Microparticles and their role in Coronary Artery Disease

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Abstract:

Despite of significant advances in prevention, medical and interventional management, Coronary Artery Disease (CAD) remains the leading cause of death worldwide. Although the number of people being diagnosed with CAD has plateaued in the western world, it is projected to increase significantly in the developing world reaching epidemic proportions, particularly in South Asia. To better stratify the risk of developing and suffering a cardiovascular event due to CAD, plasma biomarkers relating to disease burden but also disease activity in CAD are needed; this will allow targeting of appropriate management to high-risk patients for acute events. Over the last twenty years, data have emerged showing that microparticles (MPs) are involved in the pathogenesis of formation and evolution of atherosclerotic plaques causing either stable angina (SA) or acute coronary syndromes (ACS). Herein we provide an overview of our current knowledge of MP formation, composition and possible mechanisms through which they could be contributing to CAD. We also reviewed currently available treatments that can reduce MP production and hence reverse the pathological effects of MPs.

(Keywords**:** Microparticles, Coronary Artery Disease, Acute Coronary Syndrome)

1: Introduction:

Despite significant advances in the medical and interventional management of coronary artery disease (CAD) mortality and morbidity remains high with ischemic Heart Disease (IHD) being the leading cause of death worldwide in the last 5 years. Atherosclerotic disease, the hallmark of CAD, is now considered a chronic inflammatory process. Over the last twenty years, data have emerged showing that immune cells are involved in the pathogenesis, formation and evolution of atherosclerotic plaques causing either stable angina (SA) or acute coronary syndromes (ACS). Intriguingly, the clinical presentation of patients with CAD differs based on the type of atherosclerotic plaques they harbour. Whilst patients with stable plaques present with stable angina it is those with vulnerable plaques that present more acutely with ACS. Early identification of features that define possible atherosclerotic plaque instability is thus vital to improve cardiovascular risk stratification and prognosis. As our understanding of CAD pathophysiology has evolved from not just a focal but ultimately a systemic disease, approaches to identify these high-risk patients may need to combine identification of local vulnerable plaques or myocardial damage but also novel plasma biomarkers relating to cumulative atherosclerosis burden.

Microparticles (MPs) are now considered key mediators of inflammation and therefore may play a role in to both the formation and progression of atherosclerosis and subsequent plaque rupture leading to ACS. MPs were referred to as “platelet dust” when first reported in 1967 (6). The perception that MPs were merely “innocent debris” rapidly changed due to an increasing body of evidence suggesting that have potent pro-coagulant and pro-inflammatory properties (7). MPs are sub cellular particles (measuring <1 μm) derived from the plasma membrane of any eukaryotic cell. Although MPs are formed in response to various biological processes such as cellular activation and apoptosis, the evidence of their role in pathological states comes from their presence in excess numbers in disease states such as ACS, sepsis, systemic inflammation (including vasculitis), and malignancy (8). Over the last decade an increasing number of studies have explored the mechanisms of formation of MP, their content, and contribution to pathological states through a number of mechanisms such as angiogenesis, inflammation and coagulation. Pertinent to the pathology of ACS are MPs derived from platelets, representing 70% of total MP, but also those from, monocytes, endothelial cells, erythrocytes and leukocytes (9).

Herein we provide an overview of our current knowledge of MP formation, composition and possible mechanisms through which they could be contributing to CAD. We also review currently available treatments aiming to reduce MP production, and the pathological effects of MPs.

2a: MP formation and composition

The normal plasma membrane consists of a phospholipid bilayer (10). The distribution of phospholipids in the bilayer is asymmetric with the outer layer consisting of phosphatidylcholine/sphingomyelin and inner layer consisting of phosphatidylserine (PS) (11). This pattern of distribution of phospholipids are under the control of three proteins; Flippase, Floppase and Scramblase (12). Increased intracellular calcium following cell activation stimulation alters the function of these three proteins and results in movement of phospholipids towards the outer layer leading to exposed PS. This reorganisation of plasma membrane lipid bi-layer is associated with loss of asymmetry of cytoskeleton thus leading to vesicle formation; which is then cleaved by Caspases and results in MP formation (10). Caspases were further shown to play a role in the release of MPs by the cleavage of ROCK I, a Rho-kinase, during apoptosis (13). Thrombin induced Endothelial cell vesiculation has also been shown to involve nuclear factor (NF)-κβ signalling, TRAIL and ROCK II activation (14). The exposed PS is a potent pro-coagulant as it provides an excellent substrate for the pro-thrombinase complex (15).

