Role of microparticles in endothelial dysfunction and arterial hypertension

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INTRODUCTION

What are microparticles?

During cell activation, multiple eukaryotic cells, such as endothelial cells or leukocytes, but also prokaryotes, have the ability to shed little cell blebs, so called microparticles [1,2]. Microparticles consist of the cell membrane as well as of the cytoplasm of their maternal cells and can be classified by flow cytometry into for example endothelial microparticles (EMPs), leukocyte microparticles and...
platelet microparticles (PMPs). When microparticles were first described by Wolf over 40 years ago, it was suggested that they are only a kind of cellular debris. However, within the last couple of years microparticles have gained increasing interest in different medical fields and recent effort has been undertaken to investigate the biology of microparticles, as well as the impact of microparticles on different diseases. It thereby has become evident that microparticles can be used as circulating surrogate markers for several pathophysiological conditions, such as inflammation, coagulation but also metastatic diseases and additionally are important circulating biological vectors.

**Microparticles as biological vectors in circulation**

The biology of microparticles is still incompletely understood, but it is evident that microparticles have far more functions than only activating inflammatory cells and the coagulation cascade. It has recently been shown that they bind to and fuse with distinct target cells, a process that is at least partly mediated by specific interactions of microparticles with surface receptors (such as Mac-1) of the target cell. By fusion with their target cells, microparticles deliver cytoplasm as well as membrane anchored surface receptors to their destination cells. This process is frequently associated with changes of the target cells phenotype and function. Hence, microparticles are an own kind of biological vectors modulating the function of their target cells remote from the location where they initially had been released.

Elevated microparticle levels can often be found in pathological conditions which are associated with cell mediated inflammation and coagulation. To assess the inflammatory effect of platelet microparticles, which is the largest microparticle fraction in the blood, Jy et al. investigated the effect of PMPs on neutrophils. They found that microparticles released from platelets attach to neutrophils and activate those. Hence, platelet microparticles may in fact be an additional link between vascular coagulation and inflammation in cardiovascular disease.

Hypothesizing that microparticles might not only influence the phenotype but also the transcriptome of their target cells, Hunter et al. assessed whether microparticles from mononuclear cells contain microRNAs, which are small non-coding RNA molecules that regulate mRNA translation and thereby affect post transcriptional gene expression. In this ground breaking study, they found that microparticles indeed contain a broad spectrum of different microRNAs, which they might deliver to their target cells and presumably affect the target cells protein synthesis. Interestingly, when compared to microRNA patterns of their cells of origin, microparticles do not contain a random set of microRNAs of their parental cells, but are loaded with a distinct, specific selection of microRNAs. These findings suggest that microparticle release is a highly regulated process in which cell vesicles are “loaded” from their cells of origin with specific RNA molecules which might eventually be transferred to their target cells. However, to date the underlying molecular mechanisms of this loading process are not understood.

To what extent circulating microparticles are involved in intercellular signalling was demonstrated by a pivotal study of Janowska-Wieczorek et al. They found that PMPs transfer the platelet surface receptor glycoprotein (GP) Ib/IIIa to the surfaces of different lung cancer cell lines. As the GP Ib/IIIa integrin has a high affinity to (sub)endothelial antigens, tumour cells that were pre-incubated with platelet microparticles also showed increased metastasization. Hence, PMPs might be directly involved in the progression of tumour diseases.

In summary, microparticles are small cell blebs that represent a novel way of intercellular communication, which seems to be particularly relevant for inflammatory and pro-coagulatory diseases. Due to the effects on their target cells, microparticles are able to change the phenotype, the function and presumably also the transcriptome of their target cells and might be involved in the pathogenesis of several cardiovascular diseases.

**ARTERIAL HYPERTENSION**

Arterial hypertension is a strong risk factor for atherosclerosis and vascular mortality and often starts with endothelial dysfunction. Early diagnosis of impaired endothelial function is crucial to allow medical anti-inflammatory, endothelium-protective treatment at an early disease stage. Reflecting endothelial dysfunction, endothelial microparticles might be a valuable tool to assess endothelial dysfunction, particularly in asymptomatic patients.

