Published in final edited form as:

J Stroke Cerebrovasc Dis. 2015 October ; 24(10): 2313–2320. doi:10.1016/j.jstrokecerebrovasdis.2015.06.018.

The Role of Circulating Platelets Microparticles and Platelet Parameters in Acute Ischemic Stroke Patients

Yusen Chen¹, Yun Xiao¹, Zhijun Lin¹, Xiang Xiao², Caixia He¹, Ji C. Bihl^{1,2}, Bin Zhao¹, Xiaotang Ma^{1,#}, and Yanfang Chen^{1,2,#}

¹ Department of Neurology, the Affiliated Hospital of Guangdong Medical College, Zhanjiang, Guangdong, 524001, China

² Department of Pharmacology & Toxicology, Boonshoft School of Medicine, Wright State University, Dayton, OH, 45435, USA

Abstract

Background—Platelet activation and aggregation are critical in the pathogenesis of acute ischemic stroke (AIS). Circulating platelet microparticles (PMPs) and platelet parameters are biological markers of platelet function in AIS patients, however, their associations with stroke subtypes and infarct volume remain unknown.

Methods—We recruited 112 AIS patients including large artery atherosclerosis (LAA) and small artery occlusion (SAO) subtypes, and 35 controls in this study. Blood samples were collected at admission and after antiplatelet therapy. The levels of circulating PMPs and platelet parameters [mean platelet volume (MPV), platelet count (PC), plateletcrit (PCT) and platelet distribution width (PDW)] were determined by flow cytometry and hematology analysis, respectively. Infarct volume was examined at admission by magnetic resonance imaging.

Results—(1) The levels of circulating PMPs and MPV were significantly elevated in AIS patients when compared with healthy controls; (2) The level of circulating PMPs, but not platelet parameters, was decreased after antiplatelet therapy in AIS patients; (3) The infarct volume in LAA subtype was larger than that in SAO subtype. Notably, circulating PMP level was positively correlated with the infarct volume in LAA subtype. No association with infarct volume in either AIS subtype was observed for platelet parameters; (4) According to the regression analysis, circulating PMPs was an independent risk factor for the infarct volume in pooled AIS patients after adjustments of other impact factors (hypertension and diabetes).

#. Corresponding Authors: Yanfang Chen, MD, PhD, Department of Pharmacology & Toxicology, Boonshoft School of Medicine, Wright State University, 3640 Colonel Glenn Hwy, Dayton, OH, 45435, USA, Phone: (937)-775-3265, Fax: (937)-775-7221, yanfang.chen@wright.edu, Xiaotang Ma, PhD, Department of Neurology, the Affiliated Hospital of Guangdong Medical College, Zhanjiang, Guangdong, 524001, China, Phone: 13729046058, mxtgdmc@163.com.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest: There is no conflict of interest.

Ethical Approval: This study was approved by the Ethics Committee of Guangdong Medical College and was in accordance with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all participants for being included in this study. There was no delay in any of the therapeutic interventions in order to carry out the present study.

Conclusions—Our results suggest that circulating PMP level is associated with cerebral injury of AIS, which offers a novel evaluation parameter for AIS patients.

Keywords

Acute ischemic stroke; circulating platelet microparticles; infarct volume; platelet parameters

Introduction

Acute ischemic stroke (AIS) is characterized by the sudden loss of blood circulation to an area of the brain, resulting in irreversible brain injury and subsequent neurologic deficits. It caused by thrombotic or embolic occlusion of a cerebral artery and is the fourth leading cause of death in the United States. Thrombotic AIS occurs when cerebral artery is blocked by blood clots that form within the brain and represents more than 50% of all AIS cases. Whereas, embolic AIS occurs when cerebral artery is blocked by blood clots that form somewhere else in the body and travel to the brain. Increased platelet activation and aggregation are involved in the pathogenesis of AIS, and associated with stroke risk factors such as hypertension and hypercholesterolemia.¹ Platelets are known to play roles in maintaining vascular homeostasis and mediating immune responses, inflammation and atherosclerosis.²⁻⁷ The use of antiplatelet therapies in the management of AIS further emphasizes the pivotal role of platelets in the pathogenesis of AIS. Therefore, assessments of platelet function and bioactivity may be of special importance for monitoring the onset and progression of AIS. Platelet parameters include mean platelet volume (MPV), platelet count (PC), plateletocrit (PCT) and platelet distribution width (PDW). MPV is one of the most commonly studied platelet activation markers, since larger platelets are more aggregable and reactive than the smaller ones.⁸⁻⁹ Previous studies have found that MPV is significantly increased in ischemic stroke patients.^{8,10-12} PDW is used to determine the heterogeneity of platelet size.^{13,14} Platelet activation can cause both morphologic and quantitative changes of platelets. PC measures the number of platelets in the blood, and PCT is a complementary analysis of PC and measures the percentage of platelets in the blood.¹⁵ Platelet parameters are useful in evaluating the function of platelets, however, whether they also hold potential for monitoring the progression of AIS remains unclear.

