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Review

Biomarkers of cerebral microembolic signals

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ABSTRACT

Stroke is a major cause of mortality and morbidity around the world. Microembolic signals (MES), as the markers of unstable atherosclerotic plaque, can predict the occurrence and prognosis of ischemic stroke (IS). MES can also assess the efficacy of antithrombotic agents and predict the recurrence probability of IS. Unstable plaques are the main source of MES; thus, numerous biomarkers of atherosclerotic plaque instability are highly likely to predict the presence of MES. This study aims to review recent biomarker candidates for MES or microembolism. Current research indicates that the following are independent markers for positive MES: high level of serum soluble P-selectin, chemokine (C-X-C motif) ligand 16 (CXCL16) and fibrinogen, high neutrophil count, reduced ratio of CD4 + $CD25^{high}$ regulatory T cells (Tregs) and the C allele of tumor necrosis factor receptor superfamily member 11B (TNFRSF11B) rs3102735. However, a more integrated profile of biomarkers for MES is needed to improve the stratification of patients with carotid stenosis and enhance the effectiveness of therapeutic interventions and prevention for IS.

1. Introduction

Cerebral embolism is identified as a common cause of cerebrovascular disease. Large emboli in cerebral arteries can lead to ischemic stroke (IS) or transient ischemic attack (TIA). Microemboli with small diameters can result in mild focal ischemia or infarction, as revealed by diffusion weighted imaging [1]. They may also be detected only by transcranial Doppler (TCD) ultrasound monitoring [2–4]. Even smaller microemboli may result in potential damage to neurons and affect the cognitive function [5–7]. Abnormal signals of solid particles detected by TCD ultrasound in patients treated with carotid endarterectomy were initially referred to as microembolic signals (MES) [8]. Clinical evidence shows that the positive rate of MES is significantly higher in symptomatic patients or patients with stenosis ≥ 70%, patients with ulcerations in plaque and patients with hypoechoic plaque structure [9]. These clinical results suggest that MES in cerebral circulation are not only markers of unstable carotid atherosclerotic plaque [10], but also independent predictors of the risk and prognosis of IS [11]. Moreover, cerebral MES predict the recurrence probability of patients with previous IS or TIA [12]. They are also considered as clinical biomarkers to assess the efficacy of antithrombotic agents in the prevention of IS [13,14].

Recent prospective studies indicate that a number of platelet-related molecules, inflammatory cytokines, chemokines, as well as other mediator molecules are identified as biomarkers of plaque destabilization and can predict cerebrovascular events. The atherosclerotic plaque instability is the main source of MES, thus, the biomarkers of plaque destabilization are likely to be associated with the presence of microembolism. In this review, we present an overview of current potential biomarkers of microembolism. Systematic searches of the PubMed database were conducted using the keywords (“microemboli” or “microembolic” or “microembolism” or “MES”) and (“cerebral” or “carotid”).

2. Biomarkers of MES

2.1. Platelet activation markers —P-selectin

Two hypotheses regarding the composition of in vivo microemboli exist. One hypothesis describes microemboli as small particles of debris dislodged from atherosclerotic lesions [15]; the other hypothesis suggests that microemboli mainly consist of newly generated platelet aggregates, resulting from the shear stress and activation of the coagulation cascade triggered by ruptured plaques [16,17]. Ritter et al. found that P-selectin expression on the platelet surface was lower in patients with MES + symptomatic and asymptomatic carotid stenosis, and the surface expression of thrombospondin (THBS1) was lower in patients with in MES + symptomatic carotid stenosis [16]. Both P-selectin and THBS1 are platelet activation markers, which are shed from the surface of activated platelets [18]. Subsequently, the assessment of soluble P-selectin (sSELP) levels in the plasma showed that the sSELP levels were consistently higher in patients with MES + symptomatic and asymptomatic carotid stenosis. These findings suggested that activation of

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platelets is higher in MES + patients than in MES − patients, which supports the hypothesis that platelets are the key blood cellular components involved in cerebral microembolism. This theory can also be demonstrated by the indirect clinical evidence that therapies directed specifically against platelets, such as aspirin [19,20], clopidogrel [13,14] and tirofiban [21], can effectively reduce the number of MES.

2.2. Leukocyte count

Inflammation plays a key role in the initiation, progression and terminal stage of atherosclerosis (AS), plaque destabilization and eventually plaque rupture [22]. Clinically, the leukocyte count is one of the most economical, convenient, and frequently available marker of inflammation, however, its diagnostic and prognostic importance in cerebrovascular events remains unclear. A retrospective observational study in patients who underwent carotid stenting found that a higher pre-stenting leukocyte count was associated with more MES during carotid stenting [23], particularly neutrophil count, which significantly predicted the total MES. An additional study later evaluated the association of neutrophil count with spontaneous microembolism in patients with recently symptomatic carotid stenosis [24]. The results indicated that the neutrophil count can be an independent predictor of spontaneous MES, even after performing adjustments for the National Institutes of Health Stroke Scale (NIHSS) score and potential confounders, including high-sensitivity C-reactive protein (hs-CRP). According to multivariate analysis data, in both studies, no association between hs-CRP and microembolization was found and differences in monocyte and lymphocyte counts between MES + patients and MES − patients were not statistically significant.

