REVIEW

Extracellular vesicles in diabetic cardiac and cerebro-vascular pathology

Stefania Lucia Magda[1,2], Elisa Serban[2]

Abstract: Individuals with type 2 diabetes mellitus develop more frequently than healthy controls cardio- and cerebrovascular disorders. It is important to understand the mechanisms through which diabetes contributes to the development and severity of these complications. Extracellular vesicles (EV) are important mediators of cell to cell communication in several diseases, including diabetes mellitus and vascular disease. Three populations of EVs are described presently: exosomes, microvesicles and apopototic bodies. Recent studies have shown that atherosclerotic lesions of all stages contain microvesicles. Higher levels of circulating EVs have been discovered in individuals with cardiovascular risk factors, with both pro-atherogenic and anti-atherogenic effects. Regarding cerebrovascular disease, studies have shown that exosomes, especially those derived from stem cells, play an important role, preventing post-ischemic suppression. Different cell types in the heart contribute to the pathogenesis of diabetic cardiomyopathy and EV seem to be essential in the intercellular crosstalk between heart cells. According to recent research EVs could play an important role in different cardiac and cerebral regenerative therapies and could also be used as therapeutic vectors in cardiovascular medicine. Further large animal and human studies are necessary to validate EVs as diagnostic and therapeutic tools.
Keywords: Diabetes, cardiovascular disease, cerebrovascular disease, cardiomyopathy.

Rezumat: Pacienţii cu diabet zaharat de tip 2 dezvoltă mai frecvent decât indivizii sănătoşi complicaţii cardio şi cerebrovasculare. De aceea, este important să descifrăm mecanismele prin care diabetul contribuie la dezvoltarea şi severitatea acestor complicaţii. Veziculele extracelulare (EV) reprezintă mediatori importanţi ai comunicării intercelulare în diferite afecţiuni, inclusiv în diabetul zaharat şi boala vasculară. Trei populaţii de EV sunt cunoscute în prezent: exozomii, veziculele extracelulare şi corpii apoptotici. Studii recente au demonstrat că leziunile aterosclerotice de toate gradele conţin microvezicule. Nivele mai ridicate de EV au fost evidenţiate la indivizi cu factori de risc cardiovascular. EV pot avea atât efecte pro, cât şi anti-aterogenice, dependent de tipul lor. În ceea ce priveşte afectarea cerebrovasculară, studiile au indicat că exozomii, mai ales cei derivaţi din celule stem, joacă un rol important în supresia post-ischemică. Diferite tipuri de celule cardiace contribuie la patogeneza cardimiopatiei diabetice şi studiile au demonstrat că EV sunt esenţiale în dialogul intercelular cardiac. Cercetări din ultima decadă au indicat că EV sunt foarte importante în diferite terapii regenerative cardiace şi cerebrale şi ar putea fi utilizate ca şi vectori terapeutici în medicina cardiovasculară. Sunt necesare studii de dimensiuni mari pentru a valida EV ca şi instrumente diagnostice şi terapeutice.
Cuvinte cheie: diabet, boala cardiovasculară, boala cerebrovasculară, cardimiopatie.

INTRODUCTION

Diabetes mellitus is a common chronic disease all over the world, with increasing incidence and prevalence, due to contemporary lifestyle with reduced physical activity, processed food and increased obesity. A meta-analysis published in 2009, including studies from 91 countries, reported an estimate for 2010 of 285 million people with diabetes worldwide, with significant differences between populations and regions, and a predicted increase from 2010 to 2030 of 54%, corresponding to an annual growth of 2.2%, nearly twice as high as the annual growth of the total world adult population[1].

Individuals with type 2 diabetes mellitus develop more frequently than healthy controls cardiovascular disorders, including coronary heart disease, stroke,

[1] „Carol Davila"University of Medicine and Pharmacy, Bucharest, Romania
[2] Department of Cardiology, Emergency University Hospital, Bucharest, Romania

◤ **Contact address:**
Stefania Lucia Magda, MD, PhD
Department of Cardiology, Emergency University Hospital, Bucharest, Romania.

peripheral arterial disease, and diabetic cardiomyopathy, mainly through the chronic, damaging exposure of the vascular system to hyperglycemia[2]. Therefore, it is important to understand the exact mechanisms through which diabetes contributes to the development and severity of these complications.