Microparticles (MPs) are submicron membrane fragments released from stressed, activated or apoptotic cells. Circulating platelet MPs (PMPs) are the most abundant type of MPs found in human circulation and express various platelet surface markers such as CD42, CD61, CD62P.^{16,17} They can carry nuclear and cytoplasmic components from their parent cells, and transfer these information to affect neighbor or distant cells. Therefore, circulating PMPs can be considered as both biological biomarkers and effectors of platelet activation associated with pathological situations.^{18,19} Previous studies have found that the level of circulating PMPs was elevated in patients with various diseases such as hypertension, atherosclerosis, stroke, etc.²⁰⁻²⁴ However, there is no study investigating the level of circulating PMPs, and its correlation with the infarct volume in different AIS subtypes. Whether the level of circulating PMPs or platelet parameters could be used as a surrogate marker for AIS remain unclear. Our objective is to determine the potential roles and

applications of circulating PMPs and platelet parameters in the clinical management of AIS patients.

Methods

Study Subjects

The study recruited 112 AIS patients (65 males and 47 females) from the Department of Neurology at the Affiliated Hospital of Guangdong Medical College in China between 2011 and 2013. All AIS patients were admitted to the hospital within 48 hours of stroke onset. The etiological diagnosis of AIS was confirmed with magnetic resonance imaging (MRI). Medical history recording and blood sample collection were performed at admission. The recruited 112 AIS patients including 53 cases of large artery atherosclerosis (LAA) subtype and 59 cases of small artery occlusion (SAO) subtype, according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria.²⁵ The TOAST criteria have been extensively applied in studying of differences of AIS subtypes with respect to etiology, risk factors, treatment and outcomes. After admission, all patients received antiplatelet therapy (aspirin 100 mg/day plus cilostazol 200 mg/day) for a 4-week course as well as routine managements for controlling blood pressure, blood glucose and plasma lipid levels. A total of 35 healthy controls (20 males and 15 females) were recruited from the Health Examination Center of the Affiliated Hospital of Guangdong Medical College during the same time period. The control subjects completed health investigations including medical histories, blood tests and brain MRI on the examination day. Exclusion criteria of subjects for this study included any of the situations: (1) infectious disease in a previous month; (2) histories of autoimmune disorder, peripheral vascular disease or stroke; (3) transient ischemic attack, cerebral infarction and cerebral hemorrhage; (4) liver and kidney failure; (5) cardiac dysfunction and (6) medications for lipid control, inflammation suppression or immunosuppression.

The study was approved by the ethics committee of Guangdong Medical College and conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from each participant prior to enrollment in the study.

Measurement of Infarct Volume by Brain MRI

Infarct volume was measured by experienced neurologists who were blinded to all clinical and laboratorial results. To avoid a learning effect during the study, the measurements were performed after a training session. MRI was performed by diffusion-weighted imaging using a 1.5-T clinical imaging system (Philips Medical Systems), as previously described.^{26,27} Briefly, the diffusion-weighted imaging was done by using a multislice, single-shot and spin-echo echo planar sequence. Typical sequence parameters were echo time of 118 msec, matrix size of 128×128 , field view of 260×260 and 7 mm slice thickness, with no gap between slices and with a set of 20 axial slices covering the whole brain. The infarct volume was calculated by the sum of slice thickness times total infarct area. The magnetic resonance diffusion sequence at $b = 1000$ was run 3 times, with diffusion gradients applied in each of the x, y, and z planes.

Flow Cytometric Analysis of Circulating PMPs

Peripheral blood (2 mL) was collected from patients at admission and after antiplatelet therapy, or from healthy controls on the examination day. The level of circulating PMPs was determined by flow cytometry as previously described, with minor modifications.^{28,29} In brief, blood sample (anticoagulated with 3.8% sodium citrate) was centrifuged at 800×g for 30 minutes to get rid of red blood cells and obtain platelet-rich plasma (PRP). The PRP was subjected to centrifugation at 1,500×g for 15 minutes to remove cell debris and obtain platelet-poor plasma (PPP). The PPP was centrifuged at 35,000×g for 15 minutes to pellet MPs. The MP pellet was resuspended in 100 µl phosphate buffered saline and fixed with 1% paraformaldehyde, and then stained with 10 µl fluorescein isothiocyanate-conjugated anti-human CD61 (eBioscience, San Diego, CA) antibody for 30 minutes at room temperature in the dark. Isotype matched nonspecific antibody (eBioscience) served as negative control. Circulating PMPs were analyzed by for flow cytometric analysis (BD FACSCanto II flow cytometer, BD Biosciences, San Jose, CA). The flow cytometer was set to acquire 100,000 events/sample.

