Circulating microparticles as biomarkers of stroke: A focus on the value of endothelial- and platelet-derived microparticles

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Abstract
Stroke is one of the leading causes of mortality and disability worldwide. Numerous pathophysiological mechanisms involving blood vessels, coagulation and inflammation contribute to the vascular occlusion. Perturbations in these pathways can be detected by numerous methods including changes in endoplasmic membrane remodeling and rearrangement leading to the shedding of microparticles (MPs) from various cellular origins in the blood. MPs are small membrane-derived vesicles that are shed from nearly all cells in the body in resting state or upon stimulation. MPs act as biological messengers to transfer information to adjacent and distant cells thus regulating various biological processes. MPs may be important biomarkers and tools for the identification of the risk and diagnosis of cerebrovascular diseases. Endothelial activation and dysfunction and altered thrombotic responses are two of the main features predisposing to stroke. Endothelial MPs (EMPs) have been recognized as both biomarkers and effectors of endothelial cell activation and injury while platelet-derived MPs (PMPs) carry a strong procoagulant potential and are activated in thrombotic states. Therefore, we reviewed here the role of EMPs and PMPs as biomarkers of stroke. Most studies reported high circulating levels of EMPs and PMPs in addition to other cell origins in stroke patients and have been linked to stroke severity, the size of infarction, and prognosis. The identification and quantification of EMPs and PMPs may thus be useful for the diagnosis and management of stroke.

KEYWORDS
biomarkers, endothelial microparticles, platelet microparticles, stroke

1 | INTRODUCTION

Stroke is one of the most devastating neurological disorders and is considered one of the leading causes of long-term acquired disability in adults (Katan & Luft, 2018). It is considered to be the second main cause of mortality in the world after ischemic heart disease (Benjamin et al., 2018; Katan & Luft, 2018). According to the American Heart Association (AHA) latest statistical update, there were 6.3 million deaths due to cerebrovascular diseases around the world (Benjamin et al., 2018). In addition, the direct and indirect cost of stroke is very high. In the United States (between 2013 and 2014), this cost was around $40.1 billion causing thus a major economic burden (Benjamin et al., 2018). Stroke is a very heterogeneous disease and includes several etiologies and subtypes (Amarenco, Bogousslavsky, Caplan, Donnan, & Hennerici, 2009). Therefore, consistent definitions of clinical stroke and its subtypes is crucial for the proper interpretation of clinical trials and studies aiming to examine incidence, prevalence, and mortality of stroke.
within predefined populations (Sacco et al., 2013). The World Health Organization defined stroke in the 1970s as "rapidly developed clinical signs of focal (or global) disturbance of cerebral function, lasting more than 24 hr or leading to death, with no apparent cause other than of vascular origin" (Aho et al., 1980). However, since significant advances have been made in the clinical and radiographic diagnosis of stroke and its mimics, this definition is now considered outdated and does not reflect the heterogeneity and complexity of the disease. Consequently, in 2013, the AHA and the American Stroke Association developed an expert consensus document for an updated definition of stroke, taking into account the remarkable advances in brain imaging and its clinical implications on the diagnosis of stroke and its subtypes (Coupal, Thapar, Qureshi, Jenkins, & Davies, 2017; Sacco et al., 2013). This document summarized different stroke etiologies and enlisted them in a table for a better definition of stroke.

Stroke can be classified into two major types: ischemic (~80%) which is due to cerebral vessels’ blockage and hemorrhagic stroke (~20%) which is due to brain hemorrhage (Ojaghihaghighi, Vahdati, Mikaelipour, & Ramouz, 2017). Each type is further classified into subtypes according to their etiology. Ischemic stroke has been classified according to the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification into five subtypes based on clinical features and other prognostic: large artery atherosclerosis, small vessel occlusion, cardioembolism, stroke of other determined etiology, and stroke of undetermined etiology (Adams et al., 1993). Hemorrhagic stroke is divided into two subtypes based on bleeding location in the brain to; intracerebral hemorrhage (ICH) where bleeding is directly in brain parenchyma or subarachnoid hemorrhage (SAH) where bleeding takes place between arachnoid space and pia mater surrounding the brain (Ojaghihaghighi et al., 2017). However, other classifications are available for both types of stroke as well.

There is no single outcome that can accurately measure and predict postacute strokes disability and prognosis (Kasner, 2006). Many scales have been developed to measure stroke outcomes including the modified Rankin scale, the National Institutes of Health stroke scale (NIHSS), the Barthel index, the stroke impact scale, and the Glasgow outcome scale (Harrison, McArthur, & Quinn, 2013; Kasner, 2006). In addition, each scale has its advantages and pitfalls that must be taken into consideration before choosing a certain one (Kasner, 2006).

Early and accurate stroke diagnosis is crucial in patient care to adopt the right therapeutic strategies and prevent permanent disabilities (Ojaghihaghighi et al., 2017). For instance, intravenous thrombolysis with recombinant tissue plasminogen activator (rtPA) which is currently the first line drug used for acute ischemic stroke treatment, is believed to be most effective when administered within the first few hours of stroke onset (Gumbinger et al., 2014). Various clinical and radiographic tools are used to diagnose stroke (Sacco et al., 2013). Clinical manifestations and findings help clinicians to determine the type of stroke but usually, brain imaging is required (Ojaghihaghighi et al., 2017). Computed tomography and magnetic resonance imaging are used to recognize the brain lesion location, shape, size and extent and is helpful to determine the cause of stroke and exclude mimics (Sacco et al., 2013). Blood biomarkers, including $100$ calcium binding protein, brain natriuretic peptides, and matrix metalloproteinase-9 have also been evaluated in the diagnosis of acute stroke (Jickling & Sharp, 2011; Sacco et al., 2013). However, these markers lack specificity since they are generated in other diseases that can also affect the blood-brain barrier (Kim, Moon, & Bang, 2013).

