Microparticles as biomarkers of vascular dysfunction in metabolic syndrome and its individual components

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Summary

Heterogeneous in size (0.1-1 μ m), microparticles are small membrane vesicles released from activated and/or apoptotic cells. Although described since 1967 by Wolf [1], it is only since the 1990's that microparticles have been considered as biomarkers as well as potential mediators of biological messages between cells [2] by acting as paracrine and endocrine vectors. Detection of microparticles has been performed in biological fluids (blood, synovial fluid, saliva for instance) and some solid tissues but also from the culture medium [3]. Levels of circulating microparticles are enhanced in a large number of pathological states including cardiovascular and metabolic disorders associated with insulin resistance and this has been linked to deleterious effects on cells from the vascular wall, mainly, endothelial cells. This review highlights the increasing impact of microparticles in major cardiovascular pathological states stuations associated with metabolic derangements.

Introduction

Microparticles, small vesicles (0.1-1µm) released from the plasma membrane of activated and/or apoptotic cells, are considered as veritable vectors of biological information. Although generation of microparticles from cells is a universal event in cell life, little is known about the mechanisms regulating this process. Much evidence suggests that microparticles are generated at low levels by cells under physiological conditions; however, in a pathophysiological environment, increased production of microparticles is common. Recent review articles have focused on the formation and composition of microparticles as well as on their clearance and vascular effects [3, 4]. Levels of microparticles are easily measurable in body fluids using different techniques such as protein concentration, flow cytometry, or ELISA assays associated with their pro-coagulant activity. Using the two latter approaches, it is possible to quantify the total levels of microparticles as well as their origin through specific determination of their surface antigens. Moreover, microparticles can also be measured from solid tissues such as atherosclerotic plaque, liver or tumors [5, 6].

It should be noted that other types of vesicles such as exosomes and apoptotic bodies are also detectable in fluids though the mechanisms governing the release of these vesicles are different (see Table 1). Thus, exosomes of size < 0.1 μ m are released upon fusion of the limiting membrane of multi-vesicular bodies with the cell surface; their composition is completely different to that of microparticles. Indeed, exosomes are formed within endosomes by invagination of the limiting membranes resulting in the formation of multivesicular bodies. Subsequently, multivesicular bodies fuse with the plasma membrane and release exosomes into the extracellular environment [7]. The identification of exosomes is based on morphological and biochemical criteria. Thus, exosomes are obtained after high speed centrifugation (> 100,000 g) and express exosomal markers such as tetraspanins, heat shock protein (HSP)70 or HSP90.

Apoptotic bodies (size > 1µm) are formed by indiscriminate plasma membrane blebbing and possess nuclear fragments and histones [8]. These different populations of vesicles can be separated according to the physical characteristics of the shed vesicles. Serial steps of centrifugation are routinely used as sequential separation of cells, debris, apoptotic bodies, microparticles and exosome fractions. Indeed, microparticles can be isolated from the resulting supernatant after cell debris separation through additional centrifugation at < 100,000 *g* whereas exosomes are isolated after centrifugation at > 100,000 *g*. (Table 1). Differentiation between exosomes, apoptotic bodies and microparticles is also possible by electron microscopy or flow cytometry, although there are claims that exosomes would be too small to be detected by flow cytometry.

Molecular profiling approaches using proteomic techniques, performed in order to determine the protein composition of microparticles, have shown that protein composition depends not only on cell type, but also on the conditions of cell stimulation [9, 10]. Furthermore, differential lipid composition of microparticles may vary depending on the lipidic environment surrounding cells; thus it is possible that microparticles from patients with metabolic pathologies associated with lipid alterations may have different lipid composition which may account for different functional effects of microparticles on target cells. However, this hypothesis has been little studied. Thus, circulating levels of microvesicles from platelets [11] and tissue-factor-positive microvesicles from T cells or neutrophils [12] negatively correlate with plasma HDL levels. Finally, with regard to microparticle composition, it has been reported that microparticles envelope and protect mRNA and miRNA from extracellular ribonuclease degradation and in this way represent veritable cargo allowing intercellular communication [13].