**Microparticles in endothelial dysfunction and arterial hypertension**

Arterial hypertension is a multifactor disease that is strongly promoted by endothelial dysfunction. Recent data indicate that altered, activated endothelial cells release endothelial microparticles into circulation. EMPs can be used as cellular surrogate markers for endothelial dysfunction and are increased in several diseases with an altered endothelial function, such as atherosclerosis, aortic valve stenosis and pulmonary hypertension. It recently was published that endothelial microparticles are even associated with several cardiovascular risk factors in the Framingham Heart Study.

However, besides their role as surrogate markers, microparticles are furthermore involved in the progression of impaired endothelial function as well as in angiogenesis. For example Burger et al. assessed the effect of microparticles on endothelial inflammation and found that microparticles themselves induce endothelial expression of vascular cell adhesion molecule 1, platelet endothelial cell adhesion molecule and adhesion of 74A.1 cells, which is a cell line with macrophage characteristics. Along the same line of evidence, Boulanger et al. investigated the mechanisms how microparticles induce endothelial dysfunction and found that MPs from patients with myocardial infarction, but not from healthy
controls, induced endothelial dysfunction by impairing the endothelial nitric oxide transduction pathway. These data were confirmed by Martin et al. who discovered that T cell microparticles reduced endothelial nitric oxide- and prostacyclin mediated vasodilatation and decreased expression levels of endothelial nitric oxide synthase.

One of the few studies investigating the interconnection between microparticles and arterial hypertension was performed by Preston et al. They assessed the abundance of endothelial microparticles in patients with untreated severe hypertension vs those with mild hypertension compared to normotensive individuals. It was found that microparticles released from endothelial cells and platelets were significantly increased in patients with severe arterial hypertension and that endothelial microparticles correlated strongly with the level of both systolic and diastolic blood pressures. Thus, it can be suggested that EMPs and PMPs can be used as circulating markers for endothelial injury in arterial hypertension. The findings described by Preston et al. are supported by studies, in which increased levels of circulating endothelial microparticles had been found in patients with pre-eclampsia, a disease that is characterized by vascular inflammation, altered endothelial function and arterial hypertension.

The Renin Angiotensin System (RAS) plays a key role in arterial hypertension and is the target for anti-hypertensive medical treatment. It has been supposed that angiotensin II, which is the final effector of the RAS, not only affects the blood pressure but furthermore induces a pro-thrombotic state. Hypothesizing that the RAS might be involved in the generation of pro-thrombotic microparticles, Cordazzo et al. investigated the effect of angiotensin II on the release of microparticles from mononuclear cells. They found that angiotensin II indeed induces shedding of pro-thrombotic MP from mononuclear cells. The data of Cordazzo support the suggestion that microparticles might in fact be the link between the activation of the renin angiotensin system and a pro-thrombotic state, which can be found in patients suffering from arterial hypertension.

End-organ damage, such as hypertensive nephropathy with impaired kidney function, is a common complication of patients with arterial hypertension. To assess whether endothelial microparticles might be involved in impaired renal function under arterial hypertension, Hsu et al. measured endothelial microparticles, endothelial progenitor cells (EPCs) and the glomerular filtration rate in patients suffering from arterial hypertension. They found that elevated EMPs to EPCs ratios are associated with a decline of the glomerular filtration rate in hypertensive patients. These data underline the impact of endothelial damage assessed by the EMP to EPC ratio on the progression of impaired kidney functions in arterial hypertensive patients.

In conclusion, particularly endothelial microparticles can be found in several conditions that are associated with arterial hypertension. EMPs are not only valuable surrogate markers reflecting the extent of endothelial cell dysfunction but additionally might promote the progression of arterial hypertension and its complications.

