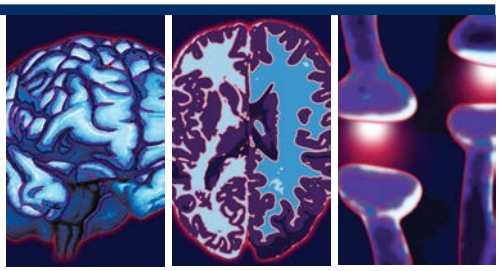

Link between Systemic and Cerebral Circulatory Levels of Microparticles and Necrosis Area of Stenotic Carotid Artery in the Patients Undergoing Carotid Stenting

Pei-Hsun Sung[1], Meng-Shen Tong[1], Chi-Hsiang Chu[2], Fan-Yan Lee[3], Hon-Kan Yip[1,4,5,6,†]

ABSTRACT

Objective: We tested the hypothesis that (1) platelet-derived activated microparticles (PDAc-MPs) and endothelial-derived activated (EDAc)-MPs were significantly higher after carotid stenting (CS) than before CS in carotid artery (CA) (defined as systemic circulation) and internal jugular vein (IJV) (defined as cerebral circulation) and (2) significantly correlated with necrotic area in CA stenosis measured by virtual histology intra-vascular ultrasound (VH-IVUS) prior to CS.

Methods: From September 2013 to 2016 July, a total of 92 consecutive patients undergoing CS were prospectively enrolled into the study. Blood samples for level of MPs were collected from CA and IJV prior to and 5-mintes after CS, respectively.

Results: The frequency of previous ischemic stroke (IS) was 45.7% (42/92). The mean stenosis prior to CS was 88.8%. The procedural success was 100% and incidences of TIA and acute IS were 3.3% (3/92) and 4.3% (4/92), respectively. No significant correlation existed between severity of CA stenosis and circulating MPs level ($p>0.5$). The PDAc-MPs was significantly higher in post-CS procedure than in pre-CS procedure in both RIJV and CA (all $p<0.007$), whereas the EDAc-MPs was significantly higher in post-CS procedure than in pre-CS procedure in CA ($p=0.003$). Post-CS PDAc-MPs and EDAc-MPs as well as ratio of post- to pre-CS PDAc-MPs were moderately correlated to necrotic area of carotid plagues (all $p<0.005$).

Conclusion: Cerebral and systemic PDAc-MPs and systemic EDAc-MPs were markedly increased in post CS than in that of prior to CS. Post-CS PDAc-MPs/EDAc-MPs and ratio of post/pre-CS PDAc-MPs were notably correlated to necrotic area of stenotic carotid plaque.

Keywords

Platelet-derived activated microparticles, Endothelial-derived activated microparticles, Carotid stenting, Necrotic area, Right internal jugular vein

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Introduction

Abundant data have shown that carotid revascularization is effective in reducing risk of ischemic stroke (IS) for patients with high-grade carotid artery (CA) stenosis [1-6]. Surgical carotid endarterectomy (CEA) is the first established gold standard treatment for symptomatic CA stenosis [1-4,7,8]. On the other hand, carotid artery stenting (CS) has emerged as a global consensus to be an alternative option for those patients who are at high risk for CEA [5,6,9-11]. However, perioperative neurological complications inevitably occurred in both interventional procedures [4-6,9-12]. Some clinical observational studies have shown that the rate of periprocedural stroke is notably higher in CS than in CEA [6,12,13]. Numerous factors, such as plaque morphology, age, technical expertise and protection devices, have been identified to be related to periprocedural IS [14-18]. Of these factors, plaque characteristics/component identified by virtual histology intravascular ultrasound (VH-IVUS) [19-21] has been established as one of the paramount factors for risk of periprocedural IS [20,22]. However, although to identify the plaque characteristics is one of better strategy for providing useful information for assessing the risk during CS, the CS procedure remains very invasive. Accordingly, to find an alternative method with less invasive and easily performed manner is eager for physicians and patients.