Microparticles are biomarkers for cardiovascular pathologies

Circulating levels of microparticles are increased in numerous cardiovascular pathologies linked to inflammatory and/or pro-coagulant states, and in other diseases such as cancer, sepsis and HIV, for instance [3]. With regard to the pathologies associated with cardiovascular complications, microparticles from platelets are the major subpopulation of circulating microparticles and in general, their levels are enhanced in cardiovascular diseases. However, other microparticle subpopulations such as those from endothelial cells, red cells or leukocytes can also be increased as described in the metabolic syndrome [14], acute coronary syndrome [15], severe hypertension [16] and type 2 diabetes [17]. All these pathologies are associated with endothelial dysfunction and microcirculation disturbances suggesting that microparticles may, at least in part, play a key role in the pathogenesis of these diseases.

When detected in blood, microparticles originate from platelets – the main subpopulation (almost 80% of total circulating microparticles) – leukocytes, erythrocytes and endothelial cells. One of the major functions of blood platelets in haemostasis is to provide a membrane surface that accelerates blood coagulation and promotes the formation of the fibrin network in the haemostatic plug [18]. By producing microparticles displaying prothrombin-converting activity, platelets are able to increase the procoagulant potential. In addition, when stimulated *in vitro*, platelets are the cells that produce microparticles most rapidly. However, although circulating levels of platelet-derived microparticles are often increased under pathological conditions, changes in other subpopulations of microparticles, such as non-platelet-derived microparticles or more specifically endothelial-derived microparticles, seems to have more significant pathological relevance [15, 19, 14]. In this respect, microparticles originating from a specific cell may be a signature of injury of that type of cells. As an example, endothelial microparticles have been considered to be predictors of endothelial dysfunction in arterial erectile dysfunction patients with insulin resistance [20].

Total circulating microparticles or those from a specific subpopulation can be correlated with the severity of different pathologies and, in this way, they can be considered as circulating biomarkers in the surveillance of disease progression. For instance, platelet-derived microparticles are positively associated with abnormal carotid and intima-media thickness and body mass index in obese patients [21]. In addition, microparticles from platelets positively correlate with plasma glucose and insulin levels whereas microparticles from T-helper cells positively and negatively correlate with body mass index (BMI) and HDL-cholesterol, respectively [12].

Recently, it has been shown that microparticles derived from endothelial and endothelialprogenitor cells (EPCs) are positively correlated with blood glucose concentration and infarct volume after ischemic cerebral injury in obese leptin-receptor-deficient *db/db* diabetic mice [22]. These findings suggest that microparticles from EPCs and endothelial cells could be used as predictive biomarkers for ischemic stroke complication in diabetes, and targeting microparticles might offer new therapeutic avenues for diabetes and ischemic stroke. Levels of endothelial-derived, but not leukocyte-derived, microparticles are higher in hypertensive diabetic patients than in those without hypertension [23]. Endothelial-derived microparticles were shown to be an independent risk factor for the presence of hypertension in diabetic patients [23].

Microparticles as effectors implicated in the maintenance of cardiovascular diseases

During the last ten years, efforts have been focused on showing that microparticles can be effectors capable of delivering biological messages to target cells. Due to its crucial localization, one of the main targets of microparticles is the endothelium, the key component of the microcirculation contributing to the local balance between relaxing and constricting mediators, that regulates hemostasis and vascular permeability. Whereas normal shear stress controls homeostasis of the endothelial monolayer, alterations in shear stress induce imbalance in the release of endothelial-derived factor leading to endothelial dysfunction. Among these alterations, a decrease of nitric oxide (NO) release and bioavailability, an increase of vasoconstrictor factors and an enhanced oxidative stress favor a disturbance of vascular reactivity, induce vascular inflammation and remodeling by up-regulating gene expression. Theoretically, all of these vascular events can be mediated by microparticles. Thus, endothelial cells can participate in microparticle production and simultaneously be target cells of endothelial-derived microparticles. Very recently, disturbed blood flow in the forearm of healthy young men was shown acutely to induce endothelial activation, as reflected by release of microparticles from endothelial cells [24]. The authors of that study suggested that in atheroprone arteries from elderly subjects and/or in subjects with risk factors such as obesity or type 2 diabetes, the effects might be even more pronounced. Moreover, that type of microparticle may participate in a vicious cycle of vascular injury by acting directly on the neighboring endothelial cells

The mechanisms by which microparticles transfer biological information to target cells are not completely elucidated, but they may involve: (i) direct ligand-receptor interaction, (ii) transfer of surface receptors, proteins, mRNA, miRNA and bioactive lipids, (iii) fusion of microparticle and target-cell membranes and (iv) microparticle internalization. Whereas direct ligand-receptor interaction leads to activation or inhibition of a transduction signal in target cells, the other three mechanisms implicate changes in the phenotype of recipient cells (for more details see [2]). For instance, microparticles carrying the morphogen Sonic hedgehog (Shh) are able to activate a cascade interacting with its receptor Patched/Smoothened in target cells and thereby to induce endothelial angiogenesis and nitric oxide production [25, 26]. However, after 2 hours of incubation, these microparticles are internalized into endothelial cells and induce overexpression of antioxidant enzymes [27]. These changes are frequently due to the horizontal transfer of the microparticle content towards target cells.