Therefore, it is necessary to explore novel effective biomarkers to be used in the identification of high-risk patients, rapid diagnosis, determining stroke etiology and pathogenesis, predicting drug response and clinical outcomes, for novel drug development and as endpoints in clinical trials (Bang, 2017). Microparticles (MPs), which are small membrane vesicles released from activated cells, are being currently heavily investigated as promising biomarkers and vectors for multiple cerebrovascular disorders including stroke.

The primary purpose of this review is to highlight the emerging role of MPs in cerebrovascular disease and specifically focusing on MPs of endothelial and platelets origins because they reflect, respectively, endothelial activation and thrombotic activity, two major contributors to the pathophysiology of ischemic stroke. We will initially review the current understanding of the roles of MPs and then focus on their importance in the understanding of the cerebrovascular disease.

2 | CELL MEMBRANE REMODELING AND MPs RELEASE

Extracellular vesicles (EVs) are submicron, intact membrane-derived vesicles that are released from cells after activation or during apoptosis (Shet, 2008). These vesicles harbor membrane constituents such as phospholipids, integral membranes, and receptors as well as cytoplasmic cargo including various proteins, DNA and RNA adopted from their cells of origin (Hargett & Bauer, 2013; Hugel, Martinez, Kunzelmann, & Freyssinet, 2005). There are several types of EVs characterized based on their size and formation/release pathways including, MPs, exosomes, oncosomes, and apoptotic bodies (Zaborowski, Balaj, Breakefield, & Lai, 2015). MPs were first discovered in 1967 by Wolf during his ongoing coagulation studies when he observed subcellular coagulant vesicles in platelet-free plasma to which he referred to as “platelet dust” (Wolf, 1967). Subsequently, numerous studies have been conducted to further investigate the structure, biogenesis, and functions of these vesicles gradually replacing it by the term MPs (Crawford, 1971; Hargett & Bauer, 2013; Webber & Johnson, 1970). Over the past 40 years, the understanding of MPs has tremendously advanced from being viewed as useless cell debris or cellular trash to one of the key cellular players regulating various physiological and pathological processes throughout the body (Hargett & Bauer, 2013). In 2005, a standard definition for MPs has been proposed by The International and Hemostasis Vascular Biology subcommittee. They described MPs as $0.1–1 \text{ μm}$ cell-derived vesicles that lack a nucleus or synthetic capacity, may contain cytoskeletal proteins, and expose some quantity of phosphatidylinerine on their surfaces (Hargett & Bauer, 2013).
3 | MECHANISMS OF MP FORMATION AND SHEDDING

The shedding of MPs from the plasma membrane is an essential part of cell membrane remodeling and is thought to be initiated by increased intracellular calcium (Shet, 2008). The phospholipid cell membrane bilayer is well-structured and each of the two leaflets has a specific lipid composition. Phosphatidylcholine and sphingomyelin are expressed on the outer leaflet while aminophospholipids (phosphatidylserine [PS] and phosphatidylethanolamine) are oriented inwards (Hugel et al., 2005). Membrane enzymes; floppase, scramblase, and aminophospholipid translocase (flippase) regulate phospholipid movements and maintain this dynamic asymmetry in a steady state. Upon cell stimulation, cytosolic calcium increases which in turn dysregulates membrane enzymatic balance and leads to the collapse of membrane asymmetry and subsequent externalization of PS. In addition, intracellular calcium activates calpain and Rho kinase which together causes cytoskeleton cleavage and rearrangement facilitating thus membrane blebbing as well as activating apoptosis via a caspase 3-mediated pathway. However, it must be noted that other stimuli that cause disruption of membrane integrity can also lead to the formation of MP (Hugel et al., 2005). Moreover, it has been shown that MPs can be released from inactivated platelets without intracellular calcium signaling and it was observed that this mechanism was dependent on αβ integrin and cytoskeleton turnover (Cauwenberghs et al., 2006). Therefore, other pathways are also most probably involved in the formation of MPs hence adding to the complexity of the biology of MP formation and release. Further research is required to fully elucidate the mechanisms governing the formation and shedding of MPs from cell membranes.

4 | PROPERTIES AND CHARACTERISTICS OF MPs

MPs have been found to be present in bloodstream of healthy individuals as well as in patients (Berckmans et al., 2001; Martinez, Tesse, Zobairi, & Andriantsitohaina, 2005). This suggests that MPs can be beneficial, maintaining homeostasis under normal physiological conditions, or deleterious if overproduced or if they carry pathogenic constituents on their surface or in cargo (Hugel et al., 2005). The concept of MPs is interesting as they are considered a circulating storage pool of bioactive molecules that if interpreted correctly can provide valuable insight about the body state and can be used as biomarkers for various pathological states (Hugel et al., 2005). In addition, MPs act as a form of effective intercellular communication system regulating various fundamental biological processes (Hoyer, Nickenig, & Werner, 2010). Studies have shown that MPs convey biological information by either direct interaction with cell receptors/integrins initiating cell signaling cascades or by direct fusion with effector cells by endocytosis and the subsequent emptying of MPs content (Hargett & Bauer, 2013). In this sense, MPs can act as signaling molecules via the expression of different proteins and lipids on their surface such as tissue factor (TF) and PS (Hoyer et al., 2010). Alternatively, MPs have been shown to transfer complete receptor proteins (e.g., CCR5 receptor), mRNA, microRNAs, proteins, and even cellular organelles to recipient cells (Deregibus et al., 2007; Hoyer et al., 2010; Mack et al., 2000; Ratajczak et al., 2006). Nevertheless, more studies are required to confirm that molecular material transferred to recipient cells are biologically functional and significantly affecting cellular responses in target cells (Zaborowski et al., 2015). MPs are very heterogeneous and vary widely depending on the stimulus and cell of origin. For example, the release of MPs in vitro can be induced using a plethora of agents and the resulting MPs vary in structure and composition (Herring, McMichael, & Smith, 2013). For instance, previous studies have shown that in vitro stimulation of human lymphoid CEM T cell line using two different pharmacological stimuli generated antigenically and functionally different MP populations. When the CEM cell line was stimulated with phytotemagglutinin for 72 hr, followed by phorbol-12-myristate-13 and actinomycin D for further 24 hr, generated MPs harbored the morphogen Sonic hedgehog (Shh) and were found to improve endothelial function both in vitro and in vivo (Agouni et al., 2007). However, when the same cell line was stimulated with an apoptotic signal using actinomycin D for 24 hr, MPs generated failed to express the morphogen Shh and caused a deleterious effect on endothelial function both in vitro and in vivo (Mostefai et al., 2008). These studies highlight the importance of the stimulus at the origin of generating MPs in conditioning the antigenic content and biological messages carried out by the shed EVs.