Extracellular vesicles (EV) represent small cell-secreted structures naturally released into the extracellular space by all eukaryotes and many prokaryotes, containing proteins, lipids, nuclear material and noncoding RNAs[2]. They were first described in the late 1960s and are important mediators of cell to cell communication in several diseases, including diabetes mellitus and cardiovascular disease. Most cell types can release vesicles into the interstitial space. These vesicles can be found in body fluids, both human and animal, such as blood, urine, tears and saliva, as well as in cell cultures[3].

Three populations of extracellular vesicles are described presently: 1. Exosomes are the smallest, with a diameter around 30-150 nm in diameter. They are released through exocytosis after fusion of multivesicular bodies with the plasma membrane; 2. Microvesicles (microparticles/ectosomes) are larger vesicles, with a diameter of 100-1000 nm, which are formed by the outward budding and scission of the extracellular membrane; 3. Apoptotic bodies are the largest subtype of microvesicles with a diameter of 1-5 μm, generated by the plasma membrane of apoptotic cells[4].

Several strategies are currently available for the quantification of extracellular vesicles, the most popular being ultracentrifugation, among others, such as: density gradient, precipitation, field flow, chromatography and affinity based capture and microfluidic techniques[4]. All available isolation methods are time intensive, require expensive equipment, and are limited by the fact that they do not purify specific populations of vesicles, probably due to lack of standardization of the techniques and methods[4]. In the last 8 years, the International Society of Extracellular Vesicles has constantly tried to update the topics of nomenclature, separation, characterization and functional analysis of EV5.

EV AND CORONARY ARTERY DISEASE

Recent studies have shown that atherosclerotic lesions of all stages contain microvesicles[6,7]. Higher levels of circulating microvesicles have been discovered in individuals with cardiovascular risk factors, such as smoking[8], dyslipidemia[9], diabetes mellitus[10] and arterial hypertension[11], probably through activation or from apoptosis of different cells being exposed to a damaging stimulus.

Data extracted from *in vitro* studies suggest that microvesicles can have both pro-inflammatory and anti-inflammatory effects, depending on different situations[3]. Microvesicles increase the release of pro-inflammatory cytokines (mainly interleukin 6 and 8) from endothelial cells and leukocytes, promoting the adhesion of monocytes to the endothelium and their migration to the atherosclerotic plaque[3,12]. Also, endothelial microvesicles can activate monocytes. Another effect of microvesicles is their interaction with the vascular endothelium and decreasing the NO production by endothelial cells-consequently impairing endothelial properties[13]. Endothelial microvesicles and platelet derived microvesicles increase endothelial permeability[14]. Microvesicles promote adhesion of monocytes to the endothelium by increasing endothelial expression of adhesion molecules[15].

Various microvesicles contribute to foam cell formation in the atherosclerotic plaque by stimulating lipid and cholesterol formation in macrophages. Macrophages and foam cell undergo afterwards apoptosis, forming a core of extracellular lipids. Increased monocytes and macrophage apoptosis contributes to increased microvesicle release in the plaque. Microvesicles of monocyte and macrophage origin are the largest population of microvesicles in human atherosclerotic lesions[16].

Infiltration of LDL particles in the vascular wall during the atherosclerotic process can induce the formation and release of tissue factor enriched microvesicles from the smooth muscle cell, microvesicles which in their turn influence smooth muscle cell proliferation and migration[17].

Extracellular vesicles of different origins, with different microRNA content, contribute to smooth muscle cell proliferation[19].

Several studies of patients with stable coronary artery disease have reported increased levels of circulating microvesicles. Specific microvesicle subpopulations, especially those of endothelial origin, characterized by CD 144+, CD 131+/annexin A5+, or microvesicles containing miR-199a and miR-126, are currently researched as interesting biomarkers for cardiovascular risk and mortality in these patients[20-22].

Calcifications present in the atherosclerotic plaque have destabilizing effect in early lesions, favoring rupture, but gain a potential protective effect in advanced

lesions with heavy calcium deposits[23]. The calcification process is based on three mechanisms: 1. Cell apoptosis, which releases microvesicles and necrotic debris, leading to nucleate apatite; 2. Deficiency of mineralization inhibitors (both tissue derived and circulating), leading to random apatite deposits; 3. An altered differentiation of vascular smooth muscle cells and stem cells, leading to bone formation[24]. As several studies have shown, atherosclerotic plaque calcifications are associated with extracellular vesicles of endothelial, smooth muscle cell and macrophage origin.