**WHAT BRINGS THE FUTURE?**

Microparticles are promising surrogate markers for a variety of pathological conditions, particularly in conditions that are associated with impaired endothelial function and arterial hypertension (Table 1). However, a lack of standardization of microparticle definitions and methods used to quantify microparticles makes it difficult to compare results from different research groups. As microparticles have a highly complex molecular architecture, they are more fragile than for example blood proteins, which are often used as clinical surrogate parameters. Hence, the way how blood samples for microparticle measurements are taken, such as the diameter and the length of the needle that was used, is critical and can significantly influences flow cytometric analysis of microparticles. Finally, even technical characteristics of the flow cytometry used to analysis microparticles can influence measurement results. Therefore, the International Society on Thrombosis and Haemostasis (www.isth.org) and the International Society for Extracellular Vesicles (http://www.isev.org) are working on recommendations for standardized protocols for microparticle measurements. Standardized studies will need to assess the diagnostic value of microparticles as surrogate markers in arterial hypertension.

As microparticles reflect a variety of different pathological changes in the vascular system (e.g., inflammation, coagulation, activation of different cell types, etc.) they might represent a broader spectrum of cellular changes in circulation than measuring only one distinct soluble marker protein. Furthermore, besides their role as vascular surrogate markers, microparticle measurement can presumably be used to monitor the success of medical treatments of diseases that are associated with vascular inflammation. However, large clinical multicentre studies are necessary to assess whether microparticles of different cellular origin can be used as surrogate markers and as tools for drug monitoring in different cardiovascular diseases.

Until now, only very few studies have investigated the effect of different drugs on circulating microparticles. Nomura et al. found that eicosapentaenoic acid, which is an omega-3 fatty acid, reduces endothelial derived microparticles in patients suffering from type 2 diabetes. Tramontano et al. described that fluvastatin has a protective effect on endothelial cells and inhibits EMP release and Morel et al. reports that vitamin C reduces endothelial and platelet derived microparticles in patients with myocardial infarction. Even if these data are promising, their results need to be confirmed by randomized multicentre studies and it needs to be assessed whether a reduction of microparticle levels is associated with a beneficial patient outcome.

In conclusion, microparticles are small cell vesicles re-
leashed by a huge variety of cells reflecting the state of activation of their parental cells. Besides functioning as surrogate markers for example for endothelial dysfunction, recent evidence indicates that they additionally influence the progression of several cardiovascular diseases. Hence, circulating microparticles might not only be valuable surrogate markers for different pathological conditions but furthermore be novel therapeutic targets by which the progression of microparticle mediated diseases might be influenced.

### REFERENCES

1. **Yuana Y**, Sturk A, Nieuwland R. Extracellular vesicles in physiological and pathological conditions. *Blood Rev* 2013; 27: 31-39 [PMID: 23261067 DOI: 10.1016/j.bre.2012.12.002]

2. **Hugel B**, Martinez MC, Kunzelmann C, Freyssinet JM. Membrane microparticles: two sides of the coin. *Physiology* (Bethesda) 2005; 20: 22-27 [PMID: 15653836 DOI: 10.1152/phsyiol.00029.2004]

3. **Wolf P**. The nature and significance of platelet products in human plasma. *Br J Haematol* 1967; 13: 269-288 [PMID: 6025241 DOI: 10.1111/j.1365-2141.1967.tb08741.x]

4. **Bernal-Mizrachi L**, Jy W, Jimenez JJ, Pastor J, Mauro LM, Horstman LL, de Marchena E, Ahn YS. High levels of circulating endothelial microparticles in patients with acute coronary syndromes. *Am Heart J* 2003; 145: 962-970 [PMID: 12796750 DOI: 10.1016/S0002-7073(03)00103-0]

5. **Fink K**, Feldbrügge L, Schwarz M, Bourgeois N, Helbing T, Fink K. Circulating annexin V positive microparticles in patients after successful cardiopulmonary resuscitation. *Crit Care* 2011; 15: R251 [PMID: 22027379 DOI: 10.1186/cc10152]

