Microvesicles – potential biomarkers for the interrelations atherosclerosis/type 2 diabetes mellitus

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Abstract
Microvesicles, also called microparticles or exosomes, are ultrastructural cellular components that have been widely researched in the past as well as present in order to establish their morphology, origin and role in physiological and pathological processes. Advanced techniques show that these microparticles have their clinical implications in the prevention and prediction in pathology and have potential in the discovery of novel therapeutic approaches to metabolic diseases such as diabetes, cardiovascular, autoimmune diseases and cancer.

Keywords: microvesicles, atherosclerosis, diabetes mellitus.

Microvesicles, which are considered “extracellular organelles” (exosomes) that vary in size from less than 50–200 nm to more than 200–1000 nm (microparticles), are released by various cell types like mast cells, epithelial cells, endothelial cells, dendritic cells, astrocytes and a large number by blood cells, especially platelets [1].

Microparticles must be distinguished from apoptotic bodies that have a diameter of 1–5 μm and whose main function are to safely remove the cell material from tissue without causing inflammation, possible horizontal transfer of oncogenes [2] and horizontal transfer of DNA [3, 4].

The mechanism by which diabetes causes atherosclerotic complications is poorly understood but is likely related to the inflammatory process, new and actual aspects as principal etiopathogenetic concept.

Histologically and chronologically, fibrous atherosclerotic lesions occur in various forms such as fatty streaks, fibrous and complicated plaques with the latter predominant in diabetes and often leading to ruptures and thromboembolism [5].

Atherotic plaques, the principal substrate for early coronary disease, have a large lipid-rich core, a thin fibrous cap and a large inflammatory content comprised mainly of macrophages [6]. The coronary arteries in diabetes mellitus present not only larger plaque burden but also a large necrotic core, as demonstrated by ultrasound analysis.

The endpoint of interest is the interrelation between atherotic plaque and the coronary artery appearance of major adverse cardiovascular events such as myocardial infarction and revascularization. The pathophysiological endpoint is the situation of decreased lipids, inflammatory and necrotic component of the atherosclerotic plaque after long-term treatment [7], acute atherothrombotic events resulting from plaque rupture or ulceration [8].

Intravascular ultrasound studies, angioscopic studies [9] and ultrafast computed tomography scans [10] have shown that diabetes is associated with accelerated atherosclerotic artery disease, cardiovascular complications and increased morbidity. The mechanism by which diabetes causes differences in coronary plaque morphology is complex and poorly understood, but likely related to the proinflammatory and prothrombotic diabetic state [11].

Hofmann et al. and Cipollone et al. [12, 13] show that RAGE mediates a novel proinflammatory axis: a central cell surface receptor b100/calgranulin polypeptides and the balance between Pgd-synthase and PgE-synthase is a major determinant of atherosclerotic plaque instability in humans.

In the past years and at present numerous articles brought convincing and detailed data in favor of accepting the concept that in atherogenic disease, blood platelets work together with T-lymphocytes and monocytes, forming a trio characteristic of the disease.

Cells present in this process are: monocytes, macrophages, platelets and lymphocytes, the pathogenesis of atheromatosis is active involving this trio cell which functions have well defined stages. Since the first events (increased vascular permeability and monocyte adhesion to endothelium) to advanced disease (appearance of foamy cells, cell necrosis and necrotic plaque formation), pathogenesis of atherosclerotic strongly implies monocyte/macrophase inflammatory complex. The presence of T-cells in combination with the macrophages indicates their effect of local inflammatory and immunological processes, which result in the development of atherosclerosis, vascular process [14–16]. These cells present numerical and organizational aspects slightly different in relation to the histological structure and function of that particular vascular area, as also reported by Pleşea et al. [17].
VanderLaan et al. [18] state that there are numerical variations of the three blood cell types due to the differences in how arteries respond to inflammation. It has been shown that in comparison to non-diabetics, the patients with type 1 and type 2 diabetes present a more accentuated infiltration of macrophages and T-lymphocytes in atherosclerotic plaques and in the larger necrotic areas [19].

The research in the last decades revealed new data and new theoretical and applied interpretations regarding the structural and biochemical organization of microvesicles, their implications in histopathology and physiology as diagnostic biomarkers, as well as the prognosis and therapy of certain diseases such as cardiovascular and metabolic diseases and tumors.

Microvesicles are heterogeneous and depend on cell type and origin (Figure 1). Their composition also varies according to the fluids they are found in, e.g., saliva, bile, urine, mucus, ascitic, cerebrospinal and bronchoalveolar fluids, lymph and blood [20].

The biochemical microvesicle analysis revealed mainly by transcriptomics, proteomics and lipidomics, are constituting basic methods of one and two-dimensional electrophoretic separation. Transmission electron microscopy brought data on the size, mass and morphology of exosomes, genetic studies on their origin, while flow cytometry analysis of microparticles circulating in blood plasma [21] (Figure 2). However, all these technologies have limitations especially in membrane analysis and should be interpreted continuously [22].

Microvesicles are microstructures found in great numbers in vasculopathies [23], metabolic diseases,
including diabetes [24], cancer, autoimmune diseases and inflammation. Due to their content, microvesicles have the potential to become physiological and pathological biomarkers. They include receptors, transporters, cyto/nuclear proteins such as annexins, cytokines, growth factors, angiogenic and heat shock proteins, GP1 anchored proteins like CD63, CD73, CD55, CD59, as well as DNA, mRNAs and microRNAs [24]. Inducers of apoptotic signals and mechanical stress such as physiological signal chemokines (adipokines and cytokines TNF-α, IL-1), hormones (insulin), oxidized LDL and Ca\(^{2+}\) are also investigated in microvesicle their interpretations, depending on their correlation with critical disease status and await further validation as biomarker [13].

