Microparticles and vascular dysfunction in obstructive sleep apnoea

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ABSTRACT Obstructive sleep apnoea (OSA) is independently associated with various cardiovascular diseases, including myocardial infarction and stroke. OSA may promote atherosclerosis risk factors such as hypertension, diabetes and dyslipidaemia, and may have direct proatherogenic effects on the vascular wall. A growing number of studies have recently focused on the role of microparticles (MPs) in the atherogenic process. MPs are small plasma membrane vesicles that can be released by a variety of vascular or blood cells, and contain both membrane and cytosolic elements. Case–control studies have shown that platelet-, endothelium- and leukocyte-derived MP levels are increased in OSA. Experimental evidence has demonstrated that MPs from OSA patients induce endothelial dysfunction, inflammation and vascular hyperreactivity when injected into mice. In this review, we provide an overview of the main characteristics of MPs, their expression in OSA and their potential role in the atherogenic process associated with OSA.

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Introduction

Obstructive sleep apnoea (OSA) is a highly prevalent disease characterised by recurrent episodes of partial or complete obstruction of the upper airways during sleep, leading to repeated falls in oxygen saturation, increased negative intrathoracic pressure and frequent arousals from sleep. There is clear evidence for an independent association between OSA and cardiovascular events [1–3]. A higher rate of incident coronary heart diseases [4] and strokes [5] was demonstrated in male patients with severe OSA. Recent data show that OSA may also contribute to cardiac systolic [6] and diastolic dysfunction [7], as well as a higher rate of cardiac arrhythmias [8]. Data from clinical and experimental studies suggest that intermittent hypoxia is the main component linking OSA to atherosclerosis [3, 9, 10]. Intermittent hypoxia induces endothelial dysfunction, systemic vascular inflammation, oxidative stress and vascular smooth cell activation, and promotes various vascular risk factors such as dyslipidaemia [11, 12], insulin resistance [13] and hypertension [14].

Activated or apoptotic cells release different types of membrane vesicles, including microparticles (MPs), exosomes and apoptotic bodies. Exosomes (<100 nm in diameter) are produced by multivesicular bodies during endocytosis. Apoptotic bodies might be generated during the final steps of programmed cell death. These different vesicles are distinguished from one another on the basis of their subcellular origin, their size, their content, the mechanisms leading to their formation and, from a practical point of view, how they are isolated. This review will focus on the role of MPs in the vascular dysfunction in OSA.

MPs are defined by their size, which ranges between 0.1 and 1 μm, and their distinctive lipid layer composition, which is rich in negatively charged phospholipids, particularly phosphatidylserine. Their composition depends on both their cellular origin and the stimuli involved during their generation. MPs may contain membrane and cytosolic proteins, transcription factors, and genetic material such as ribosomal RNA, messenger RNA and microRNA, as well as lipids or organelles from their cells of origin [15, 16]. MPs can convey biological messages to target cells through a range of pathways. Ligands carried by MPs can directly interact with receptors on target cells and induce signal transduction. In addition, membranes of MPs can fuse with the plasma membrane of target cells, leading to transfer of membrane components and delivery of MP cytoplasmic content. Finally, MPs may be phagocytosed and internalised into recipient cells [17]. Any of these interactions can result in activation or inhibition of intracellular pathways in target cells or modification of their phenotype [18].

Several recent reviews have focused on the critical role of MPs as biomarkers and communication shuttles between cells [19–21].

MPs are present under normal physiological conditions and are involved in tightly controlled biological functions, including inflammation [22], haemostasis [23] and angiogenesis [21]. The protective or deleterious effects of MPs in different clinical situations are still discussed. For example, MPs are released during cardiac stress (dobutamine echocardiography) and this rise is diminished in patients with vascular disease compared with healthy subjects, suggesting that MP increase may represent an adaptive response to a specific stimulus rather than a pathological process [24]. MPs isolated from patients with septic shock have been shown to exert a protective role in vascular function [25, 26].

