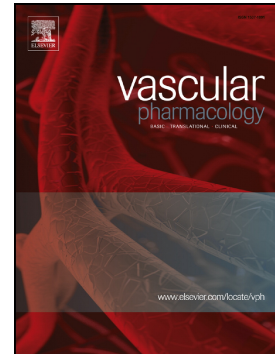# Accepted Manuscript

Vascular extracellular vesicles in comorbidities of heart failure with preserved ejection fraction in men and women: The hidden players. A mini review

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Vascular extracellular vesicles in comorbidities of heart failure with preserved ejection fraction in men and women: the hidden players. A mini review

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Abstract

Left ventricular diastolic dysfunction, the main feature of heart failure with preserved ejection fraction (HFpEF), is thought to be primarily caused by comorbidities affecting the endothelial function of the coronary microvasculature. Circulating extracellular vesicles, released by the endothelium have been postulated to reflect endothelial damage. Therefore, we reviewed the role of extracellular vesicles, in particularly endothelium microparticles, in these comorbidities, including obesity and hypertension, to identify if they may be potential markers of the endothelial dysfunction underlying left ventricular diastolic dysfunction and HFpEF.

Key words: Endothelial microparticles; HFpEF; LVD; sex; comorbidities

Endothelial microparticles

Communication in multicellular organisms is essential for appropriate signal transductions and efficient organ functioning. Although much attention has been given to paracrine and endocrine chemical signals and direct cellular interaction, the spotlight has moved onto showing that cells can communicate via small, membrane-enclosed vesicles, termed "extracellular vesicles". Eukaryotic extracellular vesicles consist of several populations of vesicles, including exosomes, microvesicles, apoptotic vesicles and oncosomes. Recently, we highlighted the differences between these vesicle populations in a position paper on the diagnosis and therapy of the ischaemic heart.(1) Now, we zoom in on one of these vesicle populations; membrane-derived microvesicles, only 100-1000nm in size and also known and widely described in literature as microparticles, in which their content, is reflective of the cell source. Microparticles, shed from endothelial cells, following activation or apoptosis, are aptly termed endothelial microparticles (EMPs). These are anuclear fragments of cellular membrane, comprising proteins, microRNAs, and enzymes specific to the cell from which they originate. The historical notion, originating from Wolf (2) over 40 years ago, that microparticles were only inert debris has been replaced with a new understanding of their possible role as a marker of underlying pathology and vascular injury. EMPs are elevated in a variety of cardiovascular-related diseases which involve impaired endothelial function such as coronary artery disease(3–5), carotid artery disease (6), type 2 diabetes (7,8) and preeclampsia.(9) EMPs have therefore subsequently adopted the role of a surrogate marker of endothelial dysfunction.(10,11) In addition, they have also been found to directly be involved with the progression of endothelial dysfunction with Boulanger et al. showing that microparticles from patients with myocardial infarction, but not from healthy

controls, induced endothelial dysfunction by impairing the endothelial nitric oxide transduction pathway.(12)

Here, the behaviour of EMPs in heart failure comorbidities in both men and women will be discussed, followed by the possible role they may play in HF, specifically the sub-type heart failure with preserved ejection fraction (HFpEF). The extracellular vesicle field is urgently looking for more uniform definitions of vesicle characteristics, including specific markers and isolation protocols. This is also true for EMPs, which we define in this overview as vesicles of 100-1000nm in size and expressing one or more endothelial specific markers, such as CD144, or as otherwise specified.

The function of the endothelium in men and women

The endothelium is made up of a single layer of cells acting as a barrier between the blood and vascular wall. It plays an important role in cardiovascular homeostasis by regulating vasomotor tone, vascular permeability, and cardiac function.(13) Impairment of the endothelium i.e endothelial dysfunction is a complex physiological event preceded by the activation of endothelial cells by cytokines under inflammatory conditions inducing a pro-inflammatory state.(14) Oxidative stress plays an important role in mediating the production and secretion of cytokines, therefore linking reactive oxygen species with inflammation and endothelial activation and dysfunction. As nitric oxide is central to the maintenance of vascular homeostasis in endothelial cells, reduction in nitric oxide bioavailability, due to reduced production or increased degradation of nitric oxide, leads to endothelial dysfunction. EMPs directly induce endothelial activation, inflammation and dysfunction and may contribute/or be involved with the increased cardiovascular risk present in a number of inflammatory

diseases which may be influenced by sex. Given that the endothelium behaves differently according to sex (15–17) one would expect the number and behaviour of circulating EMPs to also differ by sex.