Microparticles (MPs), membrane-bound vesicles, are small fragments of the plasma membrane (i.e., ranging in size from 0.1 μm to 1.0 μm). Actually, MPs which are released into extracellular space or circulation by direct budding from the plasma membrane are multiple lineages of activated and/or apoptotic cells, including platelets, endothelial cells, granulocytes, monocytes, macrophages, and lymphocytes can mediate inflammation and thrombosis [23-29]. Additionally, circulating MPs has recently emerged as a promising biomarker for predicting prognostic outcome of some disease entities [30-32]. Our previous studies have also shown that circulating platelet-derived activated MPs (PDAc-MPs) and endothelial-derived activated MPs (EDAc-MPs) were not only strongly associated with chronic kidney disease [33] and angiogenesis [34,35] but also significantly predictive of prognostic outcome in lung cancer patients [36-38]. Furthermore, leukocyte-derived MPs has been reported to constitute a promising biomarker associated with plaque vulnerability in patients with high-grade CA stenosis [39]. These aforementioned issues raise [23-39] the hypothesis that circulating MPs may be a useful biomarker with easily obtain by minimal invasive procedure (i.e., to collect the blood sample by need puncture) for an indirect assessment of carotid-plaque characteristics and the possible risk of embolic IS after CS. However, a correlation between the necrotic area of plaque (i.e., an indicator of vulnerable plaque for predictive of embolic event during the procedure) and circulating levels of PDAc-MPs and EDAc-MPs has to be confirmed prior to utilize these MPs in our daily clinical practice. Accordingly, this study assessed the necrotic area of carotid plaque by VH-IVUS and circulating levels of PDAc-MPs and EDAc-MPs during the CS procedure.

Materials and Methods

■ Study design

This clinical study was approved by the Institutional Review Committee on Human Research in Chang Gung Memorial Hospital (104-5094C) in 2013 and conducted at Kaohsiung Chang Gung Memorial Hospital. Informed consent was obtained from all study subjects.

■ Patient population, inclusion and exclusion criteria

Enrollment and exclusion criteria were based on our previous reports [40-42] with some modification, included the following: 1) in symptomatic patients, extra-cranial carotid artery (ECCA) stenosis (i.e., defined as internal or common carotid artery stenosis ≥ 60%) determined by magnetic resonance imaging (MRI)/MR angiography (MRA) and duplex ultrasound of carotid arteries prior to extra-cranial and intra-cranial angiographic studies; 2) history of stroke (> 2 months), transient ischemic attack or dizziness related to a significant ECCA stenosis; 3) In asymptomatic patients, ECCA stenosis was identified to be > 70% by MRA/duplex ultrasound of carotid arteries. Exclusion criteria were as follows: 1) history of acute or recent stroke (< 2 months), myocardial infarction, and surgery or trauma within the preceding 2 months; 2) unconsciousness or unwillingness to undergo the procedure.

For analytical purpose, the patients were categorized into group 1 (i.e., right CS group) and group 2 (i.e., left CS group), respectively.

■ Procedure and protocol of transradial, transbrachial and transfemoral arterial approach for carotid angiographic study and carotid stenting

The procedure and protocol of transradial artery (TRA) access for coronary artery and carotid artery (CA) angiographic examinations in the same procedure, and carotid artery intervention have been described in details in our previous reports [40-42]. Briefly, a high (>5 cm distance from the wrist) TRA access using a 6-French (Fr) arterial sheath is routinely applied for both diagnostic coronary and carotid angiographic studies in our hospital. However, transbrachial artery (TBA) access for carotid angiographic study is utilized if the patient is taller than 170 cm or Allen's test results are positive in both hands.

A left or right high TRA (or TBA) approach was selected at the discretion of the primary operator. Following carotid angiographic studies, the guiding catheters were exchanged, i.e., the 6-Fr Kimny guiding catheter was replaced with 7-Fr Kimny guiding catheter (Boston Scientific, Scimed, Inc. Maple Grove, MN.), for subsequent carotid stenting procedure if indicated.

If TRA or TBA approach was not feasible, traditional method of transfemoral artery (TFA) approach was utilized for those patients.