Exposure of vascular smooth muscle cells to proinflammatory cytokines can stimulate the release of exosomes which can mineralize when inhibitors of calcification are missing or not functioning[25]. Also, endothelial cells exposed to proinflammatory stimuli can release microvesicles rich in bone morphogenetic protein 2, promoting calcification in vascular smooth muscle cells[26]. Alterations in local homeostasis of calcium and phosphate lead to the formation of macrophage derived exosomes which stimulate mineralization[27].

Recent studies have also noted that in humans, advanced atherosclerotic plaques have a high content of procoagulant microvesicles, originating form leukocytes, erythrocytes and smooth muscle cells. These microvesicles can actively initiate the coagulation cascade, either through the presence of tissue factor on their surface, or by exposing phosphatidylserine, which concentrates factors VII and VIIa on their outer membrane[16,28].

Circulating leukocyte and platelet derived microvesicles can affect the clotting[29], but the actual magnitude of the prothrombotic effects of microvesicles in acute coronary syndromes is still under evaluation[3,30].

In opposition to microvesicles, exosomes have shown antithrombotic effects. In animal studies plateled-derived exosomes suppressed platelet aggregation and occlusive thrombosis[31].

Microvesicles influence different mechanisms that lead to plaque destabilization and rupture. Intraplaque hemorrhages are produced by neovascularization originating from adventitial tissue, stimulated by plaque microvesicles, such as CD40^{+} vesicles of macrophage origin. Hemorrhages are also favored by leukocyte and endothelial microvesicles with fibrinolytic activity[32,33].

Microvesicles can rise endothelial permeability 34 and also modulate inflammation in the plaque[35], promoting fibrous cap rupture. Fibrous cap weakening is associated with smooth muscle cell apoptosis, induced by the presence of microvesicles and exosomes, released in some pathological conditions[36]. Moreover microvesicles can influence breakdown of matrix structural proteins through metalloproteinase (MMP) interactions[3].

The final evolution of the atherosclerotic plaque is represented by plaque erosion or rupture with in situ thrombosis, clinically expressed as acute coronary syndrome[3].

Circulating levels of procoagulant microvesicles are higher in patients with acute coronary syndromes compared to healthy controls or patients with stable coronary artery disease[37], the origin of those microvesicles being mostly endothelial cells, leukocytes, erythrocytes and platelets[22,37]. Circulating microvesicles alterate endothelium dependent NO mediated vasodilation and endothelial microvesicles increase endothelial thrombogenicity[14,38].

Circulating microvesicles have been also investigated as prognostic markers in secondary prevention, in order to identify patients at high cardiovascular risk[21,22]. Increased levels of CD11b^{+}/CD66^{+} leukocyte derived microvesicles could be useful in identifying asymptomatic patients at high risk for plaque rupture[39], while CD3+/CD45+ microvesicles could identify individuals who will develop a major cardiovascular event[40].

In patients with acute ST elevation myocardial infarction, circulating microvesicles from the coronary arteries contain higher levels of oxidation specific epitopes, linked to inflammatory responses involved in atherosclerosis, than microvesicles from the peripheral circulation[41].

Circulating exosomes and microvesicles with specific cardiac microRNAs, increase after coronary artery by-pass. Expression of miR-208a in circulating exosomes increases in patients with acute coronary syndromes[42] and specific p-53 responsive microRNAs from plasma exosomes are predictive indicators for heart failure after myocardial infarction[43].

EV AND CEREBROVASCULAR DISEASE

Regarding cerebrovascular disease, especially stroke, studies have shown that exosomes, especially those derived from stem cells, play an important role in neurological disease, preventing post-ischemic suppression. Also, exosomes might be an interesting therapeutic resource in the field of regenerative medicine after stroke[44].

After stroke, exosomes are released from brain cells and can be detected in the peripheral blood or

the cerebro-spinal fluid[45,46]. As a response to stroke, exosomes are released also from blood cells and endothelial cells[47]. Circulating exosomes could therefore be useful biomarkers for stroke progression and recovery[44].

Exosome levels of cystatin C and CD 14 have been good predictors in studies of vascular risk in patients with coronary artery disease and also they have been associated to the progression of cerebral atrophy in patients with vascular disease[48].

Circulating exosomes can express different microRNAs in various types of cerebrovascular disease. Serum exosomal miR-9 and miR-124 levels are higher in patients with stroke compared to controls[49]. Another study has reported higher levels of miR-223 in acute ischemic stroke, correlated to stroke severity and short term outcomes[50]. Finally miR-199b-3p, miR 27b-3p, miR-130a-3p, mi-R 221-3- and miR-24-3p are more expressed in patients with asymptomatic carotid artery stenosis progression[51].

