CVD risk factors. The present review accumulates data regarding the role of circulating endothelial-derived MPs as novel marker of cardiovascular risk.

ENDOTHELIAL-DERIVED MPS AND ENDOTHELIAL DYSFUNCTION

Endothelial dysfunction is considered an early stage of CVD related to coexisting comorbidities and it frequently reflects disease progression and possible has predictive value^[24-26]. Of note, diagnosis of impaired endothelial function at early stage of CVD and in subjects with CVD risk factors is sophisticated although screening of the patients at high CVD risk is required to improve clinical outcomes and prescribe protective treatment^[27]. Elevated level of circulating endothelial-derived MPs was found in several CVD risk factors (abdominal obesity, insulin resistance, diabetes mellitus, hypertension)^[28] and it was associated with severity of vascular tone

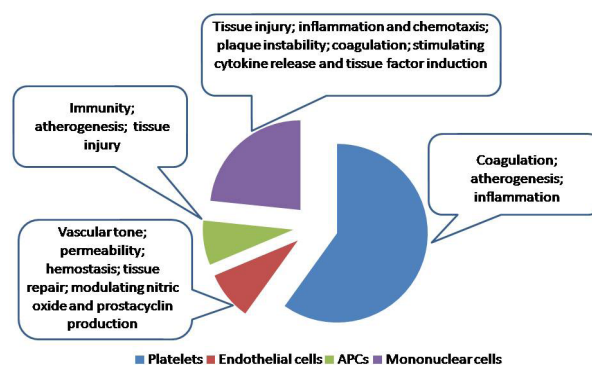


Figure 1 Biological role of microparticles originated from platelets, endothelial cells, mononuclears, and APCs. Figure shows a relevant contribution of MPs originated from in peripheral blood, as well as their predominantly biological function. Abbreviation: APCs: antigen-presenting cells; MPs: microparticles.

disorders^[29]. Endothelial-derived MPs are expressed on their surface CD144 (VE-cadherin) and CD62E (E-selectin) that used as flow cytometry markers to recognized MPs originated from endothelial cells. Therefore, endothelial-derived MPs present on the cell surface other molecules determined the biological role of the MPs and allowed them to interplay in several processes i.e. inflammation, angiogenesis, adhesion, coagulation, cell survival, tissue remodeling, tumor growth and malignancy. Endothelial-derived MPs incorporated in endothelial cells by interaction with alpha4- and beta1- integrins that are expressed on the surface of MPs. After connection with membrane of the target cells EMPs realize their biological effect by directly stimulating cells or by transferring surface receptors^[20]. It has been postulated that the final result depends closely on origin of the MPs^[30]. In fact, MPs derive from activated endothelial cells and may play a pivotal role in the angiogenesis and endothelial reparation^[31]. In opposite, endothelial-derived MPs originated from apoptotic endothelial cells are considered a direct trigger of vascular injury^[32-34]. It is still unclear whether endothelial dysfunction is result in impaired balance between activated endothelial cell-derived MPs and apoptotic endothelial cell-derived MPs or disorders of vascular tone appears to be prior altered secretion of endothelial-derived MPs. However, many investigators believed that pattern of endothelial-derived MPs

allows to access usually inaccessible tissues (endothelium, new small vessels) and understand information changing between target cells aimed personalize vascular aging and reclassify the patients at risk^[35-37]. Overall, MPs are considered a novel surrogate biological marker of endothelial injury and vascular tone disorders in general population, cardiovascular patients and subjects with wide spectrum comorbidities^[29-38].

In this context, pattern of circulating MPs may reflect CVD phenotype and be useful tool for early detection of endothelial dysfunction. Immune phenotypes of circulating microparticles in patients with cardiovascular conditions and metabolic comorbidities are reported in Table 2. Although there are studies reported the role of different immune phenotypes of endothelial cell-derived MPs as surrogate marker of endothelial dysfunction^[38-41], within last years the impact of MPs originated from activated and apoptotic endothelial cells as important circulating biological vectors in pathogenesis of inflammation, coagulation, tissue reparation, malignancy, atherogenesis, vascular aging has become more much understood^[42,43].