Determination of Platelet Parameters

Peripheral blood (2 mL) was collected from patients at admission and after antiplatelet therapy, or from healthy controls on the examination day. The blood sample was anticoagulated with ethylenediaminetetraacetic (EDTA; Wego, Shandong, China). The platelet parameters (MPV, PC, PCT and PDW) were determined using automated hematology analyzer (LXP50; Beckman Coulter, CA, USA).

Statistical Analysis

All data were expressed as mean ± standard error of the mean (SEM). Comparisons between two groups were determined by Student's t-test. Multiple comparisons were determined by one- or two-way analysis of variance (ANOVA). Comparisons for categorical characteristics were determined by Chi-square test. Correlation between two variables was analyzed using the Spearman's rank correlation test. Multivariate logistic regression analysis was used to determine the predictive factors of infarct volume in pooled AIS patients. SPSS software version 17.0 (SPSS software, IBM, Armonk, NY) was used. For all tests, $P < 0.05$ was considered statistically significant.

Results

General Characteristics of Normal Controls and AIS Patients

The general characteristics of all study subjects were summarized in Table 1. The cases of hypertension and diabetes were higher in both LAA and SAO subtypes ($P < 0.05$), when compared with healthy controls. There were no significant differences in age, sex, smoking and alcohol consumption, cholesterol, triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and homocysteine (HCY) among all experimental groups ($P > 0.05$).

The Levels of Circulating PMPs were Similarly Increased in Both LAA and SAO Subtypes, and Decreased after Antiplatelet Therapy

The basal level of circulating PMPs was higher in LAA subtype than normal controls [$(5.00 \pm 0.42) \times 10^9/\text{L}$ and $(3.17 \pm 0.20) \times 10^9/\text{L}$, LAA versus control, $P < 0.05$; Figure 1A], as well as higher in SAO subtype than normal controls [$(5.81 \pm 0.43) \times 10^9/\text{L}$ and $(3.17 \pm 0.20) \times 10^9/\text{L}$, SAO versus control, $P < 0.05$; Figure 1A]. However, there was no significant difference in the level of circulating PMPs between LAA subtype and SAO subtype [$(5.00 \pm 0.42) \times 10^9/\text{L}$ and $(5.81 \pm 0.43) \times 10^9/\text{L}$, LAA versus SAO, $P > 0.05$; Figure 1A]. After antiplatelet therapy (4-week course), the levels of circulating PMPs were decreased in both LAA and SAO subtypes [$(5.00 \pm 0.42) \times 10^9/\text{L}$ and $(4.06 \pm 0.34) \times 10^9/\text{L}$, before versus after treatment in LAA subtype, $P < 0.05$; $(5.81 \pm 0.43) \times 10^9/\text{L}$ and $(4.55 \pm 0.56) \times 10^9/\text{L}$, before versus after treatment in SAO subtype, $P < 0.05$; Figure 1B].

MPV was Similarly Increased in Both LAA and SAO subtypes

MPV was remarkably higher in both LAA and SAO subtypes when compared with normal controls (8.33 ± 0.11 fL and 6.34 ± 0.09 fL, LAA versus control, $P < 0.05$; 8.28 ± 0.10 fL and 6.34 ± 0.09 fL, SAO versus control, $P < 0.05$; Figure 2A). However, there was no difference in MPV between LAA and SAO subtypes (8.33 ± 0.11 fL and 8.28 ± 0.10 fL, LAA versus SAO, $P > 0.05$; Figure 2A). No significant alternations were found in other platelet parameters (e.g. PC, PCT and PDW) among all experimental groups ($P > 0.05$; Figure 2B, C and D). In addition, the levels of platelet parameters didn't change significantly before and after antiplatelet therapy (data not shown).

Infarct Volume Positively Correlated with Circulating PMP Level, But Not with Platelet Parameters, in AIS Patients

The infarct volume was remarkably higher in LAA subtype than SAO subtype (19.65 ± 3.10 cm³ and 7.40 ± 0.81 cm³, LAA versus SAO, $P < 0.05$; Figure 3A). Moreover, the level of circulating PMPs in LAA subtype was positively correlated with infarct volume ($r=0.34$, $P < 0.05$; Figure 3B). However, there was a relatively weak correlation between the level of circulating PMPs and infarct volume in SAO subtype ($r=0.27$, $P < 0.05$; Figure 3C). No correlations of infarct volume with MPV, PC, PCT and PDW were found in either LAA subtype or SAO subtype ($P > 0.05$; Figure 3D-K).