The sex-specific role of EMPs in endothelial dysfunction

There are conflicting results regarding the difference in levels of circulating EMPs between healthy men and women. Circulating microparticles of endothelial origin have been shown to be higher in young, healthy women as compared to aged-matched healthy men.(18,19) Toth et al. showed that this difference in circulating EMPs was most pronounced during the luteal phase of the menstrual cycle, suggesting an important hormonal influence on circulating levels.(19) In one study no difference between circulating levels of EMPs was found between middle-aged healthy men and women, although there were sex differences in the miRNA expression of the EMPs.(20) Differential expression of miRNAs has previously been shown to be involved with endothelial dysfunction and an increased risk of cardiovascular disease (CVD).(21) Therefore the sex differences in miRNA expression may actually reflect sex differences observed in CVD pathophysiology. These results add to the knowledge regarding the influence of sex hormones on the function of the endothelium (22). Thus, age and sex hormonal changes are likely to play a sex-differentiated role in the release and behaviour of circulating EMPs. The differential expression of EMPs may also represent different roles of EMPs such as them being markers of damage and also markers of repair.

It is well known that CVD manifests later on in a woman's life than it does in a man's life.(23) Men outnumber women with a higher prevalence of CVD across all ages until the age of 85 years after which women outnumber men and continue to do

so.(24) This age-related phenomenon is considered to be related to the change in sex-hormonal status with oestrogen acting as a protective force until after the menopause sequentially increasing the risk of CVD in women. These changes in endogenous circulating concentrations of sex hormones may modulate the risk for CVD via the vascular endothelium.(23,25) Oestrogen mediates the activation of endothelial nitric oxide synthase, which produces nitric oxide for vasorelaxation, and also has an antioxidant effect explaining the sex differences in EMPs we see in younger women compared to younger men but not between older men and older women.(26) In addition to an increased risk of CVD, following the menopause, susceptibility to the metabolic syndrome increases in women.(27)

Metabolic syndrome and endothelial dysfunction

Metabolic syndrome is characterised by the presence of three out of five clinical parameters including: increased waist circumference, low high density lipid-cholesterol, raised triglyceride levels, raised fasting blood glucose levels, and raised systemic blood pressure (either systolic or diastolic). Metabolic syndrome, of which obesity clearly is a key contributor, increases the risk of CVD particularly in women.(28) All components of the metabolic syndrome have adverse effects upon the endothelium and several studies have shown that endothelial function is impaired in metabolic syndrome contributing to ischaemic heart disease and myocardial dysfunction.(29–33) Increased serum EMPs have also been observed in women with polycystic ovarian syndrome, a condition known to be associated with the metabolic syndrome and a raised body mass index.(34) Obesity is characterised by a chronic low grade systemic inflammation (35) with macrophages invading the excess adipose tissue resulting in the release of inflammatory cytokines. This subsequently triggers a

systemic inflammatory response. Endothelial dysfunction also plays a role in the pathogenesis of type 2 diabetes (T2D).(8,36) An improvement in glycaemic control is reciprocated by an improvement in endothelial function.(37)

Amabile et al. demonstrated that EMPs were associated with several cardiometabolic disease risk factors including higher triglyceride levels and the metabolic syndrome (38) in a cohort free from CVD. However, this study did not show an association between frank T2D and circulating EMPs. Results regarding specific EMP populations in T2D have been conflicting. One small study found circulating CD144+ to be present in T2D patients (39) and Koga et al. showed significantly elevated levels in T2D with coronary artery disease compared with non-diabetic control patients.(7) However, Sabatier et al. found that the total microparticle population was higher in type 1 diabetes mellitus and T2D compared to controls but microparticles of endothelial origin were only higher in type 1 diabetes mellitus patients compared to controls and not T2D.(40) Thus it may be that the risk factors associated with T2D result in the increase in EMPs seen in these studies or in the case of the study by Koga et al., active coronary artery disease and not the diabetes itself. It has been suggested that the diabetic microenvironment may also influence the composition and activity of microparticles (41) with an increase in size of EMPs (42), which may account for the differences seen. Increased EMPs have also been found in obesity with studies showing an increase in EMPs in obese women as compared to lean women of a similar age.(29,43)