6. **György B**, Szabó TG, Turiáki L, Wright M, Herczeg P, Ledecci Z, Kitel A, Polgár A, Toth K, Défai L, Zelenák G, Böröcz I, Carr B, Nagy G, Vokey K, Gay S, Falsus A, Buzás EI. Improved flow cytometric assessment reveals distinct microvesicle (cell-derived microparticle) signatures in joint diseases. *PLoS One* 2012; 7: e49726 [PMID: 23185418 DOI: 10.1371/journal.pone.0049726]

7. **Horstman LL**, Minagar A, Jimenez JJ, Sheremata WA, Mauro LM. Microparticles from mononuclear cells: EMPs increase endothelial expression of VCAM-1, PCAM and adhesion of J774A.1 cells. *Cardiovasc Res* 2003; 20: 22-27 [PMID: 15653836 DOI: 10.1152/physiol.00029.2004]

8. **Pluskota E**, Woody NM, Szpak D, Ballantyne CM, Soloviev DA, Simon DI, Plow EF. Expression, activation, and function of integrin alphaMbeta2 (Mac-1) on neutrophil-derived microparticles. *Blood* 2008; 112: 2327-2335 [PMID: 18500985 DOI: 10.1182/blood-2007-12-127183]

9. **Jonas E**, Minagar A, Jimenez JJ, Sheremata WA, Mauro LM, Horstman LL, Bidot C, Ahn YS. Endothelial microparticles (EMP) bind and activate monocytes: elevated EMP-monocyte conjugates in multiple sclerosis. *Front Biosci* 2004; 9: 3137-3144 [PMID: 15353343 DOI: 10.2741/1146]

10. **Hunter MP**, Ismail N, Zhang X, Aguda BD, Lee EJ, Yu L, Xiao T, Schafer J, Lee ML, Schmittgen TD, Nana-Sinkam SP, Jarjoura D, Marsh CB. Detection of microRNA expression in human peripheral blood microvesicles. *PLoS One* 2012; 7: e3694 [PMID: 19002258 DOI: 10.1371/journal.pone.003694]

11. **Diede P**, Fricke A, Sander L, Stamm J, Bassler N, Htun N, Niemann M, Helbing T, El-Osta A, Jowett JB, Peter K. Microparticles: major transport vehicles for distinct microRNAAs in circulation. *Cardiovasc Res* 2012; 93: 633-644 [PMID: 22258631 DOI: 10.1093/cvr/cvs007]

12. **Janowoska-Wieczorek A**, Wysockynski M, Kijowski J, Marquez-Curtis L, Machalinski B, Rajtaczik J, Rajtaczik MZ. Microvesicles derived from activated platelets induce metastasis and angiogenesis in lung cancer. *Int J Cancer* 2005; 113: 752-760 [PMID: 15496615 DOI: 10.1002/ijc.20657]

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**Table 1**: Overview about studies investigating the interrelation between microparticles and endothelial dysfunction/arterial hypertension

| Study subjects | Flow cytometric MP characteristics | Findings | Ref. |
|----------------|------------------------------------|----------|-----|
| Framingham offspring cohort | CD144<sup>+</sup> CD31<sup>-</sup>/CD41<sup>-</sup> | Increased CD144<sup>+</sup> MP correlate with Arterial hypertension Elevated triglycerides Metabolic syndrome Increased CD31<sup>-</sup>/CD41<sup>-</sup> correlate with elevated triglycerides | Amabile et al<sup>[2]</sup> |
| MPs of AMI patients | Isolated blood MPs | MPs from AMI patients impair the endothelial nitric oxide pathway | Boulanger et al<sup>[3]</sup> |
| Ang II stimulated mouse aortic endothelial cells | Annexin V<sup>-</sup> | Ang II induces EMP release | Burger et al<sup>[4]</sup> |
| (Microparticles of) human mononuclear cells | CD144<sup>+</sup> | EMPs increase endothelial expression of VCAM-1, PCAM and adhesion of J774A.1 cells | Cordazzo et al<sup>[5]</sup> |
| MPs of human lymphoid CEM T cell line | Isolated cell culture MPs | Angiostatin receptor type 2 inhibitors reduce Ang II induced MP release | Martin et al<sup>[6]</sup> |
| EMPs of women with pre-eclampsia | CD62E<sup>+</sup> | Women with preeclampsia have higher EMP levels than those with gestational hypertension and controls | Gonzaález-Quintero et al<sup>[7]</sup> |
| MPs levels of patients with arterial Hypertension | CD31<sup>+</sup>/CD42<sup>-</sup> | Increased EMPs and PMPs in patients with severe arterial hypertension | Preston et al<sup>[8]</sup> |
Virdis A, Chiadoan L, Taddei S. Effects of antihypertensive treatment on endothelial function. *Curr Hypertens Rep* 2011; 13: 276-281 [PMID: 21499710 DOI: 10.1007/s11906-011-0207-x]