Microparticles as markers and mediators of biological message in metabolic syndrome and its individual components

The metabolic syndrome (MetS) is a cluster of cardio-metabolic abnormalities including visceral obesity, high blood pressure, hyperglycaemia and dyslipidaemia [28, 29], which are associated with increased risk of cardiovascular disease and type 2 diabetes [28-30]. The prevalence of MetS is increasing because of an increased incidence of overweight, obesity and physical inactivity and it continues to provide challenges for medical research beyond its clinical and public-health importance [30-32]. The pathophysiology of MetS seems to be largely attributable to insulin resistance with the implication of excessive flux of fatty acids [33, 34], but also to a pro-inflammatory state resulting from the production of cytokines from adipocytes and macrophages [34, 29, 35]. Thus, increased inflammatory factors and reactive oxygen species (ROS) are associated with detrimental cardiovascular alterations linked to the MetS. Inflammation is orchestrated by the interactions between inflammatory cells (such as leukocytes) and vascular cells (endothelial and smooth muscle cells) which under activation or apoptosis (for example), lead to the release of circulating microparticles [36, 3, 2]. Increased levels of circulating microparticles have been reported in MetS, but also in individual components of the condition. In the next sections, we will summarize the effects of microparticles of different cellular origin during cardiovascular disease associated with metabolic alterations. Table 2 summarizes the types of cardiovascular dysfunctions associated with increased levels of circulating microparticles in animals and humans in the context of MetS and its individual components.

Hypertension

Several studies have shown that circulating microparticles are increased in hypertensive patients compared to normotensive subjects and in animal models of hypertension. Most of these studies have focused on endothelial-derived microparticles as possible biomarkers of

vascular dysfunction and adverse cardiovascular risk. Preston et al. [16] compared levels of circulating endothelial- (CD31⁺/CD42⁻) and platelet-derived (CD41⁺) microparticles in patients with severe uncontrolled hypertension, untreated patients with established mild hypertension and normotensive volunteers. The two hypertensive groups were matched for coexisting risk factors. The levels of endothelial microparticles were elevated in patients with mild hypertension compared to control subjects, but were even higher in the severe hypertensive patients than in the other groups. Moreover, although platelet-derived microparticles were enhanced in severely hypertensive patients, they did not differ between mild-hypertension and control subjects. Both endothelial- and platelet-derived microparticles positively correlated with the absolute level of systolic and diastolic blood pressure; however, only endothelial microparticles positively correlated with presence of diabetes mellitus and smoking. In the study by Wang et al. [37], circulating endothelial microparticles (CD31⁺/CD42⁻) and brachial-ankle pulse wave velocity were evaluated in uncontrolled hypertensive patients, well-controlled hypertensive patients and in normotensive healthy subjects. Uncontrolled hypertensive patients exhibited higher levels of circulating endothelial microparticles and faster brachial-ankle pulse-wave velocity compared to the two other groups. Interestingly, a robust positive linear correlation was observed between amounts of circulating endothelial microparticles and values of brachial-ankle pulse-wave velocity. Nomura et al. [38] also observed that microparticles of platelet and endothelial origin were significantly increased in hypertensive patients compared to healthy controls. Another human study by Lee and colleagues [39] found that hypertensive patients with micro-albuminuria or macro-albuminuria had increased endothelial apoptotic microparticles (defined as CD31⁺/Annexin V⁺). Endothelial apoptotic microparticle levels also tended to increase in hypertensive patients with electrocardiographic left-ventricular hypertrophy which is indicative of hypertensive target-organ damage and strongly predictive of future cardiovascular morbidity and mortality [40]. Pre-hypertension, the situation that precedes