However, MPs are more usually considered independent functional pathological effectors in a large variety of diseases, including cardiovascular and metabolic disorders such as coronary heart disease [27, 28], hypertension [29] and diabetes [30], and severe pulmonary diseases such as acute lung injury [31] and pulmonary hypertension [32]. Furthermore, MPs that harbour markers of cellular activation have been shown to predict poor cardiovascular outcomes in at-risk patients [33, 34].

This article reviews clinical studies of MPs in OSA patients and experimental data supporting the potential contribution of MPs in OSA-associated vascular dysfunction.

Technical aspects of characterising circulating MPs

Characterising MPs is technically challenging because of their small size, heterogeneous densities, and overlap with other particulate structures and platelets. MPs carry antigenic markers characteristic of their parent cell, which is exploited for identifying their cellular origin, usually by fluorescently labelled monoclonal antibodies using flow cytometry or, more rarely, an ELISA.

MP levels may rise and decrease quickly after specific stimuli. A single high-fat meal is sufficient to elicit a pulse of MPs [35], as is application of a blood pressure cuff, exercise [36] or a cardiac stress (dobutamine echocardiography) [24]. Then, clearance from the circulation can occur during the next hour [24]. Therefore, the time of venepuncture is of particular interest and a morning fasting blood sample is usually preferred to facilitate further comparison between studies.

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A serious obstacle in the comparison of results from different clinical studies is the variety of methods used to recover MPs. Differences may occur at each step of the MP collection process: use of anticoagulants after venepuncture, storage conditions, length and number of freeze-thaw cycles, centrifugation, washing, and pelleting [37]. The use of these different methods accounts for the frequently divergent reports found in the literature. Several considerations are of special importance.

First, there are wide differences in pre-analytical variables, including the anticoagulant used for blood collection, the time before the first centrifugation, agitation during tube transportation and the centrifuge speeds used to eliminate whole cells or to sediment the MPs [37, 38]. Laboratories that use higher centrifuge speeds to eliminate whole cells may discard materials that are measured as MPs in other laboratories. Indeed, numerous data in the literature identify centrifugation as the main factor affecting MP analysis [39–41]. Regarding these large differences in pre-analytical protocols, the International Society on Thrombosis and Haemostasis has recently proposed recommendations for the standardisation of pre-analytical steps [42]. They recommend the use of citrated tubes, that processing should occur <2 h after blood collection, the use of two centrifugations at $2500 \times g$ for 15 min and storage at -80°C.

The second consideration is the large variety of antigenic markers employed for counting and identifying the MP cell origin. MPs from the same cell type can display multiple antigenic phenotypes, which can have differential antigen enrichment and may reflect different clinical states. For example, the counts of endothelium-derived microparticles (EMPs) can differ depending not only in the labelled markers used but also in how the cells were stimulated to release the EMPs [43]. Specifically, EMPs from cultured endothelial cells showed clear differences in their membrane markers depending on whether the cells were apoptotic or activated by TNF-α [43]. These *in vitro* findings were subsequently reflected in patient studies. In coronary artery disease, EMPs released during the acute phases of ischaemia differed from those in the chronic, stable phases. $CD31^+$ EMP levels were markedly elevated in patients with acute coronary syndrome but not in patients with stable angina. In contrast, $CD51^+$ EMP levels were increased in patients with both acute coronary syndrome and stable angina [44].

There is a mistaken view held by some investigators that MPs positive for annexin V (AV) reflect the total count of MPs, considering that all MPs contain phosphatidylserine. Recent data suggest that the fraction of MPs that bind AV varies widely, from a few percent for MPs from activated cells to >80% for MPs from apoptotic cells [45].

Another analytical variable comes from flow cytometry. Fluorescent antibody aggregates, present in commercial antibody solutions, may have the same scatter properties as MPs and contribute to the identification of false MPs during flow cytometry analysis [46, 47]. In a recent review, LACROIX *et al.* [48] suggested that centrifugation of antibodies before use may reduce this interference.