Exosomes derived from mesenchymal stem cells have enhanced in animal studies the restorative effects in the brain after stroke, reducing neurological impairment, promoting grey matter repair and white matter repair, as well as neurogenesis and reversing stroke-induced peripheral immunosuppression[52-56].

Cardiovascular dysfunction has been proposed as one of the main causes of cognitive impairment in the elderly, this association being stronger in patients with diabetes mellitus[57,58]. *In vitro* studies have shown that extracellular vesicles in diabetic microvascular disease may increase the haemato-encephalic barrier permeability[59,60].

EV AND DIABETIC CARDIOMYOPATHY (DCM)

Diabetic cardiomyopathy can be clinically defined by the presence of abnormal myocardial performance or structure in the absence of epicardial coronary artery disease, hypertension, and significant valvular disease. Hyperglycemia is the cornerstone of the pathogenesis, inducing stimuli that result in myocardial fibrosis and collagen deposition. These processes are generating altered myocardial relaxation and determine diastolic dysfunction on ultrasound imaging[61]. Over time, the progression of diabetic cardiomyopathy can lead to clinically manifest heart failure. Different cell types in the heart (such as cardiomyocytes, endothelial cells, smooth muscle cells, hematopoietic derived cells and fibroblast cells) contribute to the pathogenesis

of DCM and several studies have shown that EV are essential in the intercellular crosstalk between heart cells[2,62].

Cardiomyocyte's derived EV are implicated in diabetic cardiomyocyte steatosis. Higher levels of miR-1 and miR-133a were noted in EV derived from lipid loaded cardiomyocytes, in the serum of mice fed with a high fat diet and in the circulation of diabetic patients[63].

An animal study performed in 2014 has shown that the communication between cardiomyocyte derived EV and endothelium is altered in diabetes, inducing an altered angiogenesis[64].

Endothelial cell death and dysfunctional angiogenesis are frequent in diabetes mellitus. Several microRNA based mechanisms have been studied in order to explain vascular dysfunction in diabetes[2]. Hyperglycemia increases levels of miR-503 in the endothelium, leading to low endothelial cell proliferation and angiogenesis[65]. Also, hyperglycemia reduces miR-126 expression in extracellular vesicles derived from endothelial cell, thus impairing endothelial cell repair[66]. Reduced miR-126 expression in circulating EV and endothelial progenitor cells derived EV in patients with uncontrolled diabetes altered endothelial repair, increased apoptosis and the production of reactive oxygen species[67].

Cardiac fibroblasts are important components of the fibrotic response in diabetic cardiomyopathy. A potential mediator of the pro-fibrotic action induced by hyperglycemia in the cardiac fibroblasts is miR-21*. Inhibition of miR-21* in mice with cardiac hypertrophy suppressed the myocardial thickening[68]. Also, a model including *in vitro* cellular stretch and *in vivo* pressure overload has induced the release of extracellular vesicles form cardiomyocytes enriched with angiotensin type I receptor[69].

THERAPEUTIC POTENTIAL OF EV

According to results from studies from the last 5 to 10 years, extracellular vesicles could play an important role in different cardiac regenerative therapies and could also be used as therapeutic vectors in cardiovascular medicine.

Platelet derived vesicles induce vascular endothelial growth factor (VEGF) dependent angiogenesis and stimulate post-ischemic revascularization after chronic ischemia[70]. Also, plasma derived exosomes activate Toll like receptor 4 on cardiomyocytes and thus protect the myocardium from ischemia-reperfusion injury[71].

Mesenchymal stem cell derived extracellular vesicles could be an alternative to stem cell transplanta-

tion after myocardial ischemia, by transfer of specific microRNAs through embryonic stem cell extracellular vesicles[72].

The use of extracellular vesicles as therapeutic vectors could be done through bioengineering, either by modifying the cytosolic content of the vesicles which could be transferred to the target cell in order to influence cell metabolism; or by loading of extracellular vesicles with molecules to be delivered to target cells. Studies regarding the use of extracellular vesicles as therapeutic vectors in cardiovascular disease are few and are only on animal models. For example, administration of apoptotic bodies containing miR-126 decreased atherosclerotic plaque formation in mice[73] and stimulate vascular endothelial cell repair after vascular injury[66].