Multivariate Logistic Regression Analysis of Risk Factors for Infarct Volume in AIS Patients

The regression analysis of impact factors for infarct volume was summarized in Table 2. We found that hypertension, diabetes and circulating PMPs were risk factors associated with infarct volume in pooled AIS patients. More importantly, after adjustments of the traditional risk factors (e.g. hypertension and diabetes), we found that the level of circulating PMPs was associated with the infarct volume in AIS (OR=1.83; 95% CI=1.27-2.64; $P=0.001$). However, no association of platelet parameters with infarct volume was found ($P > 0.05$).

Discussion

There are several findings in this study. First, we demonstrated that the levels of circulating PMPs and MPV were higher in AIS patients than normal controls, however, only the level of circulating PMPs was decreased after antiplatelet therapy. Second, the infarct volume was larger in LAA subtype and associated with the elevation of circulating PMPs. Third, the level of circulating PMPs was an independent risk factor for infarct volume of AIS.

Circulating PMPs are membrane vesicles released from platelets during activation or apoptosis. They carry proteins and genetic materials from platelets, and can transfer these information to distant cells.³⁰ Therefore, circulating PMPs could be thought as novel markers for evaluating in vivo platelet function. In addition, circulating PMPs can initiate the coagulation cascade, promote leukocyte and endothelial cell adhesion and stimulate cytokine secretion in endothelial cells,^{31,32} thereby increasing the risks of atherosclerosis and ischemic stroke. In our study, we found that the level of circulating PMPs was elevated in AIS patients when compared with healthy controls. Our finding is similar to previous evidence suggesting that circulating PMP levels were increased in both acute and chronic phases of ischemic stroke.^{33,34} Additionally, we found that the level of circulating PMPs was decreased by antiplatelet therapy. This suggests that circulating PMPs are useful in evaluating the drug response and progression of AIS. Besides the circulating PMPs, platelet parameters may serve as combinational markers for evaluating the progression of AIS. Previous studies have reported that the change of platelet count or size is an early stage of platelet activation and aggregation.^{8,35} In our study, increased MPV was found in AIS patients. This suggests that increased platelet activation and aggregation may be implicated in higher tendency of prothrombotic and atherothrombotic events.^{36,37} However, no significant differences of PC, PCT and PDW were found between AIS patients and normal controls. This suggests that MPV is a more sensitive and homeostatically-maintained marker than other platelet parameters in the cases of AIS.

The infarct volume was higher in LAA subtype than that in SAO subtype. Infarct volume may depend on various factors such as age, region and the severity of ischemia, the status of collateral vessels and collateral blood flow, etc.^{42,43} This may suggest that LAA subtype has more complex pathophysiology than SAO subtype.^{37,44} Since circulating PMPs possess higher procoagulant properties than activated platelets⁴⁵ and can contribute to thrombin generation and clot formation.⁴⁶ Therefore, elevated level of circulating PMPs may involve in the progression of AIS and associate with cerebral injury in AIS. In our study, we found the level of circulating PMPs positively correlated with infarct volume in respective AIS subtypes, more evidently in LAA subtype. According to previous reports,⁴⁷⁻⁴⁹ infarct volume was inversely associated with favorable clinical outcomes in patients with ischemic stroke. Patients who achieved successful endovascular recanalization of occlusion (Thrombolysis in Cerebral Infarction 2b/3) and clinical recovery of neurologic function (modified Rankin Scale<2) had significantly lower infarct volume. Conversely, larger infarct volume was associated with higher incidence of comorbidities and lower capacity of neurorestoration. Overall, elevated level of circulating PMPs may associate with larger cerebral injury and worse clinical outcomes.