Clinical studies of MPs in OSA

Case–control studies investigating MPs in OSA are summarised in table 1 for pre-analytical procedures and table 2 for MP levels and phenotypes. All the venepunctures were performed in the morning after sleep recording in fasting patients. Some studies proposed additional venepunctures at 04:00 h [49] and 17:00 h [56] to access the night to morning or the evening to morning changes. Two studies used EDTA tubes

TABLE 1 Pre-analytical procedures involved in clinical studies of circulating microparticles (MPs) in obstructive sleep apnoea

First author [ref.]	Year	Anticoagulant	Preparation of PPP	Sedimentation of MPs	Storage	Wash step
GEISER [49]	2002	Citrate	No	No	Formaldehyde	No
JELIC [50]	2009	Citrate	160 × *g*, 10 min; 1000 × *g*, 6 min	No	-80°C	No
AYERS [51]	2009	Citrate	1550 × *g*, 20 min	18 000 × *g*, 30min	-80°C	Yes
PRIOU [52]	2010	EDTA	270 × *g*, 20 min; 1500 × *g*, 20 min	No	-80°C	No
YUN [53]	2010	Citrate	1500 × *g*, 15 min; 13 000 × *g*, 2 min	No	-70°C	No
KIM [54]	2011		2000 × *g*, 20 min; 20 000 × g, 90 min	No	-80°C	No
MARUYAMA [52]	2012	EDTA-ACD	8000 × *g*, 5 min	No	-20°C	No

PPP: platelet-poor plasma; ACD: acid–citrate–dextrose.

TABLE 2 Case–control trials investigating microparticle levels and phenotypes in obstructive sleep apnoea

First author [ref.]	Year	Patients and controls	LMPs	PMPs	EMPs
Geiser [49]	2002	12 patients with AHI ⩾10 and 6 healthy volunteers		↔$CD61^+$ ↔$CD42b^+$	
Jelic [50]	2009	16 patients with AHI ⩾5 and 16 matched volunteers free of CVRFs			↑$CD31^+CD42b^-$
Ayers [51]	2009	57 patients with $ODI_{4\%}$ ⩾7.5 and 15 matched volunteers with $ODI_{4\%}$ <5	↑$CD45^+$	↑$CD31^+CD41^+$	↔$CD31^+CD41^-$
Priou [52]	2010	35 patients with $ODI_{3\%}$ ⩾10 and 27 matched patients with $ODI_{3\%}$ <10 and no CVRFs	↑$CD62L^+$ ↑$CD66b^+$ ↔$CD45^+$ ↔$CD11b^+$	↔$CD41^+$ ↔$CD62P^+$	↔$CD146^+$
Yun [53]	2010	82 patients with AHI ⩾5 and 22 controls with AHI <5			↑$CD62E^+$ ↑$CD31^+CD42^-$
Kim [54]	2011	79 children with AHI ⩾1 and 56 children with AHI <1	↑$CD45^+AV^+$ ↑$CD11b^+AV^+$	↑$CD41^+AV^+$	↑$CD31^+CD42b^-AV^+$ ↑$CD62E^+CD42b^-AV^+$
Maruyama [55]	2012	11 patients with AHI ⩾30 and 19 controls with AHI <5		↑PMPs (ELISA)	

LMP: leukocyte-derived microparticle; PMP: platelet-derived microparticle; EMP: epithelium-derived microparticle; AHI: apnoea–hypopnoea index; CVRF: cardiovascular risk factor; $ODI_{n\%}$: *n*% oxygen desaturation index; ↑: increased; ↔: unchanged; AV: annexin V.

[52, 55] and four studies used citrate tubes [49, 50, 51, 53]. The speed of centrifugation ranged from 1000 × *g* [50] (MPs remain in the supernatant) to 20 000 × *g* [54] (MPs are pelleted) and the duration of the centrifugation ranged from 6 to 90 min. Some protocols included further centrifugations for washing MPs, which could have contributed to MP loss [51]. None of the published data on MPs in OSA included information on previous centrifugation of the antibody solution. All studies showed no difference in the total number of MPs but marked differences were observed in their cellular origin.