Lüscher TF, Vanhouthe PM, Raji L. Antihypertensive treatment normalizes decreased endothelium-dependent relaxations in rats with salt-induced hypertension. *Hypertension* 1987; 9: III193-III197 [PMID: 3596786 DOI: 10.1161/01.HYP.9.6.Pt.2.III193]

Parissis JT, Korovesis S, Giazitzoglou E, Kalivas P, Katritsis D. Plasma profiles of peripheral monoocyte-related inflammatory markers in patients with arterial hypertension. Correlations with plasma endothelin-1. *Int J Cardiol* 2002; 83: 13-21 [PMID: 11959378 DOI: 10.1016/S0167-5273(02)00021-9]

Schiffrin EL, Park JB, Intengan HD, Touyz RM. Correction of arterial structure and endothelial dysfunction in human essential hypertension by the angiotensin receptor antagonist losartan. *Circulation* 2000; 101: 1653-1659 [PMID: 10738046 DOI: 10.1161/01.CIR.101.14.1653]

Diehl P, Nagy F, Sossong V, Helbing T, Beyersdorf F, Olschewski M, Bode C, Moser M. Increased levels of circulating microparticles in patients with severe aortic valve stenosis. *Thromb Haemost* 2008; 99: 711-719 [PMID: 18392329 DOI: 10.1160/TH07-05-0334]

Shantsila E, Kamphuisen PW, Lip GY. Circulating microparticles in cardiovascular disease: implications for atherosclerosis and atherothrombosis. *J Thromb Haemost* 2010; 8: 2358-2368 [PMID: 20695980 DOI: 10.1111/j.1538-7836.2010.0007x]

Bakouboula B, Morel O, Faure A, Zobairi F, Jesel L, Trinh A, Zupan M, Canuet M, Grunebaum L, Brunette A, Desprez D, Chabot F, Weitenblum E, Freysinet JM, Chauot A, Toti F. Procoagulant membrane microparticles correlate with the severity of pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2008; 177: 536-543 [PMID: 18006886 DOI: 10.1164/rccm.200706-840OC]

Preston RA, Jy W, Jimenez JJ, Mauro LM, Horstman LL, Vallez M, Ame G, Ahn YS. Effects of severe hypertension on endothelial and platelet microparticles. *Hypertension* 2003; 41: 211-217 [PMID: 12574084 DOI: 10.1161/01.HYP.0000049760.15764.D2]

Amabile N, Cheng S, Renard JM, Larson MG, Ghorbani A, McCabe E, Griffin G, Guerin C, Ho JE, Shaw Colen KS, Vasan RS, Tedgui A, Boulanger CM, Wang TJ. Association of circulating endothelial microparticles with cardiometabolic risk factors in the Framingham Heart Study. *Eur Heart J* 2014; 35: 2972-2979 [PMID: 24742886 DOI: 10.1093/eurheartj/ehu153]

Brodsky SV, Zhang F, Naslletti A, Goligorsky MS. Endothelium-derived microparticles impair endothelial function in vitro. *Am J Physiol Heart Circ Physiol* 2004; 286: H1910-H1915 [PMID: 15072974 DOI: 10.1152/ajpheart.0172.2003]