ENDOTHELIAL-DERIVED MPS AND CARDIOVASCULAR DISEASES

Hypothetically, circulating level of MPs released from activated and / or apoptotic endothelial cells may link to deleterious effects on target cells from the vascular wall, adipose tissue, antigen-presenting cells, as well as secretion of cytokines, active molecules, signal proteins, pro-coagulant factors, etc.^[44,45]. The data obtained within last decade are controversial and are frequently under speculation. Increased CD31+/CD42+ subset of MPs was found in hypertensive subjects^[39]. Moreover, CD41+ endothelial-derived MPs level correlates positively with blood pressure, aortic pulse wave velocity, cholesterol level, and negatively with circulating level of endothelial progenitor cells in patients with arterial hypertension^[39,46]. González-Quintero *et al*^[47] reported that the number of both CD31+ and CD62+ (E-selectin) MPs in patients with mild-to-moderate gestational hypertension was significantly higher than healthy control, but CD41+ subset was not. It has suggested that endothelial-derived MPs, which realized a consequence of vascular endothelial cell damage, may have proatherogenic potent and induce tissue injury. Indeed, Huang *et al*^[48] found that hypertensive patients with microalbuminuria or macroalbuminuria had significantly increased CD31+/annexin V apoptotic MPs, which contribute in tissue injury through increased free radical production, cellular senescence and inducing apoptosis^[49]. Therefore, elevated level of CD31+/annexin V+ apoptotic MPs were found in coronary artery disease patients and it was positively correlated with endothelial dysfunction and CVD risk factors i.e. such as smoking, age, male sex, hypertension, dyslipidemia, and diabetes^[50,51]. Therefore, MPs shedding from endothelial progenitor cells labeled as CD34+/KDR+ subset were predictors of aortic stiffness, independent of the Framingham risk^[52]. CVD risk patients with overweight/obesity, diabetes, insulin resistance demonstrate impaired inflammatory state and endothelial microvascular function, hypercoagulation, increased arterial stiffness associated with elevated endothelial-derived MPs labeled as CD31+/CD42+, CD31+/CD41-, CD31+/CD62+, and CD144+/CD146+^[53-56]. Surprisingly, elevation of MPs endothelial origin was associated with obesity independently of metabolic abnormalities^[57] and the prognostic significance of these findings are required further studies.

Overall, optimal pool of MPs cells identified using combinations of antigenic markers that reflects imbalance in vascular tone

Table 2 Immune phenotypes of circulating microparticles in patients with cardiovascular conditions and metabolic comorbidities.

Conditions or dysmetabolic states	Immune phenotypes of endothelial-derived MPs
Hypertension	↑CD31+CD42-
Type two diabetes mellitus	↑CD31+CD42-; ↑CD144+; ↑Annexin-V
Metabolic syndrome	↑CD31+ CD42b; ↑CD31+CD42-; ↑CD144+
Obesity	↑CD31+ CD42b
Heart failure	↑CD31+ annexin V+
Asymptomatic atherosclerosis	↑CD105+; ↑CD31+ annexin V+
Stable coronary artery disease	↑CD31+CD42-; ↑CD144+;
ACS / MI	↑CD31+CD42-; ↑CD144+; ↑CD31+ annexin V+
Ischemic stroke / TIA	↑CD62E+

MPs: microparticles; ACS: acute coronary syndrome; MI: myocardial infarction; TIA: transient ischemic attack.

enhances and severity of endothelial dysfunction is not determined. Whether levels of circulating MPs relates to aging, sex, gestation, and metabolic comorbidities, such as insulin resistance, obesity, increased fasting glucose, and particularly dyslipidaemia, rather than early stages of cardiovascular disorders is still exactly not clear. Amabile *et al*^[28] reported that each tertile increase in the Framingham risk score corresponded to a 9% increase in log-CD31+/CD41- circulating MPs. Authors suggested that elevated MPs labeled CD31+/CD41- are considered the potential surrogate marker regarding the influence of high-risk metabolic profiles on endothelial integrity, which mediates endothelial dysfunction with reduced vascular repair capacity. Of note, an association of elevated endothelial-derived MPs with CVD risk was seen independently age, sex, and ethnicity. The involvement of MPs in the pathogenesis of endothelial dysfunction has defined currently, but predictive value of these findings was not obviously. However, data of recent investigations appear to be optimistically regarding that the imbalance between different immune phenotypes of circulating endothelial-derived MPs originated from activated and apoptotic endothelial cells may have more utility for risk stratification in subjects with CVD risk factors and documented CVD^[58-60].