Platelet-derived MPs in OSA

The characteristic surface markers that indicate a platelet origin are specific glycoproteins of platelets and megakaryocytes, such as CD41, CD62P (P-selectin), CD42b and CD61. Platelet-derived microparticles (PMPs) represent 60% of all MPs. Studies investigating PMP levels in OSA have reported conflicting results (table 2). In 2002, Geiser *et al.* [49] showed that although the percentage of platelets with the activation-dependent epitope CD62P was increased during sleep (at 04:00 h) in OSA patients compared with controls, no significant difference was observed in $CD42b^+$ or $CD61^+$ PMP levels at either 04:00 h or 07:00 h. A recent case–control study by Priou *et al.* [52] also found no increase in $CD41^+$ or $CD62P^+$ PMP levels in OSA patients. In contrast, Ayers *et al.* [51], Maruyama *et al.* [55] and Kim *et al.* [54] found a significant increase in circulating $CD31^+CD41^+$ and $CD41^+AV^+$ PMP levels in OSA patients. Furthermore, Maruyama *et al.* [55] and Kim *et al.* [54] found a positive correlation between PMP levels and OSA severity, as measured by the apnoea–hypopnea index (AHI). The effect of OSA treatment on PMPs is also controversial. Maruyama *et al.* [55] reported a decrease in PMP levels after 1 month of continuous positive airway pressure (CPAP) treatment in an uncontrolled study. In contrast, a randomised controlled study [57] showed no change in PMP levels after short-term CPAP withdrawal.

As discussed earlier, methodological differences could have contributed to the discordant results regarding PMP levels in OSA, as the centrifugation speeds were higher (the MPs were in the pellet) in studies demonstrating an increase in PMP levels in OSA patients, compared with the studies that investigated MPs in the supernatant. Discrepancies might also be due to the clinical characteristics of included patients. Indeed, Priou *et al.* [52] included patients and controls who were free from any cardiovascular disease or

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risk factors, whereas AYERS *et al.* [51] included patients with high body mass index (BMI), of whom 40% were hypertensive and 10% had coronary heart disease. In the study by MARUYAMA *et al.* [55], the OSA and control subjects were not matched for BMI or cardiovascular risk factors. It can be hypothesised that metabolic comorbidities, also known to be associated with increased PMP levels [58], may have contributed to these conflicting results.

Little is known about the pathological relevance of changes in circulating PMP levels specifically in OSA. PMPs have been involved in the pathogenesis of inflammation and atherosclerosis [59] and to have a major role in blood coagulation [60, 61]. PMP levels have been shown to be significantly associated with the vascular dysfunction linked to OSA in children [54] and could potentially account for the increased risk of altered endothelial function. In addition to their potential implication in the atherogenic process, PMPs could play a role in the increased blood coagulability described in OSA [62]. However, the clinical use of PMPs as reliable biomarker indicators of vascular risk will have to await further studies.

Leukocyte-derived MPs

Leukocyte-derived microparticles (LMPs) are derived from all leukocyte types, including neutrophils, monocytes, and B- and T-lymphocytes [63]. There are numerous characteristic surface markers that indicate a leukocyte origin, including: CD45 for pan-leukocyte-derived LMPs; CD66b for granulocyte-derived LMPs; CD11b for monocyte-, granulocyte-, macrophage- and natural killer cell-derived LMPs; and CD62L for activated leukocyte-derived LMPs.

LMP levels were increased in OSA patients compared with the controls in all published studies (table 2). In children with moderate-to-severe OSA, both $CD11b^+$ and $CD45^+$ LMP levels were increased, and the $CD11b^+$ LMPs were positively correlated with OSA severity ($r=0.334$, $p<0.001$) [54]. High levels of $CD45^+$ LMPs were also found in minimally symptomatic adults with OSA, compared with control subjects matched for the main anthropometric data [51]. In OSA patients with marked nocturnal desaturation, PRIOU *et al.* [52] described an increased level of granulocyte-derived LMPs expressing CD66b and activated leukocyte-derived LMPs expressing CD62L. Moreover, the $CD62L^+$ MP levels were correlated with the oxygen desaturation index.