5 | FUNCTIONS OF MPs

MPs are involved in various vital biological functions such as hemostasis, coagulation, inflammation, and angiogenesis (Herring et al., 2013). It has been shown that some MPs may have procoagulant activity due to the externalization of PS which acts as a catalytic surface for enzymatic coagulation complexes assembly (Mooberry & Key, 2016). In addition, some MPs harbor TF on their surface which is considered to be the main initiator of the coagulation cascade in the extrinsic pathway (Meziani, Tesse, & Andriantsitohaina, 2008). Remarkably, one study showed that platelet-derived MPs (PMPs) have 50-100 more procoagulant activity when compared to activated platelets (Sainudriz et al., 2007). In addition, MPs have been shown to play a role in hemostasis disorders such as Scott Syndrome, hemophilia A and Von Willebrand disease (Mooberry & Key, 2016). There is also evidence showing that MPs may have anticoagulant and fibrinolytic activity as well (Mooberry & Key, 2016). MPs have been shown to carry TF pathway inhibitor on their surface and to promote the activity of the anticoagulant protein C (Koshiar, Somajo, Norstrom, & Dahlback, 2014; Kushak et al., 2005; Steppich et al., 2005; Tans et al., 1991). Therefore, the role of MPs in regulating coagulation is complex and depends on the context of their release. Similarly, MPs are involved in inflammation by facilitating the interaction between immune cells,
providing a source of aminophospholipids, and participating in the release of cytokines (Meziani et al., 2008; Puddu, Puddu, Cravero, Muscari, & Muscari, 2010). Furthermore, numerous studies have shown that MPs can regulate angiogenesis and modulate several steps in blood vessels’ formation (Martinez & Andriantsitohaina, 2011). The effects of MPs on the vascular system have been investigated intensively as well. Although it has been shown that some MPs have positive effects on endothelial function, many studies demonstrated that MPs can induce endothelial dysfunction (Agouni et al., 2007; Mostefai et al., 2008). Some of the mechanisms by which MPs induce endothelial dysfunction include, reducing nitric oxide concentration, inducing inflammation, promoting coagulation, altering angiogenesis and apoptosis (Lovren & Verma, 2013). A cellular MP and its proposed functions on coagulation, angiogenesis, inflammation, and vascular function are illustrated in Figure 1.

6 | CHALLENGES WITH THE ANALYSIS OF MPs IN CLINICAL PRACTICE

Given the importance of MPs as potential biomarkers of disease and vectors of biological messages, they have attracted attention from the scientific community; however, various challenges related to the methods of detection and analysis of MPs are hindering the wide application of MPs in clinical use. Such challenges include:

1. Methods to isolate MPs are not fully developed and standardized yet. Most of the current isolation methods allow enrichment rather than separation of EV subtype populations (Zaborowski et al., 2015). Each of the methods available has its pitfalls and there is no standard protocol for MP isolation. For instance, a series of ultra-centrifugations yields are means of good separation of EVs; however, it is often contaminated with high molecular weight protein complexes and cannot differentiate between EV subtypes and cells of origin. Immunoaffinity-based assays provide better enrichment of EV-specific subtypes and provide more homogenous EV profiles, but the overall yield is low. Many MPs subtypes share the same CD pattern of expression and therefore, combining multiple markers to exclude possible subpopulation contamination has been used to overcome this issue. For example, Simak, Gelderman, Yu, Wright, and Baird (2006) used cell-specific antibodies combinations to ensure the detection of endothelial MPs (EMPs) while making sure to eliminate MPs of platelet, leukocyte or erythrocyte origins.

2. MP quantification is highly challenging. Maas et al. (2015) conducted a thorough comparison between three of the most common methods used to quantify MPs namely; nanoparticle tracking analysis, tunable resistive pulse sensing and flow cytometry and found substantial differences in EVs counts among these techniques. The study also reiterated the importance of

FIGURE 1  Structure and functions of MPs. MPs are released from endothelial and platelet cells in resting state and upon stimulation. After membrane rearrangement, MPs externalize phosphatidylserine and harbor other cell surface molecules from their parent cells such as tissue factor and adhesion molecules (i.e. integrins, selectins, and cadherins). In addition, they carry cargo including proteins, DNA, RNA, microRNAs, and cytokines. Based on numerous factors, released MPs may play a role in various biological processes such as coagulation, angiogenesis, inflammation, and vascular endothelium function regulation. The figure shows surface antigens expressed by EMPs and PMPs and the major biological processes they are involved in. EMPs: endothelial MPs; GP: glycoprotein; ICAM-1: intercellular adhesion molecule; MCAM: melanoma cell adhesion molecule; MPs: microparticles; PECAM: platelet and endothelial cell adhesion molecule; PMPs: platelet-derived MPs; VCAM-1: vascular cell adhesion molecule; VE-cadherin: vascular endothelial cadherin [Color figure can be viewed at wileyonlinelibrary.com]
proper technical knowledge of the instruments and their settings and their effect on correct data interpretation (Maas et al., 2015).

3. Handling and processing of blood samples are very crucial and may introduce variability when comparing different studies. Aspects of sample collection, including needle size, the use of a tourniquet, the use of syringe versus vacutainer, and the type of anticoagulant used. In addition, special attention should be given to speeds of centrifugation, number of centrifugations, freeze/thaw cycles and the duration of MPs storage (Shet, 2008).