A previous study has found that the level of circulating PMPs was significantly elevated in thrombotic rather than cardioembolic AIS.⁵⁰ In this study, we firstly demonstrated that the level of circulating PMPs was increased in two subtypes of thrombotic AIS. This is in agreement with previous reports suggesting that elevated circulating PMP level could serve as a potential biomarker for thrombotic AIS.^{42,43,50} In order to further confirm the association of circulating PMPs with cerebral injury in AIS, we performed the logistic regression analysis of risk factors for infarct volume in AIS, and found that hypertension, diabetes and circulating PMPs were main impact factors for infarct volume in pooled AIS patients. However, after clinical managements of hypertension and diabetes in AIS patients, the level of circulating PMPs was significantly associated with the infarct volume. This suggests that the level of circulating PMPs could be an independent risk factor for infarct volume in AIS. It is well-known that blood vessel lesion, hemodynamic and blood component changes associate with the onset and progression of AIS. In consistence with previous reports,³⁸⁻⁴¹ we found that the cases of hypertension and diabetes were significantly high in both subtypes of AIS than control subjects, we believe that co-morbidities of hypertension and/or diabetes may contribute to elevation of circulating PMPs in AIS patients. However, when we further compared the association of circulating PMP level with infarct volume between LAA subtype and SAO subtype, we found that elevated level of circulating PMPs was evidently associated with larger infarct volume found in LAA subtype. Since hypertension, diabetes or other co-morbidities were similarly distributed in LAA subtype and SAO subtype, their influence on circulating PMP levels should be similar in both AIS subtypes.

There are some limitations need to be addressed in our study. First, NIHSS and modified Rankin Scale were not evaluated in this study, therefore, the role of circulating PMPs in predicting the clinical outcome or severity of AIS remains undetermined. Second, the nature history and kinetic profile over time of the infarct volume in both AIS subtypes are needed to be determined in our future study. Third, our study has not included all ischemic stroke subtypes. Further studies with larger samples and all AIS etiologic types would better elucidate the clinical significances of circulating PMPs. We found that the level of circulating PMPs, but not platelet parameters, was decreased after antiplatelet therapy.

Conclusion

In conclusion, our study suggests that the level of circulating PMPs could be used to evaluate cerebral injury and drug response in AIS patients, therefore it may hold clinical implications for AIS.

Acknowledgements

This work was supported by China Natural Science Foundation (NSFC, #81270195) and Guangdong Provincial Natural Science Foundation (S2011010004879, 2011B031800249) and the National Institute of Health (NIH, HL098637 to YC).

References

1. Jung KW, Shon YM, Yang DW, et al. Coexisting carotid atherosclerosis in patients with intracranial small- or large-vessel disease. *J Clin Neurol*. 2012; 8:104–108. [PubMed: 22787493]