2b:MP function

MPs carry out various biological functions depending up on the cell they are derived from (16) (Table-1). MP lipid and protein composition varies depending up on the parent cell and the stimulus that triggered their formation (17). Broadly they have a role in inflammation, coagulation, endothelial dysfunction, and angiogenesis. Evidence is also emerging of their role in microvascular dysfunction (MvD; see below). The pro-coagulant potential of MPs is largely secondary to PS exposure that acts as a platform for the assembly of several pro-coagulant factors along with Tissue Factor expression (TF)(15). MPs also act as communicators or messengers carrying cytokines, mRNA and viruses (18). In addition MPs also act as transporters of specific messenger RNAs (miRNAs), of particular relevance to cardiovascular diseases (19). Importantly, not all functions of MP appear to be detrimental as anticoagulant and fibrinolytic functions have also been been reported (15) (20). In addition they also contain increased concentrations of oxidized phospholipids and caspases when compared to parent cell thus playing a role in cellular waste processes (21).

Cell type	Surface marker	Biological function
Platelet (PMP)	CD31,CD42a,CD42b,CD61	Inflammation, Thrombogenesis, Angiogenesis⊗
Endothelial cell (EMP)	CD105,CD31,CD146,CD51 ,CD54,CD62E,CD144, CD34,CD18	Inflammation, Thrombogenesis, Angiogenesis∗, Endothelial dysfunction
Monocytes (MMP)	CD105,CD14,CD11a, TF+, CD40 L	Inflammation, Thrombogenesis, Angiogenesis
Leukocyte (LMP)(22)	CD45	Inflammation, Thrombogenesis, Endothelial dysfunction
Erythrocyte (23)	CD235a	Thrombogenesis
Neutrophil (NMP)	CD15,CD64,CD66b,CD66e ,CD11b, MPO	Inflammation, Thrombogenesis, Anti-inflammatory effect on Macrophages

Table 1: Biologic function of various microparticles (MPs) and indicative surface markers based on cellular origin. Note there may be overlap on expressed surface markers: CD146 has been found on activated T-cells; CD54 (Inter- Cellular Adhesion

Molecule-1; ICAM-1) is also expressed by leukocytes; and CD51 is present on monocytes/macrophages and platelets. PMP – Platelet derived MP, EMP – Endothelial derived MP, MMP – Monocyte derived MP, LMP – Leukocyte derived MP, NMP – Neutrophil derived MP. ⁎Low levels of EMP shown to promote angiogenesis where as high levels abolish angiogenesis (24), ⊗ in vitro only (25)

2c: Quantification and phenotyping of MPs

There are currently a number of different ways of quantifying MPs (Table-2). Flow cytometry remains the most commonly used method (26). Staining with flurochrome-conjugated Annexin V (AnV), which binds to PS, is commonly used to identify MP of mixed cellular origin with flow cytometry (27). However some MPs don't bind with AnV. Whether this is a reflection of low PS content and/or a limitation of the technique using AnV staining to identify all MPs; or whether truly these AnV-negative MPs exist and have other functions remains to be established (28). As MP carry parent cell proteins and receptors, fluorochrome-conjugated antibodies directed at these components allow us to quantify the specific type of MP using flow cytometry (Table-1). The procoagulant function of MPs can be confirmed in vitro by number of coagulation assays such as Thrombin Generation assay (TGA) (29) (Table -2).

Method	Detailed Methodology	Advantages	Disadvantages
Flow Cytometry	Detects surface antigens specific to the cellular origin. An-V can be used as a general marker for PS+ MPs	Widely used Can simultaneously detect two or more MP antigens	Inability to quantify PMPs sized approximately 400–500 nm (30)
Microscopy 1. TEM 2. AFM (30)	TEM Visualizes isolated MPs and determines their structure AFM is a type of scanning probe microscopy, allows high-resolution topographic imaging of PMPs	AFM can detect 1000-fold more PMPs than FC, mostly those of a small size	The time needed for sample handling and preparation renders current AFM procedures unsuitable for the screening of many samples
Dynamic Light Scattering (31)	Analyzes PMPs exposed to monochromatic light from a laser	Small sample volume (<0.4 ml) suffices	MPs can be significantly smaller post-filtration. Monodisperse system required
Nanoparticle tracking analysis (32)	Analyzes particle movements by Brownian motion under a laser beam, with counting in real time	Small sample volume required	Studies of large vesicles (>500 nm) alone may be better carried out by flow cytometry Inability to accurately resolve heterogeneous mixtures of vesicles
Tunable resistive pulse sensing (33)	A high-resolution technique used to monitor individual and aggregated particles, of 50–1000 nm or more, as they move through tunable nanopores	Small sample volume (<0.1 ml) required	Limited data
Immunological & Procoagulant based assays (28, 34)	These assays determine the procoagulant activity, in relation to the presence of circulating phospholipid or exposure to PS, tissue factor (TF), or other PMP sur- face	Large number of samples can be screened	Limited to the capture system used soluble antigens