Tual-Chalot S, Gagnadoux F, Trzezipur W, Priou P, Andrianisitohaina R, Martinez MC. Circulating microparticles from obstructive sleep apnea syndrome patients induce endothelium-mediated angiogenesis. *Biochim Biophys Acta* 2014; 1842: 202-207 [PMID: 24275556 DOI: 10.1016/j.bb claimed.2013.11.017]

Burger D, Montezano AC, Nishigaki N, He Y, Carter A, Touyz RM. Endothelial microparticle formation by angiotensin II is mediated via Ang II receptor type 1/NADPH oxidase/Rho kinase pathways targeted to lipid rafts. *Arterioscler Thromb Vasc Biol* 2011; 31: 1898-1907 [PMID: 21597004 DOI: 10.1161/ATVBAHA.110.2222703]

Boulanger CM, Scoazec A, Ebrahimian T, Henry P, Mathieu E, Tedgui A, Mallat Z. Circulating microparticles from patients with myocardial infarction cause endothelial dysfunction. *Circulation* 2001; 104: 2649-2652 [PMID: 11723015 DOI: 10.1161/01.CIR.0000124065.31216.EE]

Martin S, Tesse A, Hugel B, Martinez MC, Morel O, Freysinet JM, Andrianisitohaina R. Shed membrane particles from T lymphocytes impair endothelial function and regulate endothelial protein expression. *Circulation* 2004; 109: 1653-1659 [PMID: 15023873 DOI: 10.1161/01.CIR.0000124065.31216.EE]

Marques FK, Campos FM, Sousa LP, Teixeira-Cardavalo A, Dusse LM, Gomes KB. Association of microparticles and pre-eclampsia. *Mol Biol Rep* 2013; 40: 4553-4559 [PMID: 23645085 DOI: 10.1007/s11033-013-2536-0]

González-Quintero VH, Smarksusky LP, Jiménez JJ, Mauro LM, Jy W, Hortsman LL, O’Sullivan MJ, Ahn YS. Elevated endothelial plasma microparticles: preeclampsia versus gestational hypertension. *Am J Obstet Gynecol* 2004; 191: 1418-1424 [PMID: 15507796 DOI: 10.1016/j.ajog.2004.06.044]

Cordazzo C, Neri T, Petreni S, Lombardi S, Balla C, Cianchetti S, Carmazzi Y, Paggiano P, Pedrinelli R, Celi A. Angiotensin II induces the generation of procoagulant microparticles by human mononuclear cells via an angiotensin type 2 receptor-mediated pathway. *Thromb Res* 2013; 131: e168-e174 [PMID: 23414567 DOI: 10.1016/j.thromres.2013.01.019]

Hsu CY, Huang PH, Chiang CH, Leu HB, Huang CC, Chen JW, Lin SJ. Increased circulating endothelial apoptotic microparticle to endothelial progenitor cell ratio is associated with subsequent decline in glomerular filtration rate in hypertensive patients. *PLoS One* 2013; 8: e68644 [PMID: 23874701 DOI: 10.1371/journal.pone.0068644]

Nomura S, Shouzu A, Osmo S, Inami N, Ueba T, Urase F, Maeda Y. Effects of eicosapentaenoic acid on endothelial cell-derived microparticles, angiopoietins and adiponectin in patients with type 2 diabetes. *J Atheroscler Thromb* 2009; 16: 83-90 [PMID: 19403992 DOI: 10.5551/jat.0091]

Tramontano AF, O’Leary J, Black AD, Muniyappa R, Cutai MV, El-Sherif N. Statin decreases endothelial microparticle release from human coronary artery endothelial cells: implication for the Rho-kinase pathway. *Biochem Biophys Res Commun* 2004; 320: 34-38 [PMID: 15207698 DOI: 10.1016/j.bbrc.2004.05.127]

Morel O, Jesel L, Hugel B, Douchet MP, Zupan M, Chauvin M, Freysinet JM, Toti F. Protective effects of vitamin C on endothelial damage and platelet activation during myocardial infarction in patients with sustained generation of circulating microparticles. *J Thromb Haemost* 2003; 1: 171-177 [PMID: 12871555 DOI: 10.1046/j.1538-7836.2003.00101.x]

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