Different cardiovascular medications can influence the level of circulating microvesicles. Antiplatelet agents (ticlopidine, abiciximab) inhibit platelet activation and also the release of platelet-derived microvesicles[74-76]. Antihypertensive agents (such as angiotensin II receptor inhibitors, beta blockers and calcium channel blockers) lower the circulating levels of platelet and monocyte derived microvesicles[77]. The effects of statin treatment on circulating microvesicles of platelet and endothelial origin are still under debate[31,78]. Statins and antihypertensive medication are able to modify the properties of *in vivo* generated endothelial microvesicles and their effect on the expression of endothelial adhesion molecules, inhibiting the adhesion of monocytes to endothelial cells and improving endothelial function[79].

Exosomes from various cell types (such as embryonic stem cells, neural stem cells and mononuclear stem cells), have been tested as treatment for stroke in addition to mesenchymal stem cell derived exosomes and showed good results in animal models of stroke, with improvement in neurological scores and reduction in lesion volume and tissue loss[44], showing a promising clinical applicability regarding neurological restorative effects and meeting also important safety considerations.

CONCLUSIONS

Extracellular vesicles are vectors of biological information that could influence cardio- and cerebro-vascular disease in diabetic patients, by transferring beneficent or negative mediators/stimuli. Also, they have strong therapeutic potential, especially regarding regenerative medicine. The issue of correct isolation of extracellular vesicles from circulation, liquid biopsies and different tissue still limit current knowledge on this subject. Also, there is still little information about in vivo dynamics of extracellular vesicles. Further large cohort animal and human studies are necessary to validate extracellular vesicles as diagnostic and therapeutic tools.

References

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010;87:4–14.
2. Gustafson D, Veitch S, Fish JE. Extracellular Vesicles as Protagonists of Diabetic Cardiovascular Pathology. Front Cardiovasc Medicine 2017; 4: 71. Doi: 10.3389/fcvm.2017.00071.
3. Boulanger CM, Loyer X, Rautou E and Amabile N. Extracellular vesicles in coronary artery disease. Nat Rev Cardiol 2017;14:259-72.
4. Anouar H, Daskalopoulou S. Extracellular vesicles characteristics and emerging roles in atherosclerotic cardiovascular disease. Metabolism.2018; 85: 213-22.
5. https://www.isev.org
6. Heusermann W, Hean J, Trojer D, et al. Exosomes surf on filopodia to enter cells at endocytic hot spots, traffic within endosomes, and are targeted to the ER. J. Cell Biol 2016; 213: 173–84.
7. Bobryshev, YV, Killingsworth, MC, Orekhov, AN. Increased shedding of microvesicles from intimal smooth muscle cells in athero-prone areas of the human aorta: implications for understanding of the predisease stage. Pathobiology 2013; 80: 24–31.
8. Gordon C, Gudi K, Krause A et al. Circulating endothelial microparticles as a measure of early lung destruction in cigarette smokers. Am. J. Respir. Crit. Care Med. 2011; 184: 224–32.
9. Ferreira AC, Peter AA, Mendez AJ, et al. Postprandial hypertriglyceridemia increases circulating levels of endothelial cell microparticles. Circulation 2004; 110: 3599–603.
10. Sabatier F, Darmon P, Hugel B, et al. Type 1 and type 2 diabetic patients display different patterns of cellular microparticles. Diabetes 2002; 51: 2840–45.
11. Preston RA, Jy W, Jimenez JJ, et al. Effects of severe hypertension on endothelial and platelet microparticles. Hypertension 2003; 41: 211–17.
12. Nomura S, Tandon NN, Nakamura T, et al. High-shear-stress-induced activation of platelets and microparticles enhances expression of cell adhesion molecules in THP1 and endothelial cells. Atherosclerosis 2001; 158: 277–87.
13. Boulanger CM, Scoazec A, Ebrahimian T, et al. Circulating microparticles from patients with myocardial infarction cause endothelial dysfunction. Circulation 2001; 104: 2649-652.
14. Edirissi H, Schock SK, Hakim AM, Thompson CS. Microparticles generated during cerebral ischemia increase the permeability of microvascular endothelial barriers in vitro. Brain Res 2016: 1634: 83-93.
15. Barry OP, Practico D, Savani R, et al. Modulation of monocyte endothelial cell interactions by platelet microparticles. J Clin Invest 1998; 102: 136-144.
16. Leroyer AS, Isobe H, Lesèche G, et al. Cellular origins and thrombogenic activity of microparticles isolated form human atherosclerotic plaques. J Am Coll Cardiol 2007; 49: 772-77.
17. Brosseau C, Morissette G, Fortin J, et al. Tumor cells expressing tissue factor influence the migration of smooth muscle cells in a catalytic activity dependent way. Can J Physiol Pharmacol 2009; 87: 694-701.
18. Sarkar A, Mitra S, Mehta S, et al. Monocyte derived microvesicles deliver a cell death message via encapsulated caspase 1. PloS ONE 2009; 4: e7140

19. Jansen F, Yang X, Proebsting S, et al. MicroRNA expression in circulating microvesicles predicts cardiovascular events in patients with coronary artery disease. J Am Heart Assoc 2014; 3: e001249

20. Sinning JM, Losch J, Walenta K, et al. Circulating CD 31+/Annexin V + microparticles correlate with cardiovascular outcomes. Eur Heart J 2011; 32: 2034-41.