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clinical hypertension, is a very frequent condition associated with increased cardiovascular risk and is a component of many cases of MetS [41, 42]. Giannotti and co-workers [43] demonstrated that although in vivo endothelial repair capacity of early EPCs was dramatically impaired in patients with newly diagnosed prehypertension and hypertension, this was not associated with increased levels of circulating endothelial apoptotic microparticles. By investigating only endothelial apoptotic microparticles (CD31⁺/Annexin V⁺ particles), the authors may have underestimated the total number of circulating endothelial microparticles. Indeed, several other studies indicate that fewer than 50% of microparticles actually expose phosphatidylserine (Annexin V^+) [14, 44]. The authors did not search other microparticle subpopulations which can be altered in pre-hypertension state. Further studies are therefore needed to draw a complete picture of how microparticle phenotype evolves with the progression and establishment of essential hypertension. Other studies in experimental models of hypertension have also shown increased levels of microparticles in hypertension. Recently, López Andrés et al. [45] found that aldosterone-salt hypertensive rats had increased circulating levels of total microparticles, platelet (CD61⁺)-, endothelial (CD54⁺)- and erythrocyte (CD235⁺)-derived microparticles than control animals. The elevation in microparticle levels was accompanied by increases in aortic stiffness, oxidative and nitrosative stress and microvascular endothelial dysfunction as well as induction of apoptosis in the vessel wall. Interestingly, treatment of rats with red-wine polyphenols prevented changes in microvascular endothelial dysfunction, oxidative and nitrosative stress and apoptosis, but perhaps more importantly, normalised the levels of circulating microparticles. Conventional hypertension treatments were shown in several human studies to modulate the numbers and phenotype of circulating microparticles. The angiotensin II blocker losartan was found to decrease both systolic and diastolic blood pressure in hypertensive patients with and without type 2 diabetes mellitus in addition to reducing circulating levels of monocytederived microparticles [18]. The combination of losartan treatment with a statin (simvastatin)

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further decreased the levels of monocyte-derived microparticles in these patients [18]. Another study found that patients with stage 1 and 2 hypertension had high levels of activated platelet microparticles compared to controls and that eprosartan treatment (600 mg per day, for one or two months) normalised patient' microparticle levels [46]. Long term treatment of diabetic hypertensive patients with the calcium antagonist, nifedipine, normalized levels of circulating monocyte- and platelet-derived microparticles [47]. These results suggest that microparticles may be implicated in the pathogenesis of hypertension and that changes in their circulating levels may be indicative of treatment efficacy in hypertension.

Diabetes

Accumulating evidence in the literature indicate that circulating microparticle levels are higher in diabetic patients and in animal models of diabetes and that these microparticles may play an important role in diabetic vascular complications. Sabatier et al. [48] explored the number and the pro-coagulant activity of cell-derived microparticles in type 1 and 2 diabetic patients and found that both groups of patients had increased levels of circulating microparticles. Nevertheless, diabetic patients differ by the pro-coagulant activity and the cellular origin of their circulating microparticles. Type 1 diabetic patients exhibited significantly increased levels of platelet-, and endothelial-derived and pro-coagulant microparticles (Annexin V⁺), and increased levels of microparticle-associated pro-coagulant activity than non-diabetics or type 2 diabetics. In type 2 diabetes patients, only pro-coagulant microparticles were significantly increased over those of control subjects without an increase in their pro-coagulant activity [48]. A more recent study by Feng et al. [49] showed, however, that type 2 diabetic patients presented higher circulating levels of pro-coagulant, platelet, leukocyte, and endothelial microparticles than did non-diabetic volunteers. In that cohort, the authors also showed that among the increased sub-types of microparticles, only those derived from the endothelium were associated with vascular dysfunction in diabetic patients. Endothelial microparticles correlated positively with glycated haemoglobin (HbA1c) and brachial-ankle pulse-wave velocity, but negatively correlated with flow-mediated dilation (FMD) [49]. A study by Nomura and co-workers [50] also reported increased plasma levels of microparticles of endothelial, monocyte and platelet origins in patients with type 2 diabetes compared to control subjects. This research also demonstrated that the presence of other comorbidities, such hypertension, with diabetes further increased the numbers of circulating microparticles [50]. Other studies also showed association between endothelial microparticle levels and diabetic complications. For instance, Koga et al. [51] found a two-fold increase in endothelial-microparticle (CD144⁺) levels in diabetic patients in comparison to healthy subjects. Endothelial-microparticle levels were significantly higher in diabetic patients with coronary artery disease than in those without. In that cohort, the elevated endothelial microparticle levels were found to be a significant independent predictor for the presence of coronary artery disease in patients with diabetes mellitus. Another study by Bernard et al. [52] investigated the levels of endothelial and platelet microparticles and the presence of coronary non-calcified plaques in 56 type 2 diabetic patients. These authors found that endothelialderived microparticles (CD144⁺) were significantly increased in patients with coronary noncalcified plaque. Recently, Tsimerman and co-workers [53] characterised the cell origin and pro-coagulant profiles of microparticles from 41 healthy volunteers and 123 type 2 diabetics with coronary artery disease, retinopathy and foot ulcers. The authors observed that diabetic patients had increased amounts of microparticles which were predominantly of platelet origin. Patients with severe diabetic foot ulcers expressed the highest levels of pro-coagulant (Annexin V^+) microparticles and those originating from platelets and endothelial cells. They also found that the microparticle pro-coagulant activity was higher in diabetics with coronary artery disease and foot ulcers than in healthy subjects and in patients with diabetic retinopathy. Interestingly, microparticles from diabetic patients increased endothelial cell coagulation activity and the most pro-coagulant microparticles were those from patients with severe diabetic foot ulcers, indicating that these microparticles may participate in thrombosis observed in diabetes. Furthermore, diabetic microparticles impaired the angiogenic capacity of human umbilical vein endothelial cells (HUVECs) grown on a matrigel. HUVECs incubated with microparticles from diabetics with coronary artery disease were unable to form tube-like networks, whereas cells incubated with microparticles from diabetics with coronary artery disease were unable to form tube-like networks, whereas cells incubated with microparticles from diabetics with retinopathy or foot ulcers induced branched-tube networks that were unstable and quickly collapsed [53]. These results suggest that microparticles may be implicated, at least in part, in the failed angiogenesis described in diabetic patients. Microparticles from other origins were also associated with diabetes complications. Platelet- and monocyte-derived microparticles for example were reported to increase with the progression and severity of diabetic retinopathy [54, 55]. In animal models of diabetes, it has been recently observed that db/db diabetic mice had increased circulating endothelial-derived microparticles and impaired cerebral microvascular density [22]. The authors also reported that circulating microparticles from db/db diabetic mice impaired EPC functions and infusion of EPCs pre-incubated with db/db microparticles failed to reduce ischemic damage in db/db mice [22].