Hypertension and endothelial dysfunction

Hypertension is one of the most important contributors to the development of HFpEF. Oxidative stress and vascular inflammation are increasingly being shown to be

involved in the pathogenesis of hypertension.(44–46) The association between hypertension and endothelial dysfunction is well established.(47–49) The management of hypertension, including dietary sodium restriction and antihypertensive medications, has also been shown to improve the endothelial dysfunction initially caused by hypertension.(44) Not only is endothelial dysfunction a consequence of hypertension, but it is also known to precede hypertension.(50,51) Amabile et al. found that hypertension was associated with an increase in EMPs in men and women free from CVD.(38) This relationship has also been observed in other studies with one study showing increased EMP levels in patients with severe hypertension.(52)

Hypertensive disorders of pregnancy and endothelial dysfunction

3%-8% of all pregnancies are complicated by hypertensive disorders.(53) Due to an increase in prevalence of obesity and metabolic syndrome among women of childbearing age, these numbers are only rising.(53) Evidence of an association between hypertensive disorders of pregnancy and increased cardiovascular risk later in life is accumulating. Preeclampsia occurs in 1-2% and has been shown to be associated with an increased risk of heart failure, with a predilection for HFpEF.(53,54) Preeclampsia, a multisystem hypertensive disorder of pregnancy characterised by endothelial dysfunction, a systemic inflammatory response and increased vascular resistance, occurs in 1-2% of pregnancies.(53) The results from one study identified 10 different overlapping biomarkers (including C-reactive protein and cardiac troponin I) that differentiated HFpEF and preeclampsia from their respective controls (non-HFpEF and women with normal pregnancies respectively).(55) This suggests that there are common pathophysiologies between the

two diseases involving a pro-inflammatory state, disturbances in myocardial function/structure, and unfavourable lipid metabolism.

One study looking at EMPs found that not only are levels higher in hypertensive patients compared to normotensive patients but that they are also higher in women with PE compared to women with gestational hypertension.(9) Placentae from patients with preeclampsia have reduced levels of endothelial nitric oxide synthase and thus less nitric oxide. Syncytiotrophoblast extracellular microvesicles and exosomes, carry signals from the syncytiotrophoblast to the mother.(56) One study found a significantly higher concentration of total exosomes and placenta-derived exosomes in maternal plasma of preeclamptic women compared to those without preeclampsia.(57) Circulating plasma syncytiotrophoblast extracellular microvesicles from placenta also carry functional endothelial nitric oxide synthase. Preeclamptic women have lower endothelial nitric oxide synthase expression in syncytiotrophoblast extracellular microvesicles compared to women with normal pregnancies suggesting that functional syncytiotrophoblast extracellular microvesicles derived endothelial nitric oxide synthase-mediated nitric oxide production is compromised in preeclampsia which may contribute to the vascular dysfunction seen in preeclampsia.(56)

Thus, there is accumulating evidence that placenta derived microvesicles and exosomes play an important role in maintaining cardiovascular health in pregnant women. Disturbances in placenta homeostasis seem to alter these protective microvesicles leading to increased vascular resistance not only in the placenta but also in the systemic vasculature of the mother. This increased high blood pressure seems to promote diastolic dysfunction and HFpEF during acute preeclampsia and also

increases the risk of HFpEF in the longterm in women who have previously experienced preeclampsia.

Atrial Fibrillation and endothelial dysfunction

Atrial fibrillation commonly coexists with heart failure, in particularly HFpEF, occurring in up to 1/3rd of patients with HFpEF.(58) A high body mass index is also associated with atrial fibrillation.(59) Endothelial dysfunction has previously been recognised in atrial fibrillation, with an improvement seen following restoration of sinus rhythm.(60,61) This impairment of endothelial dysfunction is worse in the presence of hypertension or T2D.(61) Although studies involving EMPs in atrial fibrillation are limited, increased levels of EMPs have been found in patients with either permanent or persistent atrial fibrillation compared to controls without any cardiovascular risk factors.(62)