As increased LMP levels have been described in diseases such as diabetes and hypertension [64], these comorbid conditions may have contributed to the increased LMP levels in OSA. However, some investigators included OSA patients and controls who were closely matched for anthropomorphic data and cardiovascular risk factors [51] and/or free from any cardiovascular disease or risk factors [52]. TRZEPIZUR *et al.* [56] investigated the evening to morning changes in $CD62L^+$ LMP levels. Whereas a decrease in $CD62L^+$ LMP levels was observed in control subjects during the night, a significant increase was seen in patients with moderate-to-severe OSA, suggesting a nocturnal release of leukocyte-derived MPs in response to sleep disordered breathing. Furthermore, the authors found a significant correlation between the evening to morning change in $CD62L^+$ MP levels and the AHI [56]. In a randomised controlled trial investigating the effect of 2 weeks of CPAP withdrawal in OSA patients, AYERS *et al.* [57] found a significant increase in granulocyte ($CD66b^+$) LMP levels in the withdrawal group. Altogether, these findings provide strong evidence in support of a direct link between sleep disordered breathing and LMP levels.

As described more in detail later, *in vitro* incubation of endothelial cells with MPs from OSA patients was associated with a decrease in nitric oxide production that was correlated with circulating levels of $CD62L^+$ LMP [52]. In children with OSA, LMP levels correlated positively with vascular dysfunction [54]. Altogether, these findings suggest a specific role of LMPs in OSA-associated endothelial dysfunction.

Endothelium-derived MPs

The characteristic surface markers that indicate endothelial cell origin are numerous and disparate, and the majority are not specific for endothelial expression. Strategies that use a combination of multicolour antibodies have been proposed to overcome these difficulties. The various epitopes used are the presence of CD146 (S-endo), CD31 (platelet/endothelial cell adhesion molecule 1) and CD62E (E-selectin), associated with the absence of platelet characteristics (CD41 or CD42b).

Three groups have reported an increase in circulating levels of EMPs in OSA patients (table 2) [50, 53, 54]. JELIC *et al.* [50] found that $CD31^+CD42b^-$ EMP levels were four-fold higher in patients with OSA compared with their matched controls and were negatively correlated with endothelial function, as assessed by flow-mediated vasodilation. Furthermore, CPAP therapy tended to decrease $CD31^+CD42b^-$ EMP levels in patients with OSA compared with their baseline values. In the study by YUN *et al.* [53], $CD31^+CD42b^-$ and $CD62E^+$ EMP levels were significantly higher in OSA than in non-OSA patients. The AHI and the intima-media thickness were positively correlated with $CD31^+CD41^-$ EMP levels but not with $CD62E^+$ EMP levels. In 21 patients who consented to CPAP therapy, the authors observed a significant decrease in $CD62E^+$ EMP

levels but not in $CD31^+CD42b^-$ EMP levels [53]. In the randomised controlled study by AYERS *et al.* [57], 2 weeks of CPAP withdrawal were associated with an increase in $CD62E^+$ EMP levels but not in $CD31^+CD41^-$ EMP levels.

Two studies found no increase in EMP levels in OSA [52, 55]. The lack of increase in $CD146^+$ EMP levels in the study from PRIOU *et al.* [52] may be explained by the fact that CD146, also known as the melanoma cell adhesion molecule, is a constitutive marker of the endothelial cell lineage and may be less adequate when investigating vascular damage than other markers such as CD31 and CD62E, which are associated with endothelial apoptosis and activation, respectively. The lack of increase in $CD31^+CD41^-$ EMPs in the study by AYERS *et al.* [51] was observed in a selected population of minimally symptomatic OSA patients that may differ from sleepy OSA patients in terms of vascular and metabolic profile [65–67].