	markers		
Proteomics (35)	Exploration of the nuclear material in PMP by processing and by various analytical methods such as 2D-electrophoresis and tandem mass spectrometry (MS), spectral count analysis	Potential prognostic biomarker role in future in various cardiovascular disorders	Requires sophisticated equipment with high maintenance
Cellular assays or cell culture (36)	MP function evaluated by looking at the impact on the tissue	Can be used to elucidate therapeutic role	Requires time

The detailed methodologies of various assays mentioned in the table are beyond the scope of this review. References are provided as a guide to readers. TEM – Transmission electron microscopy, AFM – Atomic force microscopy.

3: Biologic function of MPs pertinent to CAD.

MPs may contribute to formation and progression of atherosclerosis through a number of mechanisms such as angiogenesis, inflammation, coagulation, endothelial dysfunction, and microvascular dysfunction (Fig-1). These are considered in more detail below.

3a: Angiogenesis

Vulnerable atherosclerotic plaques (VP) have characteristic features such as increased necrotic core, increased apoptotic macrophages and vasa vasorum (37). Atherosclerotic plaques develop their own microcirculation as they grow and this process is driven by angiogenic factors such as vascular endothelial growth factor (VEGF). These micro vessels provide an avenue so that leukocytes and erythrocytes can enter and exit the atheromatous plaque, supplying oxygen and nutrients thus promoting the growth of the plaque. These micro-vessels are not stable and can rupture easily leading to intra-plaque haemorrhage (38). More recently the "PROSPECT" study concluded that atherosclerotic plaque may expand significantly before the acute presentation based on Intra Vascular Ultrasound (IVUS) observation of all three coronary arteries at baseline, and following occurrence of an acute event (39). It is currently not clear what leads to this expansion, but as proposed by Virmani et al this could be secondary to intra-plaque haemorrhage and subsequent alterations in the intra-plaque milieu (40). MPs may play a role in this angiogenesis-related plaque instability. Here we discuss studies demonstrating the pro-angiogenic properties of MPs.

<u>In Vitro studies:</u>

Kim et al. demonstrated how PMPs can promote the proliferation and survival, migration, and tube formation in vitro of human umbilical vein endothelial cells (HUVEC) (25). When PMPs were treated with activated charcoal, a procedure known to remove the lipid growth factors, the MP angiogenic activity was significantly reduced. These results suggest that the lipid components of the PMP may be major activating factors of protein components. In addition, PMPs stimulated proliferation, chemotaxis and tube formation of HUVEC, a process that was mediated via the Pertussis toxin-sensitive G protein, extracellular signal-regulated kinase and the phosphoinositide 3-kinase pathways. In pathological states such as a growing tumour, PMPs shed from the circulating platelets may reach adequate concentrations contributing to florid neoangiogenesis (25). In another elegant in vitro study Leroyer et al demonstrated the potential role played by MPs in intra-plaque angiogenesis and thus plaque vulnerability. MPs were isolated from carotid endarterectomy specimens surgically obtained from 26 patients and further characterized by PS exposure and identification of cellular origin. Plaque MPs (93% macrophage in origin) increased both endothelial proliferation and stimulated in vivo angiogenesis in Matrigel assays performed in mice,

whereas circulating MPs had no effect. MPs from symptomatic patients expressed more CD40L and were more potent in inducing endothelial proliferation, when compared with asymptomatic plaque MPs. MP-induced endothelial proliferation was impaired by CD40L or CD40-neutralizing antibodies and abolished after endothelial CD40-ribonucleic acid silencing. In addition, the proangiogenic effect of plaque MPs was abolished in Matrigel assays performed in the presence of CD40L-neutralizing antibodies or in CD40-deficient mice (41).

In Vivo study:

In a clinical study LMP levels were found to be elevated in patients with high – grade carotid stenosis with underlying unstable plaque. Forty two asymptomatic patients with > 70% stenosis due for endarterectomy had LMP levels quantified by high sensitive flow cytometry before surgery and plaque analysis post surgery. Based on the morphology plaques were classified into stable and unstable as per American Heart Association (AHA) criteria (42). Plaques with disrupted endothelium and intra-plaque hemorrhage are classified as unstable. After logistic regression, the neurologic symptoms and the level of CD11bCD66b+ MPs independently predicted plaque instability thus underpinning the role of LMPs as a promising biomarker in predicting neurologic events (43).