Heart failure and endothelial dysfunction

The heart failure syndrome consists of three distinct phenotypes, categorised according to the ejection fraction: preserved (HFpEF, EF≥50%), mid-range (HFmrEF, EF: 40-49%) and reduced (HFrEF, EF: <40%).(63) Approximately 50% of heart failure patients suffer from HFpEF.(13) In line with our ageing society, HFpEF is expected to become the more dominant form of heart failure in the Western world (64,65), rising in prevalence at a rate of ~1% per year.(66) Interestingly, as compared to HF(m)rEF which commonly affects men, women are more prone to developing HFpEF, with women outnumbering men in a 2:1 ratio.(63,67–70) The prevalence of HFpEF is also higher in women in screening populations suggesting that women are more likely to have unrecognised HFpEF than men.(70) Left ventricular diastolic

dysfunction encompasses asymptomatic cardiac abnormalities that are related to left ventricular stiffening and to a decline in left ventricular relaxation, both whilst preserving the ejection fraction.(68,71) Left ventricular diastolic dysfunction is considered to be a precursor of HFpEF (72) but it may also feature in HF(m)REF and other cardiovascular diseases such as atrial fibrillation and stroke.(73) Unlike HFpEF, the prevalence of left ventricular diastolic dysfunction has been found to be similar in men and women.(70) HFpEF, as compared to HFrEF has a high prevalence of comorbidities including hypertension, T2D, obesity and atrial fibrillation.(74,75) As we have seen in this review, endothelial dysfunction is common to all of these comorbidities. It is these comorbidities that have taken center stage in the recently hypothesized explanation of the underlying mechanism of HFpEF. It has been proposed that they cause a systemic microvascular endothelial inflammatory response which triggers coronary endothelial and microvascular dysfunction leading to diastolic stiffness, concentric left ventricular modelling and interstitial but also myocyte fibrosis.(76) Women with HFpEF are more likely to suffer from these comorbidities and be older than men with HFpEF.(77) Therefore one may postulate that endothelium dysfunction may play a bigger role in women with HFpEF than men with HF, HFpEF/HFrEF and thus EMPs may play a role in HFpEF in women (Figure 1).

CD144+ EMPs have been previously shown to be high in patients with heart failure and were found to be predictive of cardiovascular events.(78) However data concerning EMPs and heart failure have mainly focused on HFrEF as opposed to HFpEF.(79) Berezin et al. studied the differences in patterns of circulating EMPs in HFrEF vs. HFpEF.(80) This study interestingly found that out of a number of different EMPs, only CD14+, from a monocytic origin were associated with HFpEF.

Chiang et al. found that EMPs were in fact downregulated in HFpEF suggesting it was indicative of impaired endothelial turnover.(81) This highlights the complexity of the different classes of microparticles and their origins in different disease states. The specificity of individual microparticle populations for specific disease states is unclear. It is likely that microparticles of each type are elevated in multiple pathologies. It has also been suggested that they may be shed from more than one cell origin.(80) For example, CD31+ may be shed from both endothelial cells and platelets. Therefore elevations in circulating microparticles may identify a more generalised stress/injury rather than a specific pathological state.

Other barriers to the use of EMPs as markers of disease pathology are as EMPs are identified via flow cytometry using a panel of markers, endothelial cell markers vary between studies. Some markers detect a sub-population of EMPs, for example detecting EMPs from activated endothelial cells only, which may give an inaccurately lower value when comparing to a different marker. The process of identifying and quantifying EMPs is long and complex. Individual stages involved may again differ by study such as differences in blood collection and differences in the storage of blood. This clearly leads to a culmination of variety throughout the whole process. Indeed, some have suggested a standardised set of guidelines should be employed.(82,83)

Using the methodology used previously by Amabile et al.(38), we found that EMPs were higher in patients with HFpEF or left ventricular diastolic dysfunction compared to individuals without HF and left ventricular diastolic dysfunction, although absolute numbers measured were low. However, this was not different between men and women. We did not find any associations between EMPs and echocardiography parameters reflecting left ventricular diastolic dysfunction in multivariable analyses.

We did show that EMPs were reflective of a high body mass index (beta estimate 1.10 [95% CI 1.02-1.20]) and of atrial fibrillation (beta estimate 2.23 [95% CI 1.43-3.48]) (Figure 2). Our findings did not differ according to sex.

To conclude, EMPs do play a role in the various comorbidities including the cardiometabolic comorbidities associated with HFpEF, but do not seem to carry predictive value above and beyond these co-morbidities in HFpEF. Results from studies have also pointed to a sex-specific role of the endothelium and thus the behaviour of EMPs. However our understanding of EMPs in HFpEF is not yet fully clear and more standardised methods must be operational before these microparticles can be considered a marker of disease pathology of endothelial damage, left ventricular diastolic dysfunction and HFpEF.