■ Blood sampling for microparticle levels in carotid artery and internal jugular vein

To assess the MP levels of right internal jugular vein (RIJV), femoral vein (FV) puncture was performed by an 18# puncture needle. A 6-Fr vein sheath was then successfully inserted into the FV after the needle puncture, followed by a 6-Fr pigtail was advanced into the RIJV for blood samplings. The blood samplings were collected from RIJV for two times, i.e., prior to and 5 minutes after CS.

Similarly, blood samplings from the CS treatment site (i.e., CA) were drawn prior to and 5 minutes after CS.

■ Categorization of circulating microparticles into four types

The circulating MPs were categorized into: (1) platelet-derived activated MPs (PDAc-MPs) ($CD31^+$ $CD42b^+$ $AN\text{-}V^-$) and (2) endothelial-derived activated MPs (EDAc-MPs) ($CD31^+$ $CD42b^-$ $AN\text{-}V^-$) based on our previous reports [33,38].

■ Blood samples for flow cytometric analysis of microparticle levels

The procedure and protocol of flow cytometric analysis for determining the MP levels in RIJV (i.e., cerebral circulation) and carotid artery (i.e., systemic circulation) have been described in our previous reports [33,38]. In details, blood samplings were collected in acid citrate dextrose (ACD) vacutainer tubes. The blood sample (1.5 mL) was centrifuged at 2500 g at 4°C for 15 min without acceleration or break to prepare platelet-rich plasma. The 250 µl plasma samples were thawed and centrifuged for 10 min at 19,800 g at 4°C, and then collected for investigation of microparticles (MPs) smaller than 1.0 µm.

Size calibration was conducted with 1.0 µm beads (Invitrogen, Carlsbad, CA). The MP pellet was re-suspended with 150 µl of Annexin-V binding buffer (BD Biosciences). All buffers were sterile-filtered with a 0.2 µm filter. The 100 µl MPs were then incubated in a TruCOUNT tube (BD Biosciences) with fluorescent monoclonal antibodies: (1) phycoerythrin (PE)-labeled anti-CD31(BD Biosciences); (2) fluorescein isothiocyanate–labeled anti-Annexin-V (BD Biosciences) and; (3) phycoerythrin-Cy5(PE-Cy5)-labeled anti-CD42b (BD Biosciences). The samples were incubated in the dark for 15 min at room temperature. The samples were then analyzed on a FC500 flow cytometer (Beckman Coulter) after 400 µl Annexin-V binding buffer was added. The absolute count of MPs was measured setting the stop condition for TruCount beads at 10,000 events.

■ The procedure and protocol of virtual histology intravascular ultrasound (vh-ivus)

After wiring across stenotic lesion and using a filter for distal embolic protection, VH-IVUS (Volcano, Philips) was placed at stenosis for complete lesion assessment. Four different histological components of plaque, i.e., dark green (fibrous), yellow/green (fibrofatty), white (calcified), and red (necrotic lipid core), was immediately provided by VH-IVUS with lumen colored black. Besides, stenosis (%) was calculated as minimum lumen area (MLA, mm^2) divided by reference vessel area (RVA, mm^2).

■ Medications

Patients were pretreated with aspirin 100 mg/day or clopidogrel 75 mg/day orally. Clopidogrel (300 mg loading dose prior to the procedure, then 75 mg/day) was administered to patients

for at least 3 months following stenting, and aspirin (100 mg/day) was administered orally to each patient indefinitely.