Several human studies evaluated the modulation of circulating microparticle levels by diabetes treatments. Recently, Esposito *et al.* [56] compared the effects of pioglitazone and metformin on circulating levels of endothelial microparticles and EPCs in patients with newly diagnosed type 2 diabetes. Authors reported that 24-week pioglitazone treatment significantly reduced numbers of endothelial microparticles and increased those of EPCs [56]. The treatment of patients with type 2 diabetes for four months with the oral anti-diabetic drug miglitol (150 mg/day) was shown to decrease significantly plasma levels of platelet-derived microparticles [57]. Another oral anti-diabetic drug, acarbose, was also found to reduce plasma levels of platelet-derived microparticles in patients with type 2 diabetes [58]. Statin treatment was also reported to alter microparticles in diabetic patients. Sommeijer *et al.* [59]

found that statin, pravastatin, treatment did not change numbers of plasma microparticles from different origins in type 2 diabetics; however, it had an effect on the membrane composition of platelet-derived microparticles. The authors evaluated the intensity of Annexin V, tissue factor and Glycoprotein IIIa (GPIIIa) staining per microparticle. They found that GPIIIa expression per platelet-derived microparticle was significantly decreased after pravastatin therapy indicating reduced platelet activation [59].

These findings highlight the importance of circulating microparticles as markers of the severity of diabetes and support their potential use as tools to monitor the efficacy of treatments.

Dyslipidaemias

Few studies have investigated the levels and cellular origins of circulating microparticles in subjects with dyslipidaemia alone and not in conjunction with other risk factors. The work by Pirro *et al.* [60] characterized circulating endothelial-derived microparticles, circulating EPCs and aortic pulse-wave velocity, a marker of aortic stiffness, in 50 patients with newly diagnosed never-treated frank hypercholesterolemia and 50 normo-cholesterolaemic control subjects. In that study, subjects with secondary hyperlipidaemia caused by diabetes or another disease, history of cardiovascular disease, hypertension, evidence of atherosclerotic plaques, or with any clinical or laboratory evidence of inflammation were excluded. The authors found that hyper-cholesterolaemic subjects, as a result of both an increased number of endothelial microparticles and reduced peripheral EPCs. Endothelial microparticles and EPCs levels correlated negatively in this cohort. Furthermore, endothelial microparticle levels robustly and positively correlated with both LDL-cholesterol and aortic stiffness. A multivariate linear regression analysis showed that high plasma cholesterol and a high ratio of endothelial microparticles to EPCs independently predicted an increased aortic pulse-wave velocity [60].