4. Proper identification of MPs is also very challenging. Arraud et al. (2014) conducted an interesting study to examine MPs morphology and size in which they found that MPs (which were mainly of platelet and erythrocyte origins) are spherical and tubular in shape. Although tubular MPs constituted a small percentage, the total membrane surface area was of the same order of magnitude as spherical MPs. In addition, they found that the majority of MPs in their study did not bind Annexin V which is in contrast with the theory that MPs expose PS when they are formed. They have concluded that these MPs might be formed via a mechanism that maintains the membrane phospholipid asymmetry such as direct cell fragmentation or breakdown from PS-negative tubular MPs (Arraud et al., 2014).

Since not all studies take these challenges into account, especially older ones, it is very hard to compare and analyze findings reported from various studies.

7 | MPs AND STROKE

Almost all cell types are capable of producing and shedding MPs (Shet, 2008). Circulating MPs have been shown to be released from platelets, erythrocytes, leukocytes, endothelial cells, and smooth muscle cells (Chiva-Blanch et al., 2016). Numerous studies reported elevated levels of MPs in cardiovascular diseases and ischemic disorders (Agouni, Andriantsitohaina, & Martinez, 2014). MPs have been linked to multiple pathological states including, atherosclerosis, diabetes, myocardial infarction, metabolic syndrome, hypertension, cancer, pre-eclampsia, and sepsis (Herring et al., 2013; Lovren & Verma, 2013; Meziani et al., 2008).

Several studies are now being conducted to investigate the potential role of MPs in the context of stroke. It was recently reported in a systematic review that circulating MPs from various cell origins increase in ischemic stroke patients (Wang et al., 2018). Since endothelial dysfunction and thrombotic dysregulation are two major contributors to the pathophysiology of stroke, in this review we focused on the role of endothelial-derived MPs (EMPs), as key biomarkers of endothelial activation and dysfunction, and PMPs, as biomarkers of thrombotic state, in the development of stroke. We also reviewed their value as biomarkers in the onset and progression of the disease. Surface antigens that characterize EMPs and PMPs and their functions are summarized in Table 1.

8 | EMPs AND STROKE

Endothelial dysfunction is evident early and is recognized as an important initiator of cerebrovascular disease (Lovren & Verma, 2013). MPs shed from endothelial cells are involved in inflammation, procoagulation, and angiogenesis and thus are considered as biomarkers of endothelial cell activation and injury (Martinez et al., 2005). Profiling of EMPs in stroke may provide an understanding of the pathogenesis of the disease and reflect its prognosis. Distinct EMPs phenotypes are released depending on the type of stimulus the cells are subjected to. When endothelial cells were activated by tumor necrosis factor (TNF)-α in vitro, CD62E+ (E-selectin) MPs were released. E-selectin participates in leukocyte and platelet recruitment and therefore its release is a marker of ongoing inflammation and endothelial activation. On the other hand, apoptotic endothelial cells shed MPs with specific markers such as CD31+ platelet and endothelial cell adhesion molecule (PECAM-1) and thus used as a marker for apoptosis (Jimenez et al., 2003). EMPs released from activated cells are CD62E+ (E-selectin), CD54+ intercellular adhesion molecule (ICAM-1), and CD106+ vascular cell adhesion molecule (VCAM-1), whereas EMPs released upon apoptosis include CD31+ (PECAM-1), CD105+ (endoglin), CD146+ melanoma cell adhesion molecule (MCAM), and CD144+ vascular endothelial cadherin (VE-cadherin; Deng, Wang, & Zhang, 2017). Despite differences in settings between the different studies reported, some conclusions can be drawn. CD62E+ (E-selectin) has been measured in several stroke studies (Chiva-Blanch et al., 2016; Jung et al., 2009; Lackner et al., 2010; Lee et al., 2012; Li & Qin, 2015; Williams, Jauch, Lindsell, & Campos, 2007). It has been shown that CD62E+ EMPs are increased in ischemic stroke patients when compared to healthy controls and remained high when assessed after 7 and 90 days (Chiva-Blanch et al., 2016). High levels of CD62E+ EMPs have also been associated with recent ischemic attacks and increased risk of cardiovascular events; however, no association has been found between CD62E+ EMPs and stroke onset or recurrence (Chiva-Blanch et al., 2016; Jung et al., 2009; Lee et al., 2012). Since E-selectin is a member of the adhesion molecule family that facilitates inflammatory cells’ adhesion and rolling, these findings suggest the following hypotheses. First, high levels of CD62E+ EMPs without being correlated to the time of stroke onset demonstrate continuous endothelial cell activation. Second, endothelial dysfunction in ischemic stroke is more likely to be due to cell activation rather than apoptosis. Finally, the lack of correlation between CD62E+ EMPs and stroke recurrence while its association with cardiovascular events and hospitalization may suggest general endothelial dysfunction rather than pathology of cerebral ischemic origin (Chiva-Blanch et al., 2016; Jung et al., 2009; Lee et al., 2012).

There is some suggestion that changes in EMPs may be used as a biomarker of stroke severity (Chiva-Blanch et al., 2016; Jung et al., 2009; Li & Qin, 2015; Simak et al., 2006). Higher levels of EMPs have been found in patients with moderate/severe stroke versus mild stroke patients. Stroke severity was assessed using NIHSS scores where a score greater than five defined moderate/severe stroke
while scores less than five indicated a mild stroke. Li and Qin (2015) have shown a positive correlation between CD144+ (VE-cadherin) EMPs and stroke severity. Another study using the same scale found an increase in some EMPs (including CD144+) in moderate to severe patients compared to mild stroke patients (Simak et al., 2006). Therefore, CD144+ EMPs have the potential to be used to assess stroke severity but further evidence is needed to confirm these findings. In addition, EMPs can also be used in stroke diagnosis and identification of its etiology and subtypes. Simak et al. (2006) have shown some correlation between EMPs counts (CD105+ and CD54+) and brain lesion which can be useful to identify the extent of brain damage. In addition, Li and Qin (2015) showed a mild correlation between CD62E+ EMPs and OCSP classification which categorizes stroke subtypes according to ischemic lesion location. Therefore, CD62E+ EMP values may predict stroke subtypes. Furthermore, the ratio of CD62E+/CD31+ (E-selectin/PECAM-1) EMPs has been shown to be able to distinguish between the cause of arterial stenosis and whether it is of intracranial or extracranial origin thus providing potential information about stroke etiology (Jung et al., 2009).