■ Statistical analysis

Continuous variables with normal distribution were expressed as mean ± standard deviation, and the difference between two groups was analyzed with independent t-test. Continuous variables which didn't meet normality were reported as median, 25 percentile and 75 percentile, and the difference in groups was examined using Mann-Whitney U test. Discrete or categorical variables between different groups were reported as percentage and number, and then analyzed with Chi-square test or Fisher's exact test. Because the level of MPs, including different types and changes before and after CS, had big variation and extreme values, we normalized the data by using ratio of post-CS to pre-CS MPs. Log_{10} of the post/pre-CS ratio of MPs were taken after excluding extreme values. The median value of paired samples was analyzed with Wilcoxon's signed-rank test. Additionally, Spearman's rank correlation was adopted to assess the relation between post/pre-CS ratios of MPs and plague necrosis or clinical events, respectively. Furthermore, stepwise multivariate linear regression analysis for different type of MPs was performed with forward selection approach to identify the potential predictive variables. In detail, we defined the post/pre-CS ratio of MPs as response variables, and then use stepwise method to screen important corresponding variables. We processed remaining data with logarithmic transformation. The outlier was then eliminated after normality test. Finally, the study subjects were enrolled for multivariate regression analysis. Statistical analysis was performed using SPSS statistical software for Windows version 22 (SPSS for Windows, version 22; SPSS, IL, U.S.A.). A value of $p<0.05$ was considered statistically significant.

Results

■ Baseline characteristics and angiographic results of 92 study subjects

The age, male gender, body height, body weight and body mass index did not differ between group 1 (i.e., right CS group) and group 2 (left CS group). Additionally, the incidences of smoking, hypertension, diabetes mellitus, hyperlipidemia, peripheral arterial occlusive disease, atrial fibrillation, old stroke, and head and neck cancer with radiation therapy were similar between groups 1 and 2. Furthermore, the prescription of medications, including antiplatelet agents (i.e. aspirin or clopidogrel), angiotensin converting enzyme inhibitors (ACEIs)/angiotensin II type I receptor blockers (ARBs) were also similar between groups 1 and 2 **(Table 1)**.

The laboratory findings showed that there were no differences in terms of white blood cell and platelet counts, percentages of segment, lymphocyte, monocyte, eosinophil and basophil, ratio of neutrophil to lymphocyte and ratio of platelet to lymphocyte. Additionally, the serum levels of creatinine, total cholesterol, and low-density lipoprotein also did not differ between groups 1 and 2.

The frequency of TRA/TBA approach for carotid intervention was significantly higher than TFA approach. The coronary artery angiographic examination showed that the rate of concomitant significant coronary and carotid artery stenosis was greater than 74%. Of them, ten percent of CAD patients had left main trunk involvement. However, the frequency of one, two or triple vessel disease and LM disease did not differ between groups 1 and 2.

The frequency of significant bilateral CA stenosis did not differ between groups 1 and 2. However, the study patients had higher rate of right-side than left-side CA stenosis.

The procedural success rate (i.e., successfully stent implantation) was 100% in both groups 1 and 2. The incidence of acute ischemic stroke or transient ischemic attack after stenting was acceptable and did not differ between groups 1 and 2.

The VH-IVUS examination for identifying the obstructive CA lesions and plaque component, and flow cytometric analysis for the level of plasma microparticles to assess the plaque morphological features, burden and stenosis of obstructive CA, VH-IVUS examination was performed prior to CS. The results showed that the percentage of stenosis, minimal lumen area, reference vascular area and component of necrotic tissue did not differ between the groups 1 and **(Table 2)**.

Flow cytometric analysis showed that the blood level of PDAc-MPs in CA (APDMPs) did not differ between groups 1 and 2 prior to and after CS. In addition, the blood level of PDAc-MPs in RIJV (VPDMPs) also did not differ between groups 1 and 2 prior to and after CS.

Table 1: Baseline characteristics of the 92 study patients undergoing carotid stenting (CS) for significant carotid stenosis. Table 1: Baseline characteristics of the 92 study patients undergoing carotid stenting (CS) for significant carotid stenosis.