Figure 2 – Sedimented cell (A–C) and isolated microvesicles (D–F) from a human postoperative drainage fluid. The sample was abundant in erythrocytes (A) while leukocytes could be found (B and C). Numerous microvesicles were found in the supernatant (D–F) as well in the sediment (A) ([4], with author permission).

The majority of circulating microvesicles originate in blood platelets or even megakaryocytes [25]. Their numbers increase in cardiovascular diseases, including atherosclerosis present in diabetes mellitus type 2 [26], and could function as indicators of vascular complications. Significant differences have been demonstrated between type 1 and type 2 of diabetes [27].

The development of endothelial dysfunction and vasculopathies such as atherosclerosis during type 2 diabetes is known to involve multifunctional processes and presents with characteristic morphopathological and clinical particularities. Atherosclerosis is prevalent among diabetics (approximately 49.3%) and postmenopausal women and its severity is clinically differentiated based on coronary, cerebral and peripheral (especially the lower limbs) localization [28]. Diabetic patients present
major dysfunctions in the arterial wall, especially the endothelium, which precede microscopic alterations. Nitric oxide (NO), the main mediator of acetylcholine-induced vasorelaxation, has reduced endothelial production and leads to disturbed function of the vascular wall, increasing the likelihood for atherosclerosis [29]. Alterations also appear in the ratio NO/endothelin 1 (vasoconstriction factor) and/or acetyl produced in relation to other factors such as VCAM (vascular cell adhesion molecule) and E-selectin, all of which are synthesized in vascular endothelia and eliminated in the bloodstream via exosomes [30]. All these factors play an important role in venous thromboembolism increased cellular adhesion [31] and elevated concentration of PAI-1 (plasminogen-activator inhibitor-1), which is of importance in atherosclerotic plaque evolution in diabetes [32].

Thus, in type 2 diabetes, there is increased pro-coagulation vasoconstriction, thrombosis via hyper-coagulation and platelet activation [11, 33]. Diabetes is also associated with accelerated development of atherosclerotic disease, which leads to increased morbidity and cardiovascular complications. A greater incidence of coronary thrombosis and more extensive calcifications are seen in diabetic patients [34].

The first morphopathological arterial modifications appear with the thickening of the vascular intima that precedes the formation of atheromatous lesions and, therefore, represent an indicator of atheromatous plaque formation and of coronary heart disease prognosis [35].

Type 1 diabetic patients present a higher number of microvesicles, most of them associated with pro-coagulant activity, suggestive of an association with impaired glucose tolerance and homeostasis [23]. In contrast, type 2 diabetes correlates with dipeptidyl peptidase IV (DPP-IV) found in microvesicles, which is considered a prediction factor for the development of vasculopathies and nephropathies in diabetes that plays a major role in glucose metabolism and it is responsible for the degradation of incretins such as GLP-1 [36].

The potential of microvesicle-associated micro-RNAs as biomarkers for type 2 diabetes can be inferred from the multiple roles of micro-RNAs in the regulation of lipid and glucose metabolism [37]. Their application in therapy awaits further evaluation. The down regulation of micro-RNA 103/107 zoom the microvesicles leads to an elevated amount of caveolin-1, a positive regulator of insulin receptor signaling. This is an example of how specific micro-RNAs can be used as biomarkers for the development of insulin resistance and the pathogenesis of type 2 diabetes.

Future experimental research needs to identify the compositional characteristics of microvesicles specific to the cells and tissues of origin involved in the pathogenesis of type 2 diabetes: pancreatic beta cells, liver, skeletal and smooth muscle, adipose tissue, endothelial cells and macrophages[24]. Recently, de Ferranti and Mozaffarian showed that adipocyte hypertrophy and hyperplasia is involved in the dramatic increase in adipose tissue mass in obese as well as type 2 diabetic patients [38].

The microvesicles released from dysfunctional and hypertrophied adipocytes modify the function of vascular endothelial cells involved in the progression of diabetes and obesity. Recent studies indicate that they mediate the transport of mRNAs coding for adiponectin, resist degradation once engulfed by cultured macrophages and induce angiogenesis [39]. Adiponectin is an important factor found in microvesicles and has a role in the progression of arterial and coronary diseases and non-insulin-dependent diabetes. It is secreted by adipocytes, is localized on chromosome 3q27 and is similar in structure to complement 1q [40]. Its antiatherogenic, anti-inflammatory and anti-remodeling effects offer promising new therapy alternatives in cardiovascular pathology, especially coronary disease and diabetes [41].

In conclusion, the level of microvesicles in vasculopathies and diabetes mellitus is positively correlated with type 2 diabetes in particular. Moreover, they accompany or even induce the development of diabetes and obesity-linked diabetes. The changes in the number of circulating microparticles occur prior to the onset of atherosclerotic or diabetic symptoms. The microvesicles participate in pathogenic processes, such as the development of diabetes and cardiovascular complications in this metabolic state [42]. Microvesicles are involved in the prediction, diagnosis and treatment of common complex multifactorial pathologies and are useful biomarkers in the identification of critical disease states.

References
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