3b: Inflammation and Coagulation:

There are various mechanisms by which MPs can act as inflammatory mediators. In vitro studies have shown that binding of MPs to endothelial cells induces the expression of pro inflammatory molecules. PMPs and EMPs induce the expression of proinflammatory ICAM-1, whereas MMP induce expression of ICAM-1 and Interleukin (IL)-8 (4). Inflammation and coagulation are linked processes in many diseases, and MMP, owing to their TF content, are intensely procoagulant. It also appears that proinflammatory molecules may induce the generation of MPs.

In Vitro studies:

Although animal studies have indicated a direct pathogenic role of CRP, the mechanism underlying this remains elusive. Dissociation of pentameric CRP (pCRP) into pro-inflammatory monomers (mCRP) may directly link CRP to inflammation. Habersberger et al. investigated whether cellular microparticles (cMPs) can convert pCRP to mCRP and transport mCRP following MI. In vitro experiments demonstrated that MPs were capable of converting pCRP to mCRP, which could be inhibited by the anti-CRP compound 1,6 bis-phosphocholine. Significantly more mCRP was detected on MPs from patients following MI compared with control groups. They further demonstrated that MPs containing mCRP were able to bind to the surface of endothelial cells and generate pro-inflammatory signals in vitro, suggesting a possible role of MPs in transport and delivery of pro-inflammatory mCRP in vascular disease (44).

One of the key features of MPs is the procoagulant potential. Aliman et al. have sought to establish the mechanism by which MPs promote thrombin generation and modulate fibrin density and stability. They isolated platelets and monocytes from healthy donors, which were then stimulated with calcium ionophore, thrombin receptor agonist peptide (TRAP) or TRAP/convulxin and lipopolysaccharide (LPS). MPs were isolated, washed by high-speed centrifugation and assessed using the following: transmission electron microscopy, Nanoparticle Tracking Analysis, flow cytometry, TF activity, prothrombinase activity, thrombin generation, and clot formation, density and stability. MMPs had TF activity, supported prothrombinase activity, and triggered shorter thrombin

generation lag times than buffer controls. Compared with controls, MMPs supported faster fibrin formation, 38% higher fibrin network density and higher clot stability. In contrast, PMPs did not have TF activity and supported 2.8-fold lower prothrombinase activity than MMPs. PMPs supported contact-dependent thrombin generation, but did not independently increase fibrin network density or stability (45).

In Vivo studies:

High levels of CRP seen in patients presenting with ACS suggest that atherosclerosis is an inflammatory process (46). Ueba et al. demonstrated positive correlation between PMP and IL6 in 464 healthy volunteers (46). Biasucci et al showed correlation of EMPs and PMPs with high sensitive C-reactive protein (hs-CRP) in patients undergoing PCI following presentation with ACS (47). Cui et al showed that EMP and PMP were significantly elevated in MI and UA, and TF+ MPs were significantly elevated in MI and UA when compared to a SA group, and correlated with IL6 and CRP level (48). Higher CRP concentrations were shown to be associated with complex, vulnerable atherosclerotic plaques (49).

More recently our group have demonstrated a positive correlation between MP expression and markers of inflammation and myocardial necrosis in patients with ACS or SA (manuscript submitted). Patients with ACS or SA undergoing PCI were studied. AnV+MP were quantified using flow cytometry from blood samples taken from the right atrium (RA) and culprit coronary artery (CO). Markers of inflammation (hs-CRP, IL-6, serum amyloid antigen (SAA) and myocardial injury (Troponin T) were measured using ELISA. Total and cell specific AnV+MP expression was higher in the ACS versus the SA group in both the CO and RA sites with CO MP levels being higher than the RA site in both groups. In the CO and RA sites of the ACS group, but not the SA group, markers of inflammation (SSA, IL-6, Troponin – T) correlated positively with AnV+MP (50).

3c: Endothelial dysfunction:

Vascular endothelium plays a very important role in regulation of vascular tone and maintenance of vascular homeostasis. Endothelial dysfunction is one of the early steps in the development of atherosclerosis. It is also implicated in plaque progression and atherothrombotic events (51).

In Vitro studies:

Rautou et al have shown that MP isolated from human atherosclerotic plaques transfer ICAM-1 to endothelial cells to recruit inflammatory cells thus promoting atherosclerotic plaque progression (5).