2. Machlus KR, Italiano JE Jr. The incredible journey: From megakaryocyte development to platelet formation. *J Cell Biol.* 2013; 201(6):785–796. [PubMed: 23751492]
3. Aukrust P, Halvorsen B, Ueland T, et al. Activated platelets and atherosclerosis. *Expert Rev Cardiovasc Ther.* 2010; 8:1297–1307. [PubMed: 20828352]
4. Ishii H, Yoshida M. Platelets, coagulation, and fibrinolysis in atherosclerosis formation. *Nihon Rinsho.* 2011; 69:50–54. [PubMed: 21226260]
5. Smyth SS, McEver RP, Weyrich AS, et al. Platelet functions beyond hemostasis. *J Thromb Haemost.* 2009; 7:1759–1766. [PubMed: 19691483]
6. Patzelt J, Langer HF. Platelets in angiogenesis. *Curr Vasc Pharmacol.* 2012; 10:570–577. [PubMed: 22338572]
7. Sachs UJ, Nieswandt B. In vivo thrombus formation in murine models. *Circ Res.* 2007; 100:979–991. [PubMed: 17431199]
8. Arevalo-Lorido JC, Carretero-Gomez J, Alvarez-Oliva A, et al. Mean platelet volume in acute phase of ischemic stroke, as predictor of mortality and functional outcome after 1 year. *J Stroke Cerebrovasc Dis.* 2013; 22:297–303. [PubMed: 22005035]
9. Vagdatli E, Gounari E, Lazaridou E, et al. Platelet distribution width: a simple, practical and specific marker of activation of coagulation. *Hippokratia.* 2010; 14:28–32. [PubMed: 20411056]
10. Yilmaz MB, Cihan G, Guray Y, et al. Role of mean platelet volume in triaging acute coronary syndromes. *J Thromb Thrombolysis.* 2008; 26:49–54. [PubMed: 17705053]
11. Demirtunc R, Duman D, Basar M, et al. The relationship between glycemic control and platelet activity in type 2 diabetes mellitus. *J Diabetes Complications.* 2009; 23:89–94. [PubMed: 18358749]
12. O'Malley T, Langhorne P, Elton RA, et al. Platelet size in stroke patients. *Stroke.* 1995; 26:995–999. [PubMed: 7762052]
13. Adibi P, Faghih IE, Talaei M, et al. Population-based platelet reference values for an Iranian population. *Int J Lab Hematol.* 2007; 29:195–199. [PubMed: 17474897]
14. Osselaer JC, Jamart J, Scheiff JM. Platelet distribution width for differential diagnosis of thrombocytosis. *Clin Chem.* 1997; 43:1072–1076. [PubMed: 9191563]
15. Akpınar I, Sayin MR, Gursoy YC, et al. Plateletcrit : A platelet marker associated with saphenous vein graft disease. *Herz.* 2014; 39:142–148. [PubMed: 23575980]
16. Zahra S, Anderson JA, Stirling D, et al. Microparticles, malignancy and thrombosis. *Br J Haematol.* 2011; 152:688–700. [PubMed: 21303355]
17. Barteneva NS, Fasler-Kan E, Bernimoulin M, et al. Circulating microparticles: square the circle. *BMC Cell Biol.* 2013; 14:23. [PubMed: 23607880]
18. Morel O, Toti F, Hugel B, et al. Cellular microparticles: a disseminated storage pool of bioactive vascular effectors. *Curr Opin Hematol.* 2004; 11:156–164. [PubMed: 15257014]
19. Stepien E, Kablak-Ziembicka A, Czyz J, et al. Microparticles, not only markers but also a therapeutic target in the early stage of diabetic retinopathy and vascular aging. *Expert Opin Ther Targets.* 2012; 16:677–688. [PubMed: 22616736]
20. Nadaud S, Poirier O, Girerd B, et al. Small platelet microparticle levels are increased in pulmonary arterial hypertension. *Eur J Clin Invest.* 2013; 43:64–71. [PubMed: 23176388]
21. Lukasik M, Rozalski M, Luzak B, et al. Enhanced platelet-derived microparticle formation is associated with carotid atherosclerosis in convalescent stroke patients. *Platelets.* 2013; 24:63–70. [PubMed: 22372532]
22. Michelsen AE, Brodin E, Brosstad F, et al. Increased level of platelet microparticles in survivors of myocardial infarction. *Scand J Clin Lab Invest.* 2008; 68:386–392. [PubMed: 18752144]
23. Kuriyama N, Nagakane Y, Hosomi A, et al. Evaluation of factors associated with elevated levels of platelet-derived microparticles in the acute phase of cerebral infarction. *Clin Appl Thromb Hemost.* 2010; 16:26–32. [PubMed: 19671561]
24. Shirafuji T, Hamaguchi H, Higuchi M, et al. Measurement of platelet-derived microparticle levels using an enzyme-linked immunosorbent assay in polymyositis and dermatomyositis patients. *Muscle Nerve.* 2009; 39:586–590. [PubMed: 19347930]