PREDICTIVE ROLE OF ELEVATED ENDOTHELIAL-DERIVED MPS

Despite endothelial-derived MPs exhibit regulated effects on endothelial cells mediated vascular damage, integrity and reparation, their predictive role as biomarkers of cardiovascular outcomes is required detail scrutinizes. It is important that the assessment of endothelial dysfunction by absolute number of circulating MPs may improve prediction of Framingham risk model that allows to identify patients vulnerable to CVD^[38]. Circulating MPs labeled CD144+ or CD31+/41- were not informative as predictive biomarkers in untreated patients with pulmonary hypertension, while subjects with elevated CD62E+ MPs demonstrated worse prognosis when compared with those who had lower level of CD62E+ MPs^[61]. Therefore, elevated level of CD31+/annexin V+ apoptotic MPs was found an independent predictor of cardiovascular events in CVD patients and may be useful for risk stratification^[62,63].

Overall there are increased numbers of microparticles as biomarker of CVD. There are original papers which showed the modified expression of membrane receptors exposed by MPs in atherosclerosis, heart failure, acute coronary syndrome, myocardial infarction, and other CVD. However, changing of their immune phenotypes was not emphasis as priority to discuss around predictive value of MPs. There is hypothesis regarding being of dysregulation of MP secretion and delivery and affected their interrelation in

circulation as causality factor of metabolic disorders in CVD. Indeed, the prognostic value of circulating endothelial-derived apoptotic MPs to mononuclear progenitor cells ratio for post-discharge heart failure patients was determined^[64]. Overall, impaired phenotype reflected an endothelial injury and worsening of integrity may be defined as individual risk factor that was defined by Sabatier *et al*^[65] as the "vascular competence". Taken together, all these data clarify that there is growth of clinical interest for assessment of individual vascular risk and monitoring of CVD- and vascular-directed therapeutic approaches. However, the predictive score models based on novel biomarker defined "vascular competence" or "impaired MP phenotype" are probably required. Such attempts were made among heart failure patient population^[66], but isolated predictive role of impaired MP phenotype beyond conventional biomarker, such as NT-pro brain natriuretic peptide galectin-3, was not defined.

FUTURE PERSPECTIVES

Currently nanoparticle tracking analysis, flow cytometry, and other methods of identification of MPs are not affordable in routine clinical practice. In future the balance and the intensity of endothelial damage, integrity and tissue repair reflected by changes in circulating endothelial-derived MPs and other types of pro-angiogenic progenitor cells might be open the novel approaches to assay the risk of CVD and identify vulnerable population before therapeutic care [67]. Probably, serial measurements of circulating MPs may be a clinical therapeutic target for optimal strategy to improve clinical outcomes in CVD patients, while reproducibility of contemporary tests applied to determination of circulating MPs has become under recognized. Therefore, cost and expenditures for analytical procedures regarding MPs are extremely high. Overall, determination of impaired phenotype of MPs encompasses responses of CVD risk factors and documented CVD appear to be attractive and it is required more efforts to understand whether the novel biological marker is useful or not.

In conclusion, impaired phenotype of circulating endothelial-derived MPs is appeared to be universal individual marker of vascular damage, integrity, endothelial dysfunction with possible predictive value. The circulating level of activated and apoptotic endothelial-derived MPs seems to be associated with CVD risk and CVD progression, and imbalance between absolute number of MPs with these immune phenotypes might be reflect individual CVD phenotype, which is considered a tool for risk stratification at vulnerable population.

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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