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Figure Titles and Legends

Figure 1. Schematic showing the impact of HFpEF associated comorbidities on the endothelium and the release of circulating endothelial cells influenced by sex

Figure 2. Box plot showing the relationship between EMP ratio and atrial fibrillation

Plot displaying log transformed EMP ratios (CD144/CD9) with standard error bars in patients with atrial fibrillation (AF) compared to patients without AF

within the outlined gated area. Any particle left from the gated area is
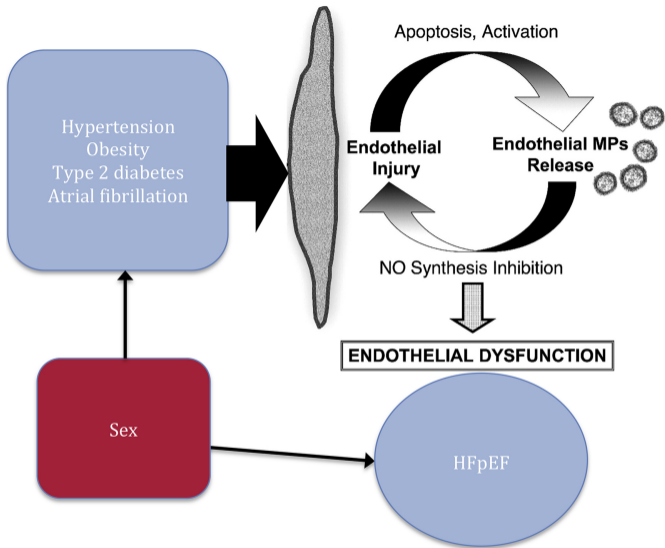
negative for CD144.

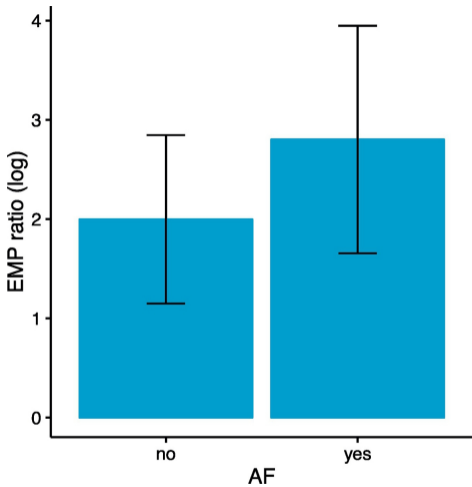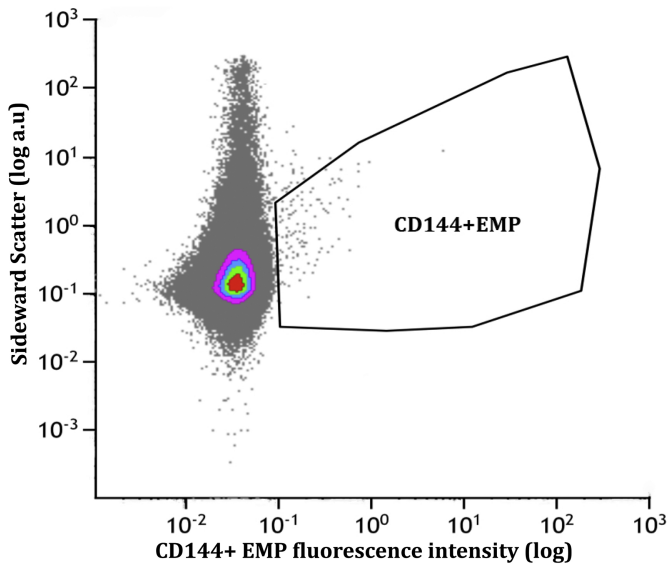Graphical Abstract
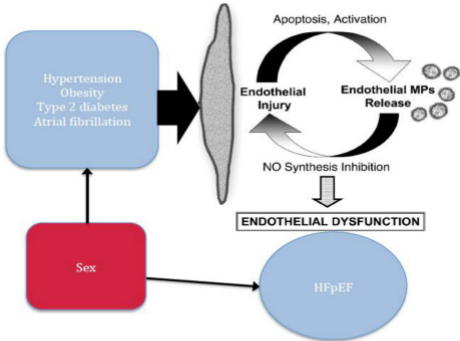
Graphics Abstract

Figure 1

Figure 2

Figure 3