EMPs may also be useful in prognosis in patients with SAH. One study conducted on patients with a hemorrhagic stroke caused by SAH showed elevated levels of EMPs positive for CD54+ (ICAM-1), CD62E+ (E-selectin), and CD106+ (VCAM-1). Severe SAH may be followed by complications including cerebral vasospasm (CVS) and cerebral infarction attributable to vasospasm (CIV). This study measured EMPs in patients with CVS and CIV and found that only CD105+ (endoglin) EMPs were upregulated. Since endoglin is considered to be a marker of apoptosis, this finding showed that apoptosis may be a cause of endothelial damage in the early onset of CVS following hemorrhagic strokes (Lackner et al., 2010). It is also worth noting that Simak et al. (2006) have observed a correlation between CD144+ (VE-cadherin) EMPs and hemorrhagic transformation that occurred in five stroke patients during the study identifying it as a potential marker of acute ICH. Further studies are warranted to prove this correlation.

Williams et al. (2007) conducted a study to evaluate the effectiveness of EMPs as biomarkers to differentiate between stroke patients and stroke mimics. They measured CD62E+ (E-selectin)
### TABLE 2 Summary of key studies that investigated EMPs and PMPs in stroke patients

| Type of stroke                        | Time of sample collection                  | MP marker                        | Findings                                                                                     | Strengths and weaknesses                                                                 | Refs.                       |
|--------------------------------------|--------------------------------------------|----------------------------------|----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|-----------------------------|
| Endothelial-derived MPs (EMPs)       | Median time of 37 hr (18.5–51.8) after onset of clinical symptoms | Endoglin: CD105"CD41α"CD45" (E'EMP) | PS^+ EMP were significantly higher in all stroke groups compared to controls, other phenotypes increased but not significantly (a marker of procoagulant endothelium) | Strengths: The use of multiple EMP antigens to avoid contamination with MPs of other origins | Simak et al. (2006)          |
| Patients with acute cerebral ischemia divided into two groups according to NIHSS scores: |                              | VE-cadherin and endoglin: CD105"CD144" (C'EMP) |                                                                                              |                                                                                           |                             |
| 1. Mild stroke; score <5             |                              | Phosphatidyl Serine: CD105"PS"CD41α (PS^+ EMP) |                                                                                              | Weaknesses: No test has been made to relate the increase of PS^+ EMP with other prothrombotic markers to prove its role in procoagulation |                             |
| 2. Moderate to severe stroke; score ≥5|                              | ICAM-1: CD105"CD54"CD45" (I'EMP) | C'E'EMP and I'EMP levels were also significantly higher in the moderate to severe group relative to the mild stroke group subjects (a marker of stroke severity) |                                                                                           |                             |
| Patients suspected with ischemic stroke versus controls at high risk of cardiovascular disease | Within 48 hr after onset of stroke | MCAM: CD146^+ | Higher EMPs counts in ischemic stroke patients compared to controls that have been sustained after 7 and 90 days with EMP showing the most increase compared to others (approximately 300-fold increase; biomarker and diagnosis) | Strengths: Measuring markers of apoptosis and activation | Chiva-Blanch et al. (2016) |
|                                      | Then after 7 and 90 days                 | E-Selectin: CD62E^+             | No correlation between CD62E^+ and the time of stroke onset (continuous cell activation) | Weaknesses: More measures should be taken to eliminate interference of MPs of other origins |                             |
|                                      |                                           |                                 | CD62E^+ (a marker of activation) more than CD146^+ (a marker of apoptosis; evidence of selective packaging of MPs) | Only PS^+ MPs have been measured |                             |
|                                      |                                           |                                 | No correlation with lesion volume                                                            | (Continues)                                                                              |                             |
| Type of stroke                                           | Time of sample collection | MP marker                        | Findings                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Strengths and weaknesses                                                                 | Refs.                                                                                           |
|---------------------------------------------------------|----------------------------|----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| Patients with acute ischemic stroke (AIS) versus age- and sex-matched healthy controls | Within 7 days of the onset of clinical symptoms | • VE-cadherin: CD144+/CD41a<sup>−</sup>  
• PECAM-1: CD31+/CD41a<sup>−</sup>  
• E-Selectin: CD62E<sup>+</sup>  
• E-Selectin PS: AV”CD62E<sup>+</sup> | • All EMPs increased in all acute stroke patients compared to controls. All EMPs were increased in mild stroke compared to controls. Only EMPs have increased in moderate to severe stroke patients compared to controls (biomarker)  
• Levels of CD144+/CD41a<sup>−</sup> (VE-cadherin) microparticles were significantly correlated with stroke severity based on NIHSS scores (stroke severity)  
• A mild degree of correlation was evident between AV” CD62E<sup>+</sup> microparticles and stroke subtype based on OCPS classification (identification of stroke subtype)                                                                 | Strengths:  
• Measuring markers of apoptosis and activation  
Correlation with stroke subtypes Weaknesses:  
• Small-sized MPs may have not been captured, hence underestimating the count. | Li and Qin (2015)                                                                 |
| Stroke patients divided into:  
1. Mild stroke score < 5  
2. Moderate to severe stroke; score ≥ 5 | | | | | |
| Acute stroke patients versus patients with vascular risk factors but no stroke events | Within 7 days of stroke symptoms onset | • PECAM-1: CD31+/CD42b<sup>−</sup>  
• AV”PECAM: CD31+/AV”  
• E-Selectin: CD62E<sup>+</sup> | • Higher levels of CD62E<sup>+</sup> were associated with recent ischemic attack and moderate to severe stroke (biomarker and severity)  
• The ratio of CD62E<sup>+</sup> to CD31+/CD42b<sup>−</sup> or CD31+/AV” EMP levels significantly discriminated extracranial and intracranial arterial stenosis (stroke etiology)  
• No follow-ups have been performed | Strengths:  
• Measuring markers of apoptosis and activation  
• Correlation with stroke etiology Weaknesses:  
• Few outcome events measured | Jung et al. (2009)                                                                 |
| Patients with stroke history at least 3 months before enrollment and compared to controls | At least 3 months before enrollment and followed-up every 3–6 months for a total period of 36 months | • PECAM-1: CD31+/CD42<sup>−</sup>  
• AV”PECAM: CD31+/AV”  
• E-Selectin: CD62E<sup>+</sup> | • A high level of CD62E<sup>+</sup> MPs is associated with cardiovascular events in patients with stroke history (prognosis and identifying high-risk patients) but not associated with stroke recurrence  
• No association between CD31+/AV” and cardiovascular events | Strengths:  
• Measuring markers of apoptosis and activation Weaknesses:  
• Heterogeneous patient population (including all types of stroke)  
• Few outcome events measured | Lee et al. (2012)                                                                 |
| Patients divided into:  
1. Low CD62E<sup>+</sup> MPs group  
2. High CD62E<sup>+</sup> MPs group | Samples were collected within 24 hr of symptoms onset and stored for 1–2 years (prospective blood banking project) | • PECAM-1: CD31<sup>+</sup>  
• E-selectin: CD62E<sup>+</sup> | • EMP levels were similar in patients with AIS and stroke mimic patients | Strengths:  
• Measuring markers of apoptosis and activation | Williams et al. (2007)                                                                 |
| Acute ischemic stroke (AIS) patients versus mimics | | | | | |
| Type of stroke                                      | Time of sample collection | MP marker                  | Findings                                                                                                                                                                                                                                                                                                                                 | Strengths and weaknesses                                                                                                                                                                                                              | Refs. |
|---------------------------------------------------|---------------------------|-----------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| Hemorrhagic stroke (subarachnoid hemorrhage SAH) versus healthy controls | Within 48 hr of stroke onset | - Endoglin: CD105⁺           | • Increased CD54⁺ (I-CAM), CD62E⁺ (E-selectin), and CD106⁺ (VCAM-1) in SAH compared to controls (markers of endothelial activation)                                                                                                                                                                                                   | • Exploring EMPs in hemorrhagic stroke                                                                                                                                                                                                     | Lackner et al. (2010) |
|                                                   |                           | - VCAM-1: CD106⁺             |                                                                                                                                                                                                                                                                                                                                                                                                  | Weaknesses:                                                                                      |                                                                                                                                                                                                                                           |