OSA is a complex disease, and various pathophysiological mechanisms are thought to contribute to the pathogenesis of the vascular impairment, including intermittent hypoxia, sympathetic overactivity, oxidative stress, systemic inflammation, hypertension, dyslipidaemia and insulin resistance. While hyperglycaemia and oxidative stress have been proposed to promote endothelial cell apoptosis [68], hypoxia and inflammation may induce endothelial cell activation [69]. Therefore, the impact of repeated apnoeas on endothelial cells may include both apoptosis and activation, and may be associated with the release of EMPs harbouring various corresponding epitopes. Some authors have suggested considering the ratio of $CD62E^+$ EMPs/$CD31^+$ EMPs in order to differentiate between activating and apoptotic EMP profiles [43]. The application of this ratio in two previous studies investigating both $CD31^+CD42b^-$ EMP and CD62E+ EMP levels [55, 56] suggests an apoptotic EMP profile in children and adults with OSA. As markers of endothelial cell apoptosis, $CD31^+CD42b^-$ EMP levels are strongly correlated with OSA severity, endothelial dysfunction and carotid intima-media thickness [53, 54], and may reflect the chronic vascular damage induced by long-term exposure to repeated apnoeas. In contrast, $CD62E^+$ EMP levels, reflecting endothelial cell activation, correlated neither with OSA nor with the severity of vascular damage but have been shown to be more sensitive to variation with CPAP initiation or withdrawal [53, 57]. $CD62E^+$ EMPs may reflect the acute impact of apnoeas on endothelial cell activation.

MPs as biological vectors of vascular dysfunction in OSA

Endothelial dysfunction

There is good evidence, both in animal models of intermittent hypoxia and in humans, that OSA is associated with endothelial dysfunction, a pivotal element in the development and progression of atherosclerosis [70–72]. Experimental data have demonstrated that MPs can promote endothelial dysfunction. MPs released *in vitro* by apoptotic T-lymphocytes impair endothelial function by stimulating oxygen free radical generation and decreasing Ser1179 phosphorylation of endothelial nitric oxide synthase (eNOS) [73, 74]. By collecting blood samples from patients suspected of OSA, PRIOU *et al.* [52] showed that MPs from OSA patients with marked nocturnal desaturation induced *ex vivo* endothelial dysfunction in the aorta and small mesenteric arteries when injected into mice. *In vitro*, incubation of endothelial cells with MPs from OSA patients for 24 h caused decreased nitric oxide production, independently of oxidative stress. Furthermore, the decrease in cellular nitric oxide production correlated with the $CD62L^+$ LMP circulating levels, suggesting a specific role of $CD62L^+$ LMPs in OSA-associated endothelial dysfunction [52].

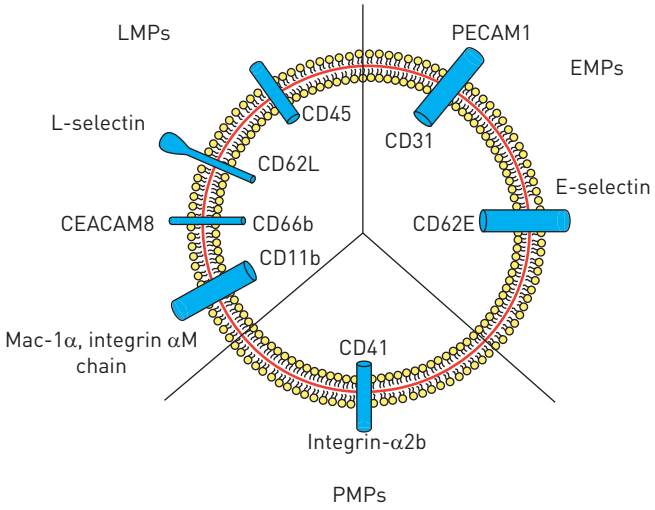

FIGURE 1 Illustration of the main epitopes harboured by microparticles from obstructive sleep apnoea patients. LMP: leukocyte-derived microparticle; EMP: endothelium-derived microparticle; PMP: platelet-derived microparticle; CEACAM: carcinoembryonic antigen-related cell adhesion molecule; Mac-1: macrophage antigen-1; PECAM: platelet endothelial cell adhesion molecule.