25. Adams HP Jr, Bendixen BH, Kappelle LJ, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke*. 1993; 24:35–41. [PubMed: 7678184]
26. Lovblad KO, Baird AE, Schlaug G, et al. Ischemic lesion volumes in acute stroke by diffusion-weighted magnetic resonance imaging correlate with clinical outcome. *Ann Neurol*. 1997; 42:164–170. [PubMed: 9266725]
27. van Everdingen KJ, van der Grond J, Kappelle LJ, et al. Diffusion-weighted magnetic resonance imaging in acute stroke. *Stroke*. 1998; 29:1783–1790. [PubMed: 9731595]
28. Orozco AF, Lewis DE. Flow cytometric analysis of circulating microparticles in plasma. *Cytometry A*. 2010; 77:502–514. [PubMed: 20235276]
29. Chen J, Chen S, Chen Y, et al. Circulating endothelial progenitor cells and cellular membrane microparticles in db/db diabetic mouse: possible implications in cerebral ischemic damage. *Am J Physiol Endocrinol Metab*. 2011; 301:E62–E71. [PubMed: 21505143]
30. Mause SF, Weber C. Microparticles: protagonists of a novel communication network for intercellular information exchange. *Circ Res*. 2010; 107:1047–1057. [PubMed: 21030722]
31. Nomura S. Function and clinical significance of platelet-derived microparticles. *Int J Hematol*. 2001; 74:397–404. [PubMed: 11794694]
32. Nomura S, Tandon NN, Nakamura T, et al. High-shear-stress-induced activation of platelets and microparticles enhances expression of cell adhesion molecules in THP-1 and endothelial cells. *Atherosclerosis*. 2001; 158:277–287. [PubMed: 11583705]
33. Cherian P, Hankey GJ, Eikelboom JW, et al. Endothelial and platelet activation in acute ischemic stroke and its etiological subtypes. *Stroke*. 2003; 34:2132–2137. [PubMed: 12907813]
34. Lee YJ, Jy W, Horstman LL, et al. Elevated platelet microparticles in transient ischemic attacks, lacunar infarcts, and multiinfarct dementias. *Thromb Res*. 1993; 72:295–304. [PubMed: 8303669]
35. Marquardt L, Ruf A, Mansmann U, et al. Course of platelet activation markers after ischemic stroke. *Stroke*. 2002; 33:2570–2574. [PubMed: 12411644]
36. Greisenegger S, Endler G, Hsieh K, et al. Is elevated mean platelet volume associated with a worse outcome in patients with acute ischemic cerebrovascular events? *Stroke*. 2004; 35:1688–1691. [PubMed: 15143290]
37. Bath P, Algert C, Chapman N, et al. Association of mean platelet volume with risk of stroke among 3134 individuals with history of cerebrovascular disease. *Stroke*. 2004; 35:622–626. [PubMed: 14976328]
38. Jain AR, Bellolio MF, Stead LG. Treatment of hypertension in acute ischemic stroke. *Curr Treat Options Neurol*. 2009; 11:120–125. [PubMed: 19210914]
39. Boden-Albala B, Cammack S, Chong J, et al. Diabetes, fasting glucose levels, and risk of ischemic stroke and vascular events: findings from the Northern Manhattan Study (NOMAS). *Diabetes Care*. 2008; 31:1132–1137. [PubMed: 18339972]
40. Rashid P, Leonardi-Bee J, Bath P. Blood pressure reduction and secondary prevention of stroke and other vascular events: a systematic review. *Stroke*. 2003; 34:2741–2748. [PubMed: 14576382]
41. Zhang C, Zhou YH, Xu CL, et al. Efficacy of intensive control of glucose in stroke prevention: a meta-analysis of data from 59,197 participants in 9 randomized controlled trials. *PLoS One*. 2013; 8:e54465. [PubMed: 23372729]
42. Cherian P, Hankey GJ, Eikelboom JW, et al. Endothelial and platelet activation in acute ischemic stroke and its etiological subtypes. *Stroke*. 2003; 34:2132–2137. [PubMed: 12907813]
43. Lee YJ, Jy W, Horstman LL, et al. Elevated platelet microparticles in transient ischemic attacks, lacunar infarcts, and multiinfarct dementias. *Thromb Res*. 1993; 72:295–304. [PubMed: 8303669]
44. Zeng L, He X, Liu J, et al. Differences of circulating inflammatory markers between large- and small vessel disease in patients with acute ischemic stroke. *Int J Med Sci*. 2013; 10:1399–1405. [PubMed: 23983602]
45. Sinauridze EI, Kireev DA, Popenko NY, et al. Platelet microparticle membranes have 50- to 100-fold higher specific procoagulant activity than activated platelets. *Thromb Haemost*. 2007; 97:425–434. [PubMed: 17334510]

46. Aleman MM, Gardiner C, Harrison P, et al. Differential contributions of monocyte- and platelet-derived microparticles towards thrombin generation and fibrin formation and stability. *J Thromb Haemost.* 2011; 9:2251–2261. [PubMed: 21883880]
47. Ribo M, Flores A, Mansilla E, et al. Age-adjusted infarct volume threshold for good outcome after endovascular treatment. *Neurointerv Surg.* 2014; 6:418–422.
48. Zaidi SF, Aghaebrahim A, Urrea X, et al. Final infarct volume is a stronger predictor of outcome than recanalization in patients with proximal middle cerebral artery occlusion treated with endovascular therapy. *Stroke.* 2012; 43:3238–3244. [PubMed: 23160876]
49. Yoo AJ, Chaudhry ZA, Nogueira RG, et al. Infarct volume is a pivotal biomarker after intra-arterial stroke therapy. *Stroke.* 2012; 43:1323–1330. [PubMed: 22426317]
50. Shirafuji T, Hamaguchi H, Kanda F. Measurement of platelet-derived microparticle levels in the chronic phase of cerebral infarction using an enzyme-linked immunosorbent assay. *Kobe J Med Sci.* 2008; 54:E55–E61. [PubMed: 18772609]

Figure 1.

The levels of circulating PMPs in normal controls and two AIS subgroups, or after anti-platelet therapy. (A) The levels of circulating PMPs were similarly increased in both LAA and SAO groups compared with normal controls. (B) The levels of circulating PMPs were decreased after antiplatelet treatment in both AIS subgroups. Data were expressed as mean $\pm$ SEM. * $P < 0.05$, versus control. # $P < 0.05$, versus before antiplatelet treatment.

Abbreviations: PMPs, platelet microparticles; AIS, acute ischemic stroke; LAA, large artery atherosclerosis; SAO, small artery occlusion.

Figure 2.