21. Nozaki T, Sugiyama S, Koga H, et al. Significance of a multiple biomarker strategy including endothelial dysfunction to improve risk stratification for cardiovascular events in patients at high risk for coronary artery disease. J Am Coll Cardiol 2009; 54: 601-608.

22. Hsu JJ, Lim J, Tintut Y, Demer LL. Cell-matrix mechanics and pattern formation in inflammatory cardiovascular calification. Heart 2016; 102: 1710-15.

23. Otsuka F, Sakakura K, Yahagi K, et al. Has our understanding of calcification in human coronary atherosclerosis progressed? Arterioscler Thromb Vasc Biol 2014; 34: 724-36.

24. Kapustin AN, Chatrou ML, Drozdov I, et al. Vascular smooth muscle cell calcification is mediated by regulated exosome secretion. Circ Res 2015; 116: 1312-23.

25. Buendia P, Montes de Oca A, Madueño JA, et al. Endothelial microparticles mediate inflammation induced vascular calcification. FASEB J 2015; 29: 173-81.

26. New SEP, Aikawa E. Role of extracellular vesicles in de novo mineralization, an additional novel mechanism of cardiovascular calcification. Arterioscler Thromb Vasc Biol 2013; 33: 1753-58.

27. Mallat Z, Hugel B, Ohan J, et al. Shed membrane microparticles with procoagulant potential in human atherosclerotic plaque: a role for apoptosis in plaque thrombogenicity. Circulation 1999; 99: 348-53.

28. Falati S, Liu Q, Gross P, et al. Accumulation of tissue factor into developing thrombi in vivo is dependent upon microparticle P selectin glycoprotein ligand 1 and platelet P selectin. J Exp Med 2003; 197:1585-98.

29. Suades R, Padro T, Vilahur G, Badimon L. Circulating and plateled derived microparticles in human blood enhance thrombosis on atherosclerotic plaques. Thromb Haemost 2012; 108: 1208-19.

30. Srikanthan S, Li W, Silverstein RI, McIntyre TM. Exosome poly-ubiquitin inhibits platelet activation, downregulates CD 36 and inhibits pro-atherothrombotic cellular functions. J Thromb Haemost 2014: 12:1906-17.

31. Leroyer AS, Rautou PE, Silvestre JS, et al. CD 40 ligand+ microparticles from human atherosclerotic plaque stimulate endothelial proliferation and angiogenesis: a potential mechanism for intraplaque neovascularization. J Am Coll Cardiol 2008; 52: 1302-11.

32. Lacroix R, Sabatier F, Mialhe A, et al. Activation of plasminogen into plasmin at the surface of endothelial microparticles: a mechanism that modulates angiogenic properties of endothelial progenitor cells in vitro. Blood 2007; 110: 2432-439.

33. Marcos-Ramior B, Garcia Weber D, Millan J. TNF induced endothelial barrier disruption: beyond actin and Rho. Thromb Haemost 2014; 112:1088-102.

34. Falk E, Nakano M, Bentzon JF, et al. Update on acute coronary syndromes, the pathologist's view. Eur Heart J 2013; 34: 719-28.

35. Janiszewski M, Do Carmo AO, Pedro MA, et al. Platelet derived exososmes of septic individuals posess propatoptotic NAD(P)H oxidase activity: a novel vascular redox pathway. Crit Care Med 2004; 32: 818-25.

36. Bernal-Mizrachi L, Jy W, Jimenez JJ, et al. High levels of circulating endothelial microparticles in patients with acute coronary syndromes. Am Heart J 2003; 145: 962-70.

37. Abbas M, Jesel L, Auger C, et al. Endothelial microparticles from acute coronary syndrome patients induce premature coronary artery endothelial cells ageing and thrombogenicity: role of the Ang II/AT1 receptor/NADPH oxidase-mediated activation of MAPKs and P13 kinase pathways. Circulation 2016; 135:280-96.