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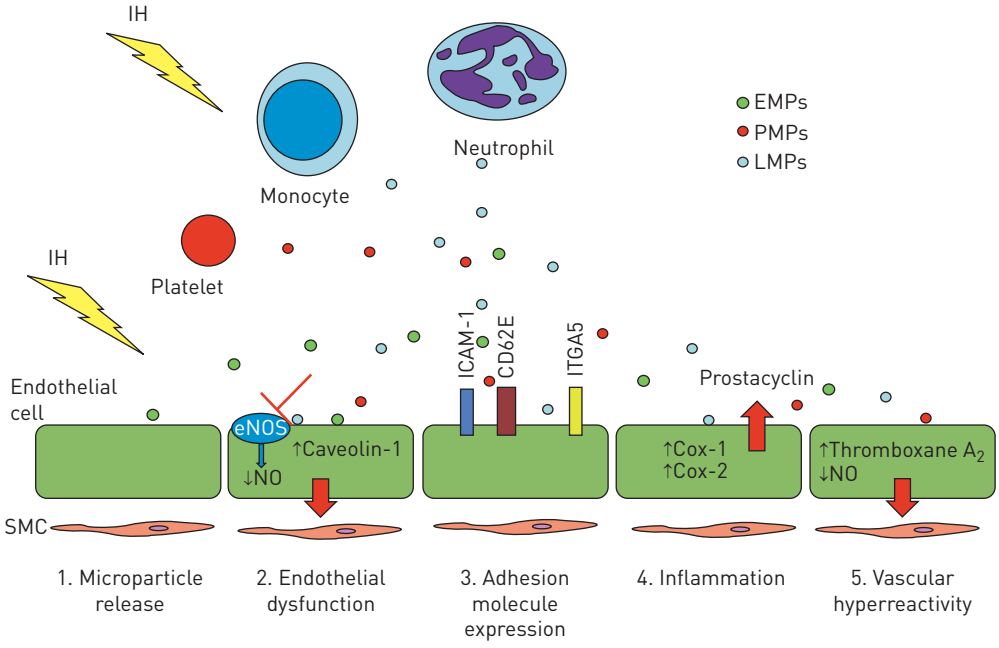

FIGURE 2 Illustration of the main pathways through which circulating microparticles (MPs) may contribute to the obstructive sleep apnoea (OSA)-associated vascular dysfunction. 1) OSAs induce intermittent hypoxia (IH), which may stimulate the release of platelet-derived microparticles (PMPs), endothelium-derived microparticles (EMPs) and leukocyte-derived microparticles (LMPs). 2) Circulating MPs interact with endothelial cells and contribute to impair endothelial function by enhancing phosphorylation of endothelial nitric oxide synthase (eNOS) at the site of inhibition and inducing expression of caveolin-1. 3) MPs promote the cell–cell interactions by increasing expression of the vascular adhesive molecules: intercellular adhesion molecule (ICAM)-1, CD62E (E-selectin) and integrin-α5 (ITGA5). 4) MPs promote vascular inflammation by increasing cyclooxygenase (Cox) expression and prostacyclin release. 5) MPs lead to vascular hyperreactivity by reducing endothelial nitric oxide (NO) release and promoting the secretion of vasoconstrictors. SMC: smooth muscle cell.

In a subsequent study [75], MPs from OSA patients injected into mice induced *ex vivo* vascular hyperreactivity in aortas with a functional endothelium but not in vessels without a functional endothelium, highlighting the mandatory role of the endothelium. Molecular investigations demonstrated that MPs from OSA patients reduced eNOS activity and subsequent nitric oxide production, increased aortic cyclooxygenase (Cox)-1 and Cox-2 expression, and increased thromboxane A_2 and prostacyclin production. Altogether, these findings support a potential implication of circulating MPs in OSA-associated endothelial dysfunction.