Analyses of MPV, PC, PCT and PDW in normal controls and two AIS subgroups. (A) MPV was similarly higher in both LAA and SAO groups than that in normal controls. (B-D) There were no significant differences in PC, PCT and PDW among all experimental groups. Data were expressed as mean $\pm$ SEM. *P < 0.05, versus control. Abbreviations: AIS, acute ischemic stroke; MPV, mean platelet volume; fL, femtolitre; PC, platelet count; PCT, plateletcrit; PDW, platelet distribution width; LAA, large artery atherosclerosis; SAO, small artery occlusion.

Figure 3.

Infarct volume positively correlated with the level of circulating PMPs but not platelet parameters in AIS Patients. (A) Infarct volume was remarkably higher in LAA group than in SAO group. Data were expressed as mean $\pm$ SEM. *P < 0.05, versus LAA group. (B, C) The correlation of infarct volume with the level of circulating PMPs in LAA and SAO group, respectively. (D-G) The correlations of infarct volume with MPV, PC, PCT and PDW in LAA group. (H-K) The correlations of infarct volume with MPV, PC, PCT and PDW in SAO group. Bold type denotes a significant result (P < 0.05). Abbreviations: PMPs, platelet microparticles; MPV, mean platelet volume; fL, femtolitre; PC, platelet count; PCT, plateletcrit; PDW, platelet distribution width; LAA, large artery atherosclerosis; SAO, small artery occlusion.

Table 1

General characteristics in normal controls and AIS patients

	Control (n=35)	LAA (n=53)	SAO (n=59)
Age (years)	65.71 ± 1.22	67.70 ± 1.42	67.95 ± 1.26
Gender, % male (n)	57.14 (20)	60.38 (32)	55.93 (33)
Gender, % female (n)	42.86 (15)	39.62 (21)	44.07 (26)
Smoking, % (n)	20.20 (7)	26.42 (14)	13.56 (8)
Alcohol consumption, % (n)	5.71 (2)	1.59 (1)	8.47 (5)
Hypertension, % (n)	34.29 (12)	81.13 (43) *	89.83 (53) *
Diabetes, % (n)	2.86 (1)	24.53 (13) *	23.73 (14) *
Cholesterol (mmol/L)	4.99 ± 0.18	5.00 ± 0.13	5.22 ± 0.16
TG (mmol/L)	1.47 ± 0.11	1.78 ± 0.17	1.53 ± 0.09
HDL (mmol/L)	1.25 ± 0.05	1.19 ± 0.04	1.29 ± 0.05
LDL (mmol/L)	3.09 ± 0.14	3.06 ± 0.12	3.29 ± 0.12
HCY (mmol/L)	11.30 ± 0.50	11.36 ± 0.45	12.11 ± 0.41

Abbreviations: AIS, acute ischemic stroke; LAA, large artery atherosclerosis; SAO, small artery occlusion; TG, triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein; HCY, homocysteine. Results were expressed as mean ± SEM or percentage.

$P < 0.05$, versus LAA.

* $P < 0.05$, versus control,

Table 2

Multivariate logistic regression of risk factors for infarct volume in AIS

	Infarct volume		
	OR	95% CI	p value
Age (years)	0.702	0.243 - 2.028	0.513
Gender, % male (n)	1.006	0.926 - 1.169	0.989
Gender, % female (n)	1.786	0.658 - 2.364	1.075
History of smoking, % (n)	1.688	0.374 - 7.629	0.496
History of alcohol consumption, % (n)	0.344	0.026 - 4.615	0.420
History of hypertension, % (n)	16.173	5.124 - 51.051	0.000
History of diabetes, % (n)	15.540	1.178 - 205.006	0.037
Cholesterol (mmol/L)	1.713	0.086 - 34.300	0.725
TG (mmol/L)	1.408	0.420 - 4.715	0.579
HDL (mmol/L)	1.257	0.042 - 39.952	0.895
LDL (mmol/L)	0.434	0.019 - 9.998	0.602
HCY (mmol/L)	1.131	0.917 - 1.397	0.250
PMPs ($\times 10^9/L$)	1.829	1.267 - 2.639	0.001
MPV (fL)	1.328	0.225 - 6.794	0.097
PC ($\times 10^9/L$)	0.680	0.063 - 9.981	0.350
PCT (%)	0.931	0.890 - 5.359	0.281
PDW (%)	1.507	1.924 - 8.451	0.076

Abbreviations: AIS, acute ischemic stroke; OR, odd ratio; CI, confidence interval; TG, triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein; HCY, homocysteine; fL, femtolitre; PMPs, platelet microparticles. Bold type denotes $P < 0.05$.