|                                                   |                           | - ICAM-1: CD54⁺              |                                                                                                                                                                                                                                                                                                                                                                                                  | Low number of patients (n=20)                                                                                                                                         |                                                                                                                                                                                                                                           |
|                                                   |                           | - E-Selectin: CD62E⁺         |                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                                           |
| Platelet-derived MPs (PMPs)                       | After 48 hr of stroke onset | β3 integrin: CD61⁺           | • Basal levels of PMPs were higher in LAA and SAO subtypes compared to controls (biomarker)                                                                                                                                                                                                                                          | Measuring other platelet parameters along with PMPs.                                                                                                                                                                                     | Chen et al. (2015) |
| Acute ischemic stroke (AIS) compared to controls  | And after 4-weeks of antiplatelet therapy |                           |                                                                                                                                                                                                                                                                                                                                                                                                  | Weaknesses:                                                                                      |                                                                                                                                                                                                                                           |
| AIS patients divided based on TOAST classification into: |                           |                             | • No significant difference in PMP levels between LAA and SAO subtypes                                                                                                                                                                                                                                                             | Using only one marker for detecting PMPs (CD61)                                                                                                                                                                                        |                                                                                                                                                                                                                                           |
| 1. Large artery atherosclerosis subtype (LAA)     |                           |                             |                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                                           |
| 2. Small artery occlusion (SAO)                   |                           |                             |                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                                           |
| Acute ischemic stroke (AIS) patients receiving recombinant tissue plasminogen activator (rt-PA) therapy divided into: | After 24 hr of stroke onset | αIIb chain: CD41⁺           | • CD41⁺ MPs were higher in patients with better recanalization outcomes                                                                                                                                                                                                                                                             | Strengths:                                                                                      | Bivard et al. (2017) |
|                                                   |                           |                             |                                                                                                                                                                                                                                                                                                                                                                                                  | Weaknesses:                                                                                      |                                                                                                                                                                                                                                           |
| Type of stroke                                                                 | Time of sample collection                  | MP marker                                               | Findings                                                                                             | Strengths and weaknesses                                                                 | Refs. |
|-------------------------------------------------------------------------------|--------------------------------------------|---------------------------------------------------------|------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|-------|
| 1. Patients with recanalization                                              |                                            |                                                         | - Total numbers of CD41+ related to reperfusion and recanalization as well as 3 months mRS (assessing clinical outcomes) | - Following-up using mRS to assess outcomes                                                                 |       |
| 2. Patients without recanalization                                           |                                            |                                                         |                                                                                                     | - Single time point measurement (at 24hr) which can be affected by many factors                |       |
| Patients suspected with ischemic stroke versus controls at high risk of cardiovascular disease | Within 48 hr after onset of stroke          | β3 integrin: CD61+/AV⁺                                 | - Higher levels of PMPs in patients at the onset of stroke compared to controls and maintained over 7 and 90 days | - No comparison with precanalization levels                                                   |       |
|                                                                                | And then after 7 and 90 days               | β3 integrin/TF: CD61⁺/CD142⁺/AV⁺                       |                                                                                                     |                                                                                                 |       |
|                                                                                |                                            | P-selectin: CD62P⁺/AV⁺                                 |                                                                                                     |                                                                                                 |       |
| Hemorrhagic stroke (subarachnoid hemorrhage SAH) versus healthy controls     | Within 48 hr of stroke onset               | αIIb chain: CD41⁺                                     | - Mild increase in PMPs compared to controls (biomarker and diagnosis)                               | - Measuring markers of apoptosis and activation                                                |       |
|                                                                                |                                            | αIIb chain/AV⁺: CD41⁺/AV⁺                             |                                                                                                     |                                                                                                 |       |
| Patients with acute phase cerebral infarction versus controls.                | Within 24 hr of stroke onset               | GbIb antibody (ELISA)                                 | - Elevated PMPs in S & L compared to control which means PMPs are higher in acute phase atherosclerotic cerebral infarction than in infarctions of cardiogenic origin | - Exploring EMPs in hemorrhagic stroke                                                              |       |
| Patients divided into:                                                        | After six months for 58 patients with small vessel occlusion and large artery atherosclerosis |                                           | - PMPs were higher in patients with stenotic lesions of the intracranial arteries                     | - Small number of patients (n=20)                                                                |       |
| 1. Small artery occlusion (S)                                                 |                                            |                                                         | - PMPs decreased after 6 months in S & L patients (more in acute than chronic phase)                 |                                                                                                 |       |
| 2. Large artery atherosclerosis (L)                                           |                                            |                                                         | - No direct relation between PMPs and infarct size                                                    |                                                                                                 |       |
| 3. Cardioembolism (CE)                                                        |                                            |                                                         |                                                                                                     |                                                                                                 |       |
| 4. Stroke of undetermined etiology (U)                                        |                                            |                                                         |                                                                                                     |                                                                                                 |       |