38. Sarlon-Bartoli G, Bennis I, Lacroix R, et al. Plasmatic level of leukocyte derived microparticles is associated with unstable plaque in asymptomatic patients with high grade carotid stenosis. J Am Coll Cardiol 2013; 62: 1436-441.

39. Chiva-Banch G, Suades R, Crespo J, et al. CD3+/CD45+ and SMA alpha+ circulating microparticles are increased in individuals at high cardiovascular risk who will develop a major cardiovascular event. Int J Cardiol 2016: 208: 147-49.

40. Bi S, Wang C, Jin Y, et al. Correlation between serum exosome derived miR-208a and acute coronary syndrome. Int J. Clin. Exp. Med 2015; 8: 4275-80

41. Matusmoto S, Sakata Y, Suna S, et al. Circulating p-53 responsive microRNAs are predictive indicators for heart failure after myocardial infarction. Circ Res 2013; 113: 322-26.

42. Ortega-Otero L, Laso-Garcia F, Gomez-de Frutos MC, et al. Role of exosomes as a treatment and potential biomarker for stroke. Transl Stroke Res 2018 Aug 13. doi: 10.1007/s12975-018-0654-7

43. Fruhbeis C, Frohlich D, KuoWP, Kramer-Albers EM. Extracellular vesicles as mediators of neuron-glia communication. Front Cell Neurosci. 2013; 7: 182.

44. Zhuang X, Xiang X, Grizzle W, et al. Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. Mol Ther. 2011;19:1769–79.

45. Kanninen KM, Bister N, Koistinaho J, Malm T. Exosomes as new diagnostic tools in CNS diseases. Biochim Biophys Acta. 2016 1862:403–10.

46. Kanhai DA, de Kleijn DPV, Kappelle LJ, et al. Extracellular vesicle protein levels are related to brain atrophy and cerebral white matter lesions in patients with manifest vascular disease: the SMART-MR study. BMJ Open. 2014;4:3824.

47. Ji Q, Ji Y, Peng J, et al. Increased brain-specific MiR-9 and MiR- 124 in the serum exosomes of acute ischemic stroke patients. PLoS One. 2016;11:163645.

48. Chen Y, Song Y, Huang J, et al. Increased circulating exosomal miRNA-223 is associated with acute ischemic stroke. Front Neurol. 2017;27:57; doi: 10.3389/fneur.2017.00057.

49. Dolz S, Górriz D, Tembl JI, et al. Circulating microRNAs as novel biomarkers of stenosis progression in asymptomatic carotid stenosis. Stroke. 2017;48:10–6.

50. Doeppner TR, Herz J, Gorgens A, et al. Extracellular vesicles improve post-stroke neuroregeneration and prevent postischemic immunosuppression. Stem Cells Transl Med. 2015;4:1131–43.

51. Xin H, Li Y, Cui Y, Yang JJ, Zhang ZG, Chopp M. Systemic administration of exosomes released from mesenchymal stromal cells promote functional recovery and neurovascular plasticity after stroke in rats. J Cereb Blood Flow Metab. 2013; 33:1711–5.

52. Otero-Ortega L, Gómez de Frutos MC, Laso-García F. et al. Exosomes promote restoration after an experimental animal model of intracerebral haemorrhage. J Cereb Blood Flow Metab 2018; 38: 767-79.

53. Otero-Ortega L, Laso-Garcia F, Gomez-de Frutos MC, et al. White matter repair after extracellular vesicles administration in an experimental animal model of subcortical stroke. Sci Rep. 2017;7:44433.

54. Chen KH, Chen CH,Wallace CG, Yuen CM, Kao GS, Chen YL, et al. Intravenous administration of xenogenic adipose-derived mesenchymal stem cells (ADMSC) and ADMSC-derived exosomes markedly reduced brain infarct volume and preserved neurological function in rat after acute ischemic stroke. Oncotarget 2016;7: 74537–56.

55. Roman GC. Vascular dementia may be the most common form of dementia in the elderly. J Neurol Sci 2002; 203-204: 7–10.

56. Vogels RL, Scheltens P, Schroeder-Tanka JM, Weinstein HC. Cognitive impairment in heart failure: a systematic review of the literature. Eur J Heart Fail 2007; 9:440–9.