Boulanger et al investigated whether or not MPs would affect endothelium-dependent responses. Rat aortic rings with endothelium were exposed for 24 hours to circulating MPs isolated from peripheral blood of 7 patients with non ischemic (NI) syndromes and 19 patients with AMI. Endothelium-dependent relaxations to acetylcholine were not affected by high concentrations of MPs from NI patients. However, significant impairment was observed in preparations exposed to MPs from patients with MI at low and high concentrations (52).

In Vivo study:

In another study endothelial-dependent vasodilatation was invasively assessed in 50 patients with CAD by quantitative coronary angiography during intracoronary acetylcholine infusion. Circulating CD31+/annexin V+ apoptotic MPs were analysed by flow cytometry in peripheral blood. Increased apoptotic MP counts positively correlated with impairment of coronary endothelial function. Multivariate analysis revealed that increased apoptotic MP counts predicted severe endothelial dysfunction independent of traditional risk factors, such as

hypertension, hypercholesterolemia, smoking, diabetes, age, or sex. Thus it was concluded that; in patients with CAD, endothelial-dependent vasodilatation closely relies on the degree of endothelial cell apoptosis, which is readily measurable by circulating CD31+/annexin V+ apoptotic MPs (53).

3d: Microvascular dysfunction:

Up to 30% of patients undergoing primary PCI (PPCI) following AMI do not achieve adequate reperfusion. This phenomenon is called “no-reflow” and may be due to microvascular obstruction (MVO) (54). Porto et al correlated PMP, EMP with indices of MVO or MvD such as thrombolysis in myocardial infarction (TIMI) flow, thrombus score, corrected TIMI frame count myocardial blush grade quantitative blush evaluator score, and 90 min ST segment resolution from sequential aortic and culprit coronary artery blood in 78 STEMI patients undergoing successful PPCI. This is the first study to suggest a direct role of MP in the pathogenesis of MVO.