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Another study by Nomura *et al.* [61] demonstrated that hyper-lipidaemic patients without type 2 diabetes had increased circulating amounts of both monocyte- and platelet-derived microparticles than normo-lipidaemic controls. A more recent study from the same group [7] evaluated the levels of circulating platelet-derived microparticles in 68 patients with hyperlipidaemia at baseline and after 6 months of treatment with pitavastatin (2 mg per day). However, the authors reported no changes in the levels of platelet-derived microparticles after treatment [7]. The levels of circulating endothelial microparticles were shown to increase very quickly in relation a postprandial increase in triglycerides in normo-lipidaemic subjects [62]. In this research, Ferreira et al. [62] showed that a single high-fat meal significantly increased endothelial microparticle levels after one and three hours and positively correlated with a postprandial elevation in serum triglycerides. Elevated postprandial plasma triglycerides are an established risk factor for cardiovascular disease [63] and several reports have indicated that increased postprandial hypertriglyceridemia can impair endothelial function in normolipidaemic subjects [64, 65]. Because cells can respond to whole-body changes by releasing circulating microparticles that are able to carry a biological message, microparticles can represent an early response to injury and may predict future adverse events in population at risk.

Obesity

Elevated levels of circulating microparticles from different cellular origins were reported in various clinical cohorts. Recently, Gündüz and colleagues [66] investigated circulating levels of endothelial-derived microparticles, carotid artery intima-media thickness and left ventricular mass index in 55 obese and overweight children and 23 healthy controls. The group reported that blood pressure, endothelial microparticle levels, and left ventricular mass index were significantly increased in the obese/overweight group compared to control subjects. Furthermore, they observed a linear correlation between blood pressure, endothelial

microparticles and overweight and obesity. This study suggested that endothelial damage starts in the early stages of childhood obesity [66]. Another cross-sectional cohort study recently evaluated the levels of different markers of platelet activation and their relationship with carotid intima media thickness along with other atherosclerotic risk factors in obese patients with or without atherosclerotic co-morbidities [21]. This work indicated that plateletderived microparticles were markedly increased in obese subjects compared to healthy volunteers and when obese patients were compared to each other; those with further cardiometabolic co-morbidities had even higher levels of these microparticles. In addition, plateletderived microparticle levels positively correlated with most parameters of cardiovascular risk such as BMI, waist circumference, fasting plasma glucose level, homeostasis model assessment (HOMA) and triglycerides. Furthermore, carotid intima media thickness had a significant and positive univariate association with platelet-derived microparticles. Multiple regression analysis showed also strong independent association between platelet-derived microparticle levels and BMI [16]. Previous work by Esposito and colleagues [67] characterized circulating levels of platelet- and endothelial-derived microparticles and FMD in 41 obese and 40 normal weight women. Obese women, when compared to lean controls, had increased circulating amounts of both platelet- and endothelial-derived microparticles and reduced values for FMD. In that study, BMI did not correlate with either platelet- or endothelial-derived microparticles, while waist-to-hip ratio significantly correlated with both types of microparticles. The impairment of FMD in obese women demonstrated a robust correlation with both platelet- and endothelial-derived microparticles; however, multivariate analysis indicated that only endothelial microparticles were an independent predictor of impaired endothelium-dependent vasodilation [67]. Strategies to reduce weight have been shown to modulate the numbers of specific sub-populations of circulating microparticles that are increased in obese subjects, further indicating a link between overweight/obesity and the elevation of circulating microparticles. For instance, Morel et al. [68] subjected 24 obese

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women to a very low-calorie diet for 90 days. Biomarkers of vascular damage and circulating levels and origins of microparticles were determined before, 30 and 90 days after starting the diet. At 90 days of diet, weight reduction was significantly associated with reduction of procoagulant, leukocyte and lymphocyte microparticles together with improved cardio-metabolic parameters such as leptin levels and blood pressure [68]. Owing to the strong correlation between microparticle circulating levels and cardiovascular risk factors in obese people, the monitoring the circulating levels of microparticles may be regarded as an interesting tool to evaluate the efficiency of life-style interventions or treatments on the reduction of cardiovascular risk in obese people.