57. Beltramo E, Lopatina T, Berrone E, et al. Extracellular vesicles derived from mesenchymal stem cells induce features of diabetic retinopathy in vitro. Acta Diabetol 2014; 51:1055. doi:10.1007/ s00592-014-0672-1 106

58. Andras IE, Toborek M. Extracellular vesicles of the blood-brain barrier. Tissue Barriers 2016; 4:e1131804. doi:10.1080/21688370.2015. 1131804

59. Aneja A, Tang WH, Bansilal S, et al. Diabetic cardiomyopathy: insights into pathogenesis, diagnostic challenges, and therapeutic options. Am J Med. 2008;121:748–757.

60. Sluijter JP, Verhage V, Deddens JC, et al. Microvesicles and exosomes for intracardiac communication. Cardiovasc Res 2014; 102: 302–11.
61. De Gonzalo-Calvo D, van der Meer RW, Rijzewijk LJ, et al. Serum microRNA-1 and microRNA-133a levels reflect myocardial steatosis in uncomplicated type 2 diabetes. Sci Rep 2017; 7:47. doi:10.1038/s41598-017-00070-6.
62. Wang X, Huang W, Liu G, et al. Cardiomyocytes mediate anti-angiogenesis in type 2 diabetic rats through the exosomal transfer of miR-320 into endothelial cells. J Mol Cell Cardiol 2014; 74:139–50.
63. Caporali A, Meloni M, Vollenkle C, et al. Deregulation of microRNA-503 contributes to diabetes mellitus-induced impairment of endothelial function and reparative angiogenesis after limb ischemia. Circulation 2011; 123:282–91.
64. Jansen F, Yang X, Hoelscher M, Cattelan A, et al. Endothelial microparticle-mediated transfer of microRNA-126 promotes vascular endothelial cell repair via SPRED1 and is abrogated in glucose- damaged endothelial microparticles. Circulation 2013;128: 2026–38.
65. Wu K, Yang Y, Zhong Y, et al. The effects of microvesicles on endothelial progenitor cells are compromised in type 2 diabetic patients via downregulation of the miR-126/VEGFR2 pathway. Am J Physiol 2016; 310:E828–37.
66. Bang C, Batkai S, Dangwal S, etal. Cardiac fibroblast-derived microRNA passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy. J Clin Invest 2014; 124:2136–46.
67. Pironti G, Strachan RT, Abraham D, et al. Circulating exosomes induced by cardiac pressure overload contain functional angiotensin II type 1 receptors. Circulation 2015; 131:2120–30.
68. Brill A, Dashevsky O, Rivo J, Gozal Y, Varon D. Platelet derived microparticles induce angiogenesis and stimulate post-ischemic revascularization. Cardiovasc Res 2005; 67: 30-38.
69. Vicencio JM, Yellon DM, Sivaraman V, et al. Plasma exosomes protect the myocardium from ischemia-reperfusion injury. J Am Coll Cardiol 2015; 65: 1525-36.
70. Ratajczak M, Ratajczak J. Horizontal transfer of RNA and proteins between cells by extracellular vesicles: 14 years later. Clin Transl Med 2016; 5:7.
71. Zernecke A, Bidzhekov K, Noels H, et al. Delivery of microRNA-126 by apoptotic bodies induces CXCL 12-dependent vascular protection. Sci Signal 2009; 2: ra81.
72. Goto S, Tamura N, Li M et al. Different effects of various antiGP IIb-IIIa agents on shear induced platelet activation and expression of procoagulant activity. J Thromb. Haemost 2003; 1: 2022-30.
73. Shouzu A, Nomura S, Seitaro O, et al. Effect of ticlopidine on monocyte derived microparticles and activated platelet markers in diabetes mellitus. Clin Appl Thromb, Haemost 2004; 10: 167-73.
74. Kagawa K, Nomura S, Nagahama M, et al. Effect of ticlopidine on platelet derived microparticles in patients with connective tissue disease. Haemostasis 1999; 29: 255-61.
75. Nomura S, Shouzu A, Omoto S, et al. Benidipine improves oxidized LDL-dependent monocyte and endothelial dysfunction in hypertensive patients with type 2 diabetes mellitus. J Hum Hypertens 2005; 19: 551-57.
76. Huang B, Cheng Y, Xie Q, et al. Effects of 40 mg vs 10 mg of Atorvastatin on oxidized low density lipoprotein, high sensitivity C reactive protein, circulating endothelial derived microparticles and endothelial progenitor cells in patients with ischemic cardiomyopathy. Clin Cardiol 2012; 35: 125-30
77. Zu L, Ren C, Bing P, et al. Endothelial microparticles after antihypertensive and lipid lowering therapy inhibit the adhesion of monocytes to endothelial cells. Int J Cardiol 2015 202: 756-59.