(Continues)
EMPs as a marker of endothelial activation and CD31+ (PECAM) EMPs as a marker of apoptosis in both groups. The ratio of CD62E+/CD31+ (E-selectin/PECAM) EMPs was more than four in both groups indicating endothelial injury. However, the study showed no difference in EMPs count between stroke and mimic groups questioning the reliability of using EMPs as a marker to discriminate between them. There were some limitations in this study including the low NIHSS score (small infarct volume) in stroke patients and the long sample storage time that may have affected the number of EMPs resulting in the underestimation of MP counts (Williams et al., 2007). Further studies may be required to confirm these findings and judge the effectiveness of EMPs as biomarkers in distinguishing between acute stroke and its mimics.

In conclusion, EMPs evaluation studies conducted on stroke patients present a number of potential benefits in clinical settings but require further research. EMPs may potentially be used as biomarkers, provide insights about stroke severity and infarct lesion, differentiate between stroke subtypes and identify etiologies thereby, helping clinicians to make more effective therapeutic decisions. The ability to differentiate between stroke and mimics may also be very useful. The main studies reported in this section are summarized in Table 2.

9 | PMPs AND STROKE

PMPs are the most abundant MPs circulating in the blood (Italiano, Mairuhu, & Flaumenhaft, 2010). Collection of PMPs requires careful processing because all platelets may not be successfully depleted from samples before measuring and thus may cause misinterpretation (Burnouf, Goubran, Chou, Devos, & Radosevic, 2014). This may be addressed by adding more centrifugation cycles which can decrease the content of platelets to up to 0.02%. PMPs are measured by many ways including physical methods such as flow cytometry and dynamic light scattering (Lawrie, Albanyan, Cardigan, Mackie, & Harrison, 2009; Shantsila, Montoro-Garcia, Gallego, & Lip, 2014). Other methods include assays that measure PMPs’ procoagulant activity, ELISA assays, and proteomics (Burnouf et al., 2014; Capriotti et al., 2013; Osumi et al., 2001). In addition, cellular assays are conducted in vitro to measure the functional impact of PMPs on cell proliferation, angiogenesis, inflammation, and the release of cytokines (Burnouf et al., 2014). Flow cytometry and ELISA are the most commonly used methods to determine levels of PMPs in clinical trials.

PMPs carry the surface antigens CD41+ (αIIb chain) and CD61+ (β3 integrin) which bind together to form glycoprotein (GP)IIa/IIb (integrin αIIbβ3) Gp, CD 42a+ (GP IX) and CD 42b+ (GP1ba) which bind together to form (GP)Iib-IX-V complex and CD62P+ (P-selectin). Since atherosclerosis and thrombosis of cranial arteries are amongst the main causes of arterial occlusion and cerebral infarction, studies explored the role of platelets and its activation in stroke (Bivard, Linz, Maquire, Parsons, & Levi, 2017; Chen et al., 2015; Chiavar-Blanch et al., 2016; Kuriyama et al., 2010; Lackner et al., 2010).
There are conflicting reports on PMPs following acute stroke or response to treatment. This may be attributed to differences in methods of detection. For instance, studies have shown elevated PMPs levels after an acute ischemic stroke that remain elevated after antiplatelet therapy. Other studies showed a significant decrease in PMPs following the initiation of antiplatelet medications (Chen et al., 2015; Chiva-Blanch et al., 2016; Kuriyama et al., 2010; Shirafuji, Hamaguchi, & Kanda, 2008). Similarly, some studies associated elevated PMPs with the acute phase of cerebral infarction while others linked it to the chronic phase (Kuriyama et al., 2010; Shirafuji et al., 2008). Nevertheless, most of the studies showed that there are higher levels of PMPs post-stroke (ischemic and hemorrhagic) which means that PMPs can be used as biomarkers for diagnosis; however, the benefits of measuring PMPs levels for assessing clinical outcomes after antiplatelet therapy remain to be elucidated (Chen et al., 2015; Chiva-Blanch et al., 2016; Kuriyama et al., 2010; Lackner et al., 2010; Pawelczyk, Baj, Chmielewski, Kaczorowska, & Klimek, 2009; Shirafuji et al., 2008). Measuring PMPs showed a potential benefit in identifying stroke etiology. Studies have shown higher levels of PMPs in patients with large artery arteriosclerosis (LAA) and small artery occlusion when compared to controls, patients with cardioembolic thrombi and other unidentified etiologies (Chen et al., 2015; Kuriyama et al., 2010). In addition, Chiva-Blanch et al. (2016) showed that there are higher CD62P⁺ (P-selectin) PMPs in LAA subtype compared to other stroke etiologies at 90 days but not at the onset of stroke. Furthermore, PMP levels were higher in patients with stenotic lesions of intracranial arteries and thickness of intima-media (Kuriyama et al., 2010). PMPs may be markers of successful treatment following an acute stroke. PMP levels were related to better reperfusion and recanalization outcomes in acute ischemic stroke patients receiving rt-PA making it a potential candidate to assess recanalization in stroke patients (Bivard et al., 2017). However, it was noted that PMP levels were not related to infarct size and only a weak correlation has been observed between PMP levels and infarct volume in LAA subtype (Chen et al., 2015; Kuriyama et al., 2010). Taken together, PMPs appear to be good biomarkers for stroke diagnosis and determining stroke etiology but subsequent measuring for follow-up purposes needs more standardization for better identification of the value of PMPs as biomarkers for stroke recurrence and prognosis. PMPs may also be of potential value in determining reperfusion in patients being treated with rt-PA. The major studies discussed in this section are summarized in Table 2.