Variables	Total (n=92)	Right CS (n=56)	Left CS (n=36)	P-value
Clinical features				
Age (years)	70.9 ± 9.8	70.7 ± 8.8	71.2 ± 11.3	0.823
Male gender	82.6% (76)	80.4% (45)	94.1% (31)	0.477
Body height (cm)	161.6 ± 6.9	161.3 ± 7.3	162.1 ± 6.2	0.611
Body weight (kg)	63.9 ± 11.1	63.8 ± 12.1	64.1 ± 9.3	0.891
Body mass index (kg/m^2)	24.4 ± 3.8	24.5 ± 4.3	24.4 ± 2.9	0.876
Smoking history	27.2% (25)	33.9% (19)	16.7% (6)	0.069
Hypertension	85.7% (78)	85.5% (47)	86.1% (31)	0.930
Diabetes mellitus	38% (35)	39.3% (22)	36.1% (13)	0.760
Dyslipidemia	56.5% (52)	58.9% (33)	52.8% (19)	0.561
PAOD	2.2% (2)	3.6% (2)	0% (0)	0.252
Atrial fibrillation	6.5% (6)	7.1% (4)	5.6% (2)	0.763
Old stroke	45.7% (42)	44.6% (25)	47.2% (17)	0.808
NPC or H/N tumor	16.3% (15)	16.1% (9)	16.7% (6)	0.940
Medication				
Antiplatelet	100% (92)	100% (56)	100% (36)	1.000
ARB/ACEI	60.9% (56)	69.6% (39)	47.2% (17)	0.032
Statin	60.9% (56)	55.4% (31)	69.4% (25)	0.177
Laboratory data				
Leukocyte count (x10^3)	7.3 ± 2.6	7.4 ± 2.8	7.2 ± 2.1	0.700
Segment (%)	63.0 ± 11.9	64.1 ± 12.5	61.3 ± 11.1	0.278
Lymphocyte (%)	26.4 ± 8.9	25.6 ± 8.4	27.6 ± 9.6	0.303
Monocyte (%)	7.9 ± 11.4	8.8 ± 14.8	6.6 ± 1.8	0.274
Eosinophil (%)	3.4 ± 3.3	2.9 ± 3.2	4.1 ± 3.3	0.090
Basophil (%)	0.3 ± 0.3	0.3 ± 0.3	0.4 ± 0.2	0.599
Platelet count (x10^3)	200.9 ± 53.7	201.4 ± 58.0	200.2 ± 47.1	0.918
NLR	2.9 ± 1.9	3.0 ± 1.5	2.9 ± 2.3	0.785
PLR	123.7 ± 50.8	125.6 ± 48.4	120.8 ± 54.7	0.664
Serum creatinine (mg/dL)	1.2 ± 0.8	1.2 ± 0.9	1.2 ± 0.8	0.853
Total cholesterol (mg/dL)	152.8 ± 33.6	150.8 ± 34.6	156.1 ± 32.3	0.542
Low density lipoprotein	88.9 ± 29.2	89.6 ± 31.9	88.0 ± 24.6	0.843
Procedural information				
Right side approach	75% (69)	98.2% (55)	38.9% (14)	<0.001
Radial/brachial access	70.7% (65)	73.2% (41)	66.7% (24)	0.501
Femoral access	29.3% (27)	26.8% (15)	33.3% (12)	0.501
Coronary angiography	97.8% (90)	98.2% (55)	97.2% (35)	0.750
CAD	74.4% (67)	78.2% (43)	68.6% (24)	0.308
Single-vessel CAD	22.2% (20)	20% (11)	25.7% (9)	0.795
Two-vessel CAD	18.9% (17)	18.2% (10)	20% (7)	0.830
Triple-vessel CAD	33.3% (30)	40% (22)	22.9% (8)	0.112
Left main trunk stenosis	10% (9)	7.3% (4)	14.3% (5)	0.302
Carotid angiography	100% (92)	100% (56)	100% (36)	1.000
Right CCA stenosis ≥50%	16.3% (15)	23.2% (13)	5.6% (2)	0.054
Righ ICA stenosis ≥50%	69.6% (64)	92.9% (52)	33.3% (12)	<0.001
Left CCA stenosis ≥50%	10.9% (10)	5.4% (3)	19.4% (7)	0.070
Left ICA stenosis ≥50%	57.6% (53)	41.1% (23)	83.3% (30)	0.001
Bilateral carotid stenosis	41.3% (38)	42.9% (24)	38.9% (14)	0.706
Procedure and clinical outcome				
Procedure success rate†	100% (92)	100% (56)	100% (36)	1.000
Transient ischemic attack	3.3% (3)	3.6% (2)	2.8% (1)	0.834
Acute embolic stroke‡	4.3% (4)	5.4% (3)	2.8% (1)	0.554
Cumulative 1-year mortality	2.2% (2)	3.6% (2)	0% (0)	0.252
Cumulative 1-year stroke	6.5% (6)	8.9% (5)	2.8% (1)	0.244