Vascular inflammation

There is evidence that OSA is associated with vascular inflammation, which is thought to be a major component of the atherogenic process. OSA patients have elevated serum levels of TNF-α, interleukin (IL)-6, IL-8, C-reactive protein [76, 77] and adhesion molecules (CD62L, soluble CD62E, CD62P, intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1) [77, 78]. OSA is also associated with delayed neutrophil apoptosis and increased levels of adhesion molecules, which could contribute to enhanced neutrophil–leukocyte interactions, and facilitate free radical production and proteolytic release [79].

MPs are key factors in the inflammatory process, and contribute to the endothelial production of various pro-inflammatory cytokines and chemokines. MPs isolated from human atherosclerotic plaques were found to stimulate the *in vitro* release of IL-1β, IL-6 and CCL2, as well as inducing the expression of ICAM-1, VCAM-1 and CD62E [16, 80]. When incubated for 24 h with endothelial cells, MPs isolated from OSA patients induced overexpression of pro-inflammatory molecules, including CD62E, Cox-2 and ICAM-1 [52]. When injected into mice, MPs from OSA patients induced overexpression of pro-inflammatory enzymes, such as Cox-1 and Cox-2 in the aorta, and overproduction of pro-inflammatory cytokines in the supernatants from the animal's aorta (thromboxane A_2, 8-isoprostane, prostacyclin and prostaglandin E_2) [75]. Altogether, these findings support a role for MPs in the vascular inflammation associated with OSA.

Oxidative stress

It has been clearly established that atherogenesis is linked to oxidative stress and lipid peroxidation [81]. In animal models of OSA, intermittent hypoxia increased reactive oxygen species (ROS) generation in the vascular wall [82], induced lipid peroxidation [83] and induced the formation of oxidised low-density lipoprotein (LDL)–cholesterol, creating a substrate for atherosclerotic plaques [84]. JELIC *et al.* [85] showed increased oxidative stress in endothelial cells harvested from the vessels of OSA patients. Increased lipid peroxidation and elevated levels of oxidised LDL were observed in OSA patients [86].

MPs are able to modulate oxidative stress. Incubation of endothelial cells with LMPs from apoptotic T-lymphocytes enhanced ROS production through a xanthine oxidase-sensitive mechanism [74, 87]. In contrast, i*n vitro* treatment of endothelial cells with MPs from patients with the metabolic syndrome reduced both nitric oxide and superoxide anion production, resulting in protein tyrosine nitration [87]. However, PRIOU *et al.* [52] studied *in vitro* superoxide anion production by endothelial cells incubated with MPs from OSA patients and controls, and found no significant differences.

Conclusion and perspectives

Clinical studies have shown that levels of MPs of various cellular origins, including platelets, endothelial cells and leukocytes, are increased in OSA patients. Figure 1 illustrates the main antigenic epitopes harboured by MPs investigated in OSA patients. Levels of MPs harbouring markers of cellular activation or apoptosis that are known to predict poor cardiovascular outcomes were found to be correlated with OSA severity as well as markers of vascular impairment, and were modified by OSA treatment. As potential biomarkers of vascular dysfunction, MPs could provide a useful tool to predict cardiovascular outcome and monitor treatment response in OSA patients. However, the clinical relevance of MP measurement is currently hampered by methodological concerns. Major discrepancies between studies investigating MPs in OSA patients may be due to the wide heterogeneity in pre-analytical and analytical processes, which need to be standardised.

Experimental data suggest that MPs may contribute to the pathophysiology of OSA-associated vascular impairment by promoting endothelial dysfunction, inflammation and vascular hyperreactivity (figure 2). Thus, MPs may emerge as a novel biological vector of vascular dysfunction adding another layer of complexity to the already multifaceted mechanisms involved in OSA-associated vascular morbidity. Further studies are required to investigate the pathways through which MPs may impair vascular function in OSA and to determine whether MPs could constitute a novel therapeutic target to improve cardiovascular outcome in OSA.

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