Metabolic Syndrome (MetS)

We have seen above that individual cardio-metabolic risk factors are associated with increased levels of circulating microparticles of different origin that correlated with the progression or severity of cardiovascular risk. The association of several cardio-metabolic risk factors within one subject and how together they influence the count and phenotype of circulating microparticles have also attracted the interest of the scientific community in recent years. With this in mind, different research groups have studied the levels and cellular origins of circulating microparticles in cohorts of subjects with MetS, a cluster of cardio-metabolic risk factors. A study by Agouni *et al.* [14] determined the count and phenotype of circulating microparticles in 43 well-characterized MetS patients and 37 healthy volunteers and assessed the effects of these microparticles on endothelial function both *in vitro* and *in vivo* in mice. The authors reported that MetS patients had increased circulating levels of microparticles of platelet, endothelial and erythrocyte origin. They also demonstrated that microparticles from MetS patients altered NO signaling pathways, decreased NO production *in vitro* in cultured endothelial cells, and impaired endothelium-dependent relaxation in response to

acetylcholine in the aorta of mice injected with these microparticles. Of particular interest, the authors found that although platelet-derived microparticles represent the predominant sub-population in MetS patients, the effects of MetS microparticles on endothelial function were driven by non-platelet microparticles [14]. More recent work from the same group [69] extended the investigation of the role of circulating microparticles in the pathophysiology of MetS to their effects on the smooth muscle. The authors observed that MetS microparticles injected into healthy mice were able to interact with smooth muscle cells causing vascular hypo-reactivity in response to vasoconstrictor agonists by triggering inflammation in the vascular wall and, particularly, by up-regulating inducible nitric oxide synthase (iNOS) and NO production. They also demonstrated that MetS microparticles interact with smooth muscle cells through the Fas/Fas-ligand death pathway to cause vascular hypo-reactivity in response to vasoconstrictors [69].

Similar findings on microparticle phenotyping have been described by Helal *et al.* [70]. They reported in a bicentric cohort of MetS patients and healthy controls that patients had significantly higher circulating levels of platelet-, erythrocyte- and endothelial-derived microparticles in addition to pro-coagulant microparticles than MetS-free controls. In that study, multivariate analysis showed that waist circumference positively influenced the level of both platelet and endothelial microparticles. BMI positively correlated with pro-coagulant and endothelial microparticles and blood pressure positively influenced the level of endothelial microparticles [70]. Previous studies have also addressed microparticles in patients with MetS. Arteaga *et al.* [71] reported endothelial-cell microparticle release, platelet and leukocyte activation and increased binding of microparticles from endothelial cells and platelets to leukocytes in patients with the MetS, whereas Ueba *et al.* [72] reported that microparticle levels were predictive of MetS. Chironi and colleagues [73] showed that MetS patients exhibited higher leukocyte-derived microparticle levels than those free of such

syndrome and, in the overall study population, that leukocyte-derived microparticle levels increased gradually in parallel with the number of components of MetS. Possible reasons for these inconsistent observations between studies are differences in methods used for microparticle isolation and quantification and in patient-selection criteria.

Altogether, these findings suggest that microparticles could be used as predictive biomarkers of cardiovascular pathologies associated with the coexistence of several cardio-metabolic risk factors. For instance, other cardiovascular risks such as smoking should be considered as factors able to modify the level and the type of circulating microparticles. Whereas Grant and collaborators [74] have described reduced levels of circulating plasma-derived microvesicles (mix of microparticles and exosomes) in smokers, other workers have found an increase in endothelial-derived microparticles [75] and unchanged levels of procoagulant microparticles [76]. Moreover, *in vitro* exposure of macrophages to tobacco smoke can directly induce the release of a mix of microparticles and exosomes carrying gelatinolytic and collagenolytic activities, mainly through the protease MMP14. These vesicles may be novel mediators of clinically important matrix destruction in smokers [77].

Future directions

As described above, levels of circulating microparticles are a factor in multiple cardiovascular diseases such that it would be possible to evaluate changes in circulating microparticle levels in response to pharmacological treatment (for review see [3]). Several studies indicate that a reduction in the levels of total, or a specific population of, circulating microparticles could indicate the efficacy of a treatment or disease regression. In addition, modulation of microparticle levels by drug treatment could reduce the deleterious effects of microparticles. For example, treatment of patients with ischemic cardiomyopathy with atorvastatin significantly decreased plasma levels of endothelial-derived microparticles together with increasing numbers of EPCs [78]. Furthermore, in patients with peripheral arterial occlusive

disease, atorvastatin treatment reduced the expression of tissue factor, GPIIIa and P-selectin in platelet-derived microparticles thus inhibiting initiation of thrombin generation [79]. Further analyses of microparticle detection and quantification for each patient could allow personalized therapy. For the moment, standardization of the method of isolation and detection of microparticles is needed [80].