Clinical evidence demonstrates that the neutrophil count is related to the presence and severity of carotid AS [25] and is a marker of unstable plaque. Animal experiments also confirm that neutrophils play an important role in the progression of AS [26]. However, it is not clarified whether the elevated neutrophil counts existed before the IS or resulted from the stress and inflammatory response that occur secondary to brain injury in IS. In the aforementioned studies, the significant relevance after adjustment for the NIHSS score and the hs-CRP level suggested that elevated neutrophil counts play a role in the pathogenesis of IS-related to carotid AS rather than act as a mere reflection of the inflammatory process in the arteries. Considering its inexpensive applicability, the neutrophil count could be a promising diagnostic marker of MES.

2.3. Chemokine −C-X-C motif chemokine ligand 16 (CXCL16)

CXCL16, expressed in both soluble and transmembrane forms, is a chemokine that guides the migration of activated Th1 and Tc1 cells. Also called SR-PSOX, it serves as a scavenger receptor for phosphatidylserine and oxidized low-density lipoprotein (ox-LDL), which mediates the internalization of ox-LDL and phosphatidylserine-coated particles [27]. CXCL16 can activate T-cells and promote the secretion of interferon gamma, limphotoxin alpha, colony stimulating factor 2, interleukin 2, which are inflammatory molecules that promote AS [28]. Clinical studies show an increased expression of CXCL16 mRNA and protein in the plaques of coronary and carotid AS [29]. In addition, our previous research revealed a significant elevation of serum CXCL16 level in patients with atherosclerotic IS [30]. Recently, we further investigated the correlation between CXCL16 and MES and found a consistently significant increase in serum CXCL16 concentrations in MES + patients than in MES − patients [31]. In addition, the high level of serum CXCL16 was accompanied by the overexpression of markers of plaque destabilization, such as matrix metalloproteins (MMPs), C−C motif chemokine ligand 2 (CCL2) and vascular cell adhesion molecule 1 [32]. These results indicated that higher levels of CXCL16 could contribute to plaque instability and be a biomarker of MES.

2.4. Other immune inflammation indicators

We previously investigated the correlation between MES and immune inflammation in patients with acute IS by using inflammatory indices, such as the proportions of $CD4^+CD25^{high}$ regulatory T cells (Tregs) and phospholipase A2 group VII (PLA2G7) [33]. The results demonstrated that increased plasma fibrinogen (Fg) level and decreased ratio of $CD4^+CD25^{high}$ Tregs were probably independent risk factors for MES +. A higher PLA2G7 level tended to associate with MES +, but the results failed in multivariate logistic regression analysis.

Tregs are important endogenous immune modulators, which suppress inflammatory responses and maintain immune homeostasis [34]. However, the role of $CD4^+CD25^{high}$ Tregs in IS remains inconclusive. Our results indicate that the decreased ratio of $CD4^+CD25^{high}$ Tregs is an independent risk factor of MES, which means the $CD4^+CD25^{high}$ Tregs play a protective role [35,36]. Animal experiments have also indicated that the Tregs adoptive therapy could be a novel and potent therapy for IS by reducing inflammatory responses, attenuating post-stroke blood-brain barrier disruption and inhibiting MMP-9 [36].

Fg contributes to platelet aggregation and fibrin formation and modulates subsequent coagulation in in plaque development, thus accelerating AS progression [37]. The high Fg level was related to coronary artery disease (CAD) and carotid AS. In addition, it is an independent predictor of the occurrence and extent of CAD [38]. Thus, according to the results described earlier, the high Fg level is also an independent predictor of carotid AS and plaque instability. These findings provide a theoretical basis for the application of defibrinogen therapy for AS.

2.5. Tumor necrosis factor receptor superfamily member 11B (TNFRSF11B) rs3102735

TNFRSF11B is a soluble glycoprotein secreted by vascular endothelial and smooth muscle cells. As a member of the tumor necrosis factor (TNF) receptor superfamily (TNFSF), it acts as a decoy receptor for TNF superfamily member 10 and the TNF superfamily member 11 [39]. Clinical evidence indicates a positive correlation between plasma TNFRSF11B levels and the presence and severity of cerebral AS [40–42]. Genetic studies also showed that the T245G, T950C, and G1181C polymorphisms of the TNFRSF11B gene correlated with a history of IS and carotid plaque instability [43,44]. The possible mechanisms of how TNFRSF11B contributes to the occurrence of IS include the promotion of the infiltration of T cells and dendritic cells in plaque, expression of angiopoietin-2, and endothelial cell adhesion [45]. Our previous study indicated that the TNFRSF11B rs3102735 polymorphism was associated with the occurrence of IS [46]. Subsequently, we explored how the rs3102735 polymorphisms relate to the MES and then evaluated their relationship with the severity of neurologic deficits [47]. The frequency of the CC + CT genotype was significantly higher in the MES + group than in the MES − group. Patients carrying the C allele showed an increased risk for the occurrence of MES than T allele carriers. However, further studies with multiple loci, larger sample size and different ethnic origins are needed to establish a potential role for TNFRSF11B as a genetic marker of microembolism and IS.

3. Summary and conclusion

Microembolism in cerebral circulation is the marker of stenotic atherosclerotic plaque instability and has important diagnostic and prognostic values. The high level of serum sSELP, CXCL16 and Fg, high neutrophil count, decreased ratio of Tregs and the C allele of TNFRSF11B rs3102735 are independent markers of positive MES (Table 1). Many other biomarkers involved in the pathogenesis of AS predict plaque instability, such as interleukin 6 and interleukin 8, ox-LDL, myeloperoxidase and CCL2. However, no direct evidence exists for