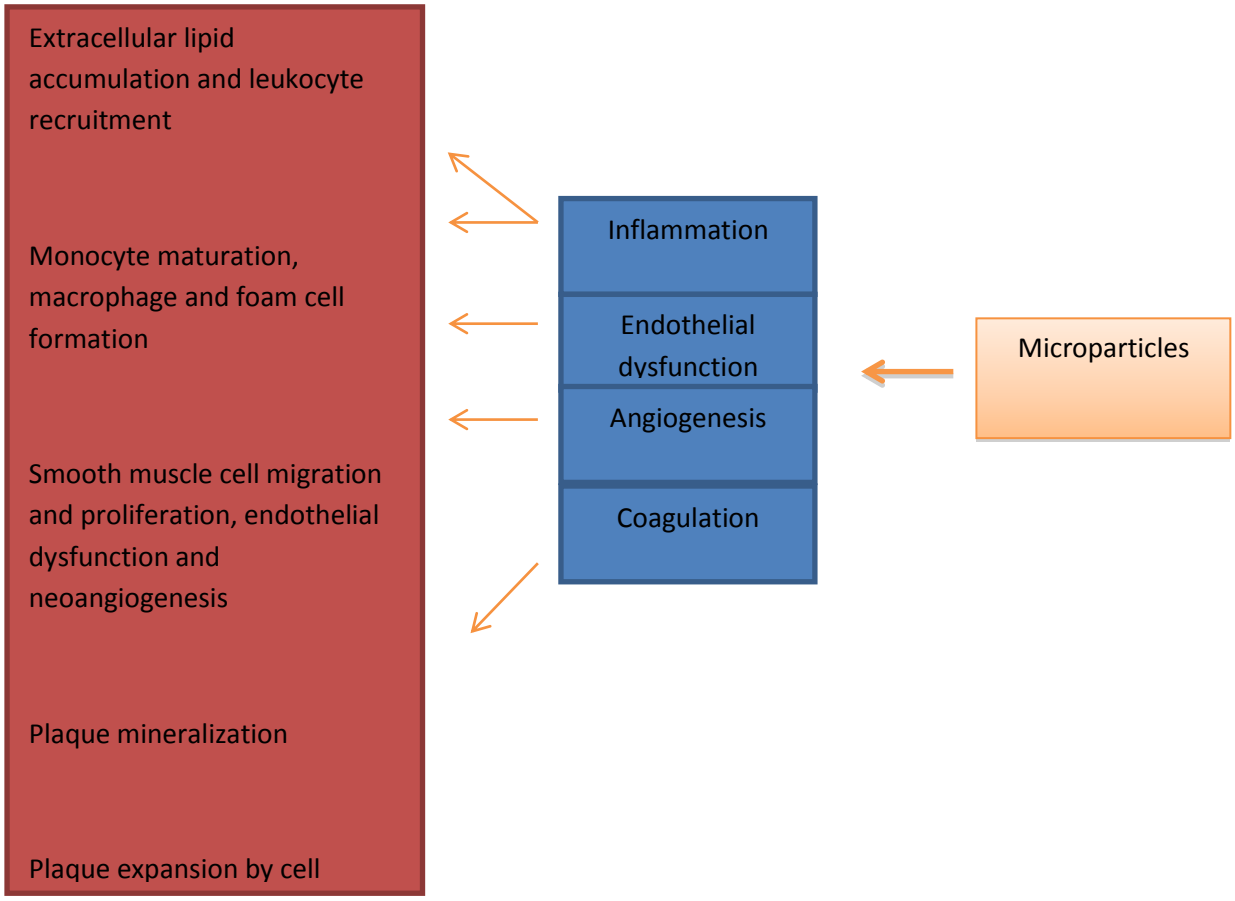

Figure1

Flow chart depicting the interplay between the biologic function of microparticles (MP) and the atherosclerotic process.

Key steps in the formation and progression of atherosclerosis

4a: MP in patients considered to be at high risk of CAD or with stable CAD:

Abnormal lipid levels, smoking, hypertension and diabetes are the major risk factors for myocardial infarction worldwide (55, 56). In a large community based study of 844 individuals from a Framingham Off spring cohort, various phenotypes of EMPs were shown to be associated with traditional risk factors in subjects without CAD. Multivariable analyses showed hypertension and metabolic syndrome to be associated with CD144+ EMPs, and hypertriglyceridemia to be associated with CD144+, CD31+/CD41- EMPs (57). Cigarette smoking is one of the major risk factors known to be associated with cardiovascular disease. The impact of brief smoking on the vasculature was assessed in a small study.12 healthy volunteers were studied following randomization to either smoking or not smoking in a crossover fashion. Blood sampling for endothelial progenitor cells (EPCs) and a panel of MPs was performed at baseline, 1, 4 and 24 hours following smoking/not smoking (58). The results showed smoking even a single cigarette generated an acute release of EPC and MPs, of which the latter contained nuclear matter reflecting injury to the vascular wall (58). Saudes et al. have shown in their cross-sectional study MP concentration and phenotype in familial hypercholesterolemia (FH) differed markedly from non-FH. Levels of AnV+-total, CD45+-pan-leukocyte and CD45+/CD3+-lymphocyte-derived circulating MPs were significantly higher in FH-patients with subclinical lipid-rich atherosclerotic plaques than fibrous plaques. Patients with life-long high LDL exposure have higher endothelial activation and higher pro inflammatory profile, even under current state-of-the-art lipid lowering therapy (LLT) suggesting long term effects of vascular damage (59). Augustine et al carried out a panel of MP assays on a cohort of patients referred for Dobutamine stress echocardiography (DSE). They found that procoagulant, platelet, erythrocyte, and endothelial but not leukocyte, granulocyte, or monocyte-derived MPs were elevated immediately after a standardized DSE and decreased after 1 hour. Interestingly in twenty-five patients who had positive DSE test the MP levels did not change during stress. Similar findings were also noticed in patients with negative DSE test but with previous vascular disease. In those who subsequently underwent coronary angiography MP rise during DSE had occurred only in those with normal coronary arteries suggesting blunted response of MP is seen in patients with or at risk of vascular disease (60). In a Computed tomographic angiography based study (CTA) correlation was found between groups with moderate calcific coronary atherosclerotic disease with MPs procoagulant activity in patients with stable CAD. Another CTA based study on post-menopausal healthy women considered to be at low risk of CAD by Framingham score, showed that EMPs, PMPs, and their TGA were greatest in women with high coronary artery calcification (CAC) scores, thus emphasizing their value in identifying women with premature CAC (62). EMPs expressing CD62E were shown to predict cardiovascular outcomes in patients with stroke history. Three hundred patients with a history of stroke in the preceding three months were recruited. EMPs were assayed by flow cytometry on recruitment. Of the 298 who completed the study according to protocol for 36 months major cardiovascular events occurred in 29 patients (9.7%). Higher major CV events rates were noted to occur in patients with high levels of CD62E+MPs (63). In an elegant study by Sinning et al CD31+/Annexin V+MPs were shown to be increased in patients with cardiovascular risk factors and impaired coronary endothelial function. They determined CD31+/Annexin V+ MP by flow cytometry in 200 patients with stable CAD undergoing angiography and correlated with cardiovascular outcomes. The median follow-up time for major adverse cardiovascular and cerebral event (MACCE)-free survival was 6.1 (6.0/6.4) years. MACCE occurred in 72 patients (37%). MPs levels were significantly higher in patients with MACCE compared with patients without MACCE. In multivariate analyses (cardiovascular risk factors, number of diseased vessels, use of angiotensin-converting enzyme-inhibitors and statins), high MP levels were associated with a higher risk for cardiovascular death, the need for revascularization, and the occurrence of a first MACCE. Inclusion of the MP level into a classical risk factor model substantially increased c-statistics from 0.637 (95% CI: 0.557-0.717) to 0.702 (95% CI: 0.625-0.780) (P=0.03). This study gives robust evidence that the level of circulating CD31+/Annexin V+ MPs is