10 | CLINICAL UTILITY OF MPs IN STROKE

Since MPs play a pivotal role in the pathophysiology of different diseases, its clinical applications have been widely investigated. One of the approaches is to use MPs to evaluate and monitor therapeutic efficacy (Chen, Li, & Liu, 2018). For example, some drugs such as statins, aspirin and anti-oxidants have been shown to reduce MPs levels in patients and thus can be used to assess treatment effectiveness (Baron, Boulanger, Staels, & Tailleux, 2012; Bulut, Becker, & Mugge, 2011; Morel et al., 2003; Suades, Padro, Alonso, Mata, & Badimon, 2013). Another approach is utilizing MPs characteristics as therapeutic agents. For instance, the ability of some MPs to stimulate the formation of new blood vessels has been shown to induce revascularization and improve mouse kidney function in an ischemia/reperfusion injury model (Ranghino et al., 2012). In addition, as stated earlier, MPs carry genetic material cargo such as DNA, mRNA, and microRNA which can be transferred to distal cells (Hugel et al., 2005). Therefore, MPs have the potential to be used in gene therapy or as nontoxic therapeutic delivery tools (Chen et al., 2018). MPs provide the advantage of being nontoxic and stable in blood; however, the effectiveness of the genetic material or drug delivery to target cells is still to be elucidated (Chen et al., 2018). Furthermore, due to MPs coagulant activity especially of PMPs, their use as prothrombotic agents has been explored to treat bleeding disorders like thrombocytopenia (Blajchman, 2003; Piccin, Murphy, & Smith, 2007). Therefore, various functions of MPs could be utilized to provide therapeutic effects. Also, one of the main promising clinical applications of MPs is their use as biomarkers and diagnostic tools. As mentioned earlier, MPs levels vary with many diseases and therefore could be used for diagnosis (Hugel et al., 2005). Researchers tried to investigate changes in circulating MPs patterns of expression that can be distinctive to certain phenotypes of disease (Berezin, 2016). For example, studies by Berezin et al. have widely investigated the imbalance between MPs released from apoptotic versus activated endothelial cells, to which has been referred to as “impaired phenotype”, to provide predictive scores for heart failure assessment and predicting cardiovascular risks (Berezin, 2016; Berezin, Kremzer, Martovitskaya, Samura, & Berezina, 2015; Berezin, Kremzer, Samura, Berezina, & Kruzliak, 2015). These scores have been shown to provide better predictive values when combined with other markers in heart failure patients (Berezin, Kremzer, Martovitskaya, Berezina, & Samura, 2015). A similar score has also been used for stroke diagnosis where a ratio between CD62E/CD31 has been used rather than absolute numbers to discriminate between endothelial activation and apoptosis and to identify the site of arterial stenosis (Jimenez et al., 2003; Jung et al., 2009). In the context of stroke, the use of MPs as diagnostic tools could provide potential value given challenges in stroke’s diagnosis. Imaging equipments are expensive, not easily available, time-consuming, and require professional interpretation (Saenger & Christenson, 2010). Thus, there is a need for novel stroke biomarkers characterized by early and steady release after infarction, improved sensitivity, and specificity over current biomarkers that could differentiate between stroke types and stroke mimics (Saenger & Christenson, 2010). MPs are being investigated as a promising candidate, but their use is faced by some challenges with regard to sample handling and reliable measurement tools as discussed earlier in this review. Nevertheless, as referred in Figure 2, promising fields include the use of MPs to predict the severity of infarct, site of injury and differentiate between different phenotypes. In addition, MPs can also be used to monitor...
therapeutic efficacy and predict survival rates as shown by Bivard et al. (2017) relating PMPs with better recanalization outcomes. More studies are warranted, however, to confirm the value of MPs in stroke treatment and diagnosis.

11 | CONCLUSION

Normal basal levels of MPs reflect a tightly controlled balance between cell survival, proliferation, and death. Based on the nature of the stimulus that causes their release, this balance is shifted to either direction with changes in the levels and composition of MPs and upon which MPs could be good or bad. Given the complexity of MPs functions and roles, it is still a matter of debate whether they are a cause or a consequence, friends or foes, active participants or passive observers. Nevertheless, MPs acting as remarkable biological sensors are very attractive targets for studying and exploring novel and personalized therapeutic strategies. In this review, we reviewed in more detail the roles of EMPs and PMPs in stroke patients (studies are summarized in Table 2). Despite the challenges, EMPs and PMPs could provide potential benefits as biomarkers, diagnostic tools and prediction of stroke severity (promising markers are illustrated in Figure 2). However, standardization of MPs detection and quantification methods is highly required to further confirm and generalize the results.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

A. A. conceptualized the idea. A. A. and H. E. wrote the manuscript. A. S. P., F. A. M., and A. S. contributed to writing selected sections and critically revised the manuscript. A. A. coordinated the writing up and the submission process. All authors approved the final version for submission.

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