Acknowledgments:

Authors are thankful to Prof Margaret Rayman (University of Surrey) for her critical reading of the manuscript. Dr Agouni's research is funded by the Physiological Society, the Royal Society and the University of Surrey.

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Figure 1. Mechanisms through which microparticles participate to the pathophysiology of cardiovascular diseases. Cell stimulation or apoptosis lead to microparticle shedding. Microparticles can interact with endothelial cells and alter the NO-pathway and reactive oxygen species production leading to reduced bio-availability of NO and impairing thus vascular relaxation. Microparticles also can stimulate smooth muscle cells, through for example Fas/FasL interaction, causing vascular hyporeactivity. Circulating microparticles may bind coagulation factors and activate platelets enhancing thus coagulation. Microparticles can stimulate EPCs and reduce their tissue damage repair capacity and can also impair the angiogenic capacity of endothelial cells. Microparticles can stimulate lymphocytes and endothelial cells to produce cytokines and other inflammatory mediators. Arrows with (-) indicate an inhibitory effect and arrows with (+) indicate a stimulatory effect.

	Apoptotic bodies	Microparticles	Exosomes	
Size	> 1 µm	0.1-1 µm	<0.1 µm	
Isolation	< 15,000 g	15,000 – 21,000 g	> 100,000 g	
Density (g/ml)	1.16-1.28	Unknown	1.13-1.19	
Methods of detection	Flow cytometry, immunofluorescence, electron and atomic force microscopies, light scattering technique, western blotting, ELISA-based tests	Flow cytometry, immunofluorescence, electron and atomic force microscopy, light scattering technique, western blotting, ELISA-based tests	Immunofluorescence, electron microscopy, light scattering technique, western blotting, ELISA-based tests	
Interaction	Transfer	Ligand-receptor	Ligand-receptor	
with target	Fusion	Transfer	Transfer	
cells	Internalization	Fusion	Fusion	
		Internalization	Internalization	

Table 1.

Component	Microparticle origin	Animal models	Humans	References
Hypertension	Endothelial		Faster brachial ankle pulse-wave velocity (<i>in vivo</i>)	[37]
	Endothelial/apoptotic		left-ventricular hypertrophy (in vivo)	[40]
	Endothelial cells , platelets, and erythrocytes	Aortic stiffness, microvascular endothelial dysfunction (<i>ex</i> <i>vivo</i>)		[45]
Diabetes	Pro-coagulant, platelets and endothelial cells		Increased pro- cogulant activity (<i>ex vivo</i>)	[48]
	Pro-coagulant, platelets, leukocytes, and endothelial cells		Faster brachial- ankle pulse-wave velocity and reduced FMD (<i>in</i> <i>vivo</i>)	[49]
	Endothelial cells		Coronary artery disease (<i>in vivo</i>)	[51, 52]
	Pro-coagulant, platelets and endothelial cells		Impaired angiogenesis (<i>ex</i> <i>vivo</i>)	[53]
	Platelets and monocytes		Progression and severity of retinopathy (<i>in</i> <i>vivo</i>)	[54, 55]
	Endothelial cells	Impaired cerebral microvascular density and impaired EPC functions (<i>in vivo</i> and <i>ex vivo</i>)		[22]
Dyslipidaemias	Endothelial cells		Aortic stiffness and decreased EPCs numbers (<i>in</i> <i>vivo</i>)	[60]
Obesity	Endothelial cells		Increased left ventricular mass index (<i>in vivo</i>)	[66]
	Platelets		Increased carotid intima media thickness (<i>in</i> <i>vivo</i>)	[21]
	Platelets and endothelial cells		Reduced FMD (<i>in vivo</i>)	[67]
MetS	Pro-coagulant, platelets, erythrocytes and endothelial cells	Endothelial dysfunction and vascular hypo- reactivity (<i>in vivo</i> and <i>ex vivo</i>)	,	[14, 69]
	Endothelial cells, leukocytes and platelets		Microparticles are predictive of MetS (<i>in vivo</i>)	[72, 73]

Table1. Summary of the cardiovascular dysfunctions associated with increased levels of circulating microparticles in animals and humans in MetS and its individual components