an independent predictor of cardiovascular events in stable CAD patients and may be useful for risk stratification by incorporating MPs into existing risk scoring models (64).

4b: MP in patients with ACS

Circulating PMPs have been shown to be associated with the risk of future atherothrombotic events. Namba et al found PMPs to be significantly higher in patients with ACS than those in the group with stable angina. In their cohort of patients (66 ACS, 126 SA and 26 patients with no CAD with a mean clinical follow-up for 11.1±2.4 months) a positive correlation was observed between arc of calcification in the culprit vessel noted on intra vascular ultrasound (IVUS) with levels of circulating PMP. Furthermore, the cohort of subjects with high load of circulating PMPs had low event free survival suggesting a potential role for circulating high PMPs in risk stratification (66). Several small studies have shown that the levels of PMPs, EMPs, MMPs, tissue factor bearing MPs (TF+MPs) were higher in patients with unstable angina (UA) and acute myocardial infarction (AMI) when compared to those with stable angina (67, 68). In addition it was noted that CD31+EMPs were particularly high in those whose index presentation was AMI than in those with recurring MI or UA. CD51+MPs and PMPs did not have such a discriminatory role (69). Procoagulant EMPs were found to be significantly elevated in the peripheral blood of ACS patients when compared to SA or patients with no CAD suggesting an important role for endothelial injury in inducing the procoagulant potential (70). Crea et al noted that the levels of EMPs and PMPs to be high in intra-coronary blood (culprit artery) when compared to systemic blood (aortic) in AMI patients undergoing primary PCI (PPCI) (47). Higher levels of MPs (CD31, CD146, CD42b and CD11a) were also demonstrated in the culprit arteries of STEMI patients when compared to systemic samples and significant reduction in MP levels post PCI thus demonstrating their role in thrombus formation (71).

High EMPs levels were also noted to be associated with high-risk angiographic lesions like eccentric, multiple irregular or thrombotic lesions (69) the so called type 2 lesions based on Ambrose classification (72). One of the problems of PCI is a subsequent clinical event due to in-stent restenosis (ISR). MP may have a role in predicting which cohorts of patients are at risk of developing ISR. Inoue et al in their study analysed circulating PMP, hs-CRP and activated Mac-1 on surface of neutrophils in 61 patients undergoing PCI. All three markers have increased in time dependent manner with maximum response at 48 hours in coronary sinus blood. Inflammation as well as platelet activation at the site of local vessel-wall injury plays an essential role in the mechanism of restenosis after PCI. Multiple regression analysis showed that each of PMP, hs-CRP and Mac-1 was an independent predictor of the late lumen loss. Coronary stenting enhanced circulating PMP in association with an inflammatory response in the injured vessel wall. PMP may be a useful marker for evaluation of stent-induced inflammatory status and a powerful predictor of restenosis equivalent to activated Mac-1 (73). Higher levels of circulating EMPs and PMPs were also shown to correlate with myocardium at risk on cardiac magnetic resonance imaging (CMRI) and anv+MP on day2 post ACS with Troponin T levels (74). The levels of circulating MPs have also been linked with the remote ischemic conditioning. Nagy et al found that PMPs levels were significantly elevated in patients treated electively with PCI compared to subjects with diagnostic catheterization alone. They showed that at 15 minutes after the completion of PCI the levels of PMPs, platelet P-selectin expression and the ratio of platelet-monocyte heterotypic aggregates were significantly ($p < 0.05$) elevated in the PCI group compared to the non-stented subjects (75). One of the inherent properties of MPs is their aforementioned procoagulant potential. Morel et al evaluated the prothrombotic status of DM and non-DM (NDM) patients at days 1 (D1) and 6 (D6) after MI, by measurement of circulating procoagulant MP and soluble GPV (sGPV), the platelet glycoprotein V major fragment released upon thrombin cleavage. AnV+MP and their

procoagulant potential quantified by TGA were significantly higher in DM and NDM patients when compared to healthy volunteers at D1 and D6. The persistent elevation of PMPs and EMPs at D6 together with DM patients with elevated MP levels at D1 presenting with further CV events at 6 month follow up suggests a prognostic potential for MP (76).

5: Treatment directed towards MP:

A number of different studies have explored the effect of various therapies on modulating circulating levels of MPs in cardiovascular disease. These treatment modalities range from dietary substitutes, prognostic medications used in CAD and mechanistic procedures such as haemofiltration. However most of these studies were carried out in small numbers of participants with no large scale randomized trial data. This is not surprising given the lack of standardization in quantifying MPs and time and effort required for their assay.

For instance, treatment with n-3 fatty acids after myocardial infarction appears to exert favorable effects on levels of PMP and MMPs as shown by Del Turco et al (77). The numbers of PMPs in plasma were significantly decreased by n-3 fatty acids, while they were unchanged in the placebo group. Total microparticle TF-procoagulant activity was also reduced in the n-3 fatty acid group compared to that in the placebo group. Similar results were also noted with dietary flavanol (78) and diet rich in oats (79).

Reducing oxidative stress by administering vitamin C also appears to decrease the levels of MPs. In a study of post MI patients, vitamin C was administered at a dose of 1g/day in addition to standard therapy. Circulating MPs were quantified by functional prothrombinase assay before and after 5 days of vitamin C administration. Vitamin C resulted in reduction in MPs, particularly in high-risk individuals with DM, dyslipidemia or more than two cardiovascular risk factors (80). Aspirin at a dose of 100 mg/day over 8 weeks was also shown to reduce the number of PMPs and EMPs by 62.7% and 28.4% respectively; but no effect was noted on flow mediated dilatation used to assess endothelial function in patients with CAD in a small study consisting of 15 patients. This effect seems to be independent of the cyclooxygenase-2 (COX-2) pathway as similar results were not achieved with Etoricoxib – a selective COX-2 inhibitor, in the control group (81).

Studies involving Lipid Lowering therapy (LLT) did not show much promise in attenuating the levels of MPs. In a cohort of patients with CAD Simvastatin/Ezetimibe combination did not change platelet aggregation, the amount of circulating endothelial and platelet microparticles, or endothelial progenitor cells. (82). However Huang et al noted reduction of levels of EMPs and increase in EPCs with high dose Atorvastatin (40 mg) when compared to lower dose Atorvastatin (20 mg) group although this did not translate into minimizing events in the higher statin group at the end of one year (83). In STEMI patients undergoing PPCI significant reduction was noticed in peripheral procoagulant MP levels in the group who were given Abciximab (potent platelet Group 2b/3a inhibitor) prior to PCI when compared to the group who did not get Abciximab. In this cohort of 86 patients 30 patients received Abciximab as per operator discretion. The group receiving Abciximab showed significant reduction of circulating MPs, EMPs and PMPs after PCI (84).

Epoprostenol, a synthetic prostacyclin clinically used in pulmonary hypertension prevented the formation of platelet mixed conjugates with PMN or monocytes, platelet PAC-1 and P-selectin expression and platelet microparticle generation at nanomolar concentrations in vitro (85). In an in vitro study Abdelhafeez et al have shown that a standard CVVH model can decrease EMP levels. MPs generated from HUVECs were circulated through a standard CVVH filter (pore size 200 mum, flow rate 250 mL/hr) for a period of 70 minutes. A 50% reduction in EMPs was noted within the first 30 minutes. EMPs entering the dialysate after 4 hours were 5.7% of the EMP original concentration (86). In an earlier study Hong et al demonstrated filtration by standard therapeutic

size filter not only removed NMPs but also abolished the pro-inflammatory effects in a group of children with vasculitis (36). However large-scale clinical studies are required before the results can be extrapolated to patient with CAD.

Future research directions

6:Conclusions

In this article we have provided an overview of the role of MPs in CAD and how current therapies may target MPs. Even though strong evidence suggests that MPs are important biological mediators in the development and progression of CAD the lack of standardized methods for enumeration and identification restricts their current use in routine clinical practice. What is evident is that MPs are not only implicated in the pathogenesis of disease but they may have a role as biomarkers predicting acute events and perhaps represent a systemic signal which may help in identifying the patients with vulnerable plaques. However, it remains to be shown whether specific targeting of MPs is an effective means for reducing cardiovascular morbidly and mortality. Further research is required beyond associative data to directly implicate MPs in disease pathogenesis whilst developing novel inhibitor molecules targeted against MP and testing these in the clinical setting.

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