Data are expressed as mean ± SD or % (n).
Abbreviation: CS, carotid stenting; PAOD, peripheral arterial occlusive disease; NPC, nasopharyngeal cancer; H/N, head and neck; ARB/ACEI, angiotensin II type I receptor blocker/angiotensin converting enzyme inhibitor; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; CAD, coronary artery disease; CCA, common carotid artery; ICA, internal carotid artery.
†The procedure success of carotid stenting is defined as residual <30% stenosis on final carotid angiography.
‡Procedure-related stroke within one week after carotid stenting
Continuous and categorical variables between two groups were compared with independent t test and Chi-square test, respectively.

Table 2: Data description of VH-IVUS, platelet-derived and endothelium-derived MPs in carotid arterial and right internal jugular venous samples before and after CS.

Variables	Total (n=92)	Right CS (n=56)	Left CS (n=36)	P-value[1]
Plague on lesion site				
Stenosis (%)	87.1 ± 8.9	86.8 ± 10.7	87.6 ± 5.7	0.691
Minimum lumen area (mm^2)	5.4 ± 2.4	5.4 ± 2.4	5.4 ± 2.4	0.976
Reference vessel area (mm^2)	44.7 ± 6.0	44.9 ± 5.9	44.4 ± 6.1	0.734
Necrosis component (%)	24.7 ± 10.2	24.2 ± 10.9	25.3 ± 9.1	0.642
Microparticles				
APDMPs-Pre	84951.2 (39543.5, 229764.5)	95528.5 (41933.8, 231137.3)	77480.1 (29844.0, 208873.4)	0.662
APDMPs-Post	218473.1 (87788.5, 378584.9)	207492.0 (74940.8, 344341.5)	298648.6 (124991.3, 413318.8)	0.281
Post/Pre-CS ratio†	2.09 (1.17, 4.64)	2.10 (1.18, 4.37)	2.09 (0.92, 6.35)	0.783
P-value[2]	<0.001			
VPDMPs-Pre	56796.0 (23956.0, 117491.3)	69336.1 (35769.3, 120343.3)	40404.0 (18294.0, 119147.9)	0.262
VPDMPs-Post	89355.2 (41792.5, 208611.2)	75623.8 (40164.2, 663220.3)	106061.9 (44705.9, 208611.2)	0.659
Post/Pre-CS ratio	1.37 (0.59, 3.90)	1.23 (0.49, 4.03)	1.23 (0.63, 3.75)	0.713
P-value[2]	0.006			
AEDMPs-Pre	3317.8 (1110.1, 10104.1)	3229.4 (1637.0, 8000.3)	3317.8 (697.2, 14553.1)	0.750
AEDMPs-Post	5274.3 (1807.8, 15263.4)	5948.5 (2035.1, 18375.8)	4739.0 (1663.1, 12142.7)	0.124
Post/Pre-CS ratio	1.40 (0.67, 3.33)	1.39 (0.67, 3.25)	1.54 (0.65, 4.70)	0.938
P-value[2]	0.003			
VEDMPs-Pre	1553.5 (765.9, 5863.6)	1985.4 (1003.6, 6832.9)	1229.8 (594.1, 5544.4)	0.097
VEDMPs-Post	2517.8 (709.7, 7230.2)	2309.8 (856.2, 9269.5)	3147.6 (612.1, 6670.4)	0.533
Post/Pre-CS ratio	0.93 (0.50, 3.60)	0.92 (0.43, 3.82)	0.99 (0.56, 3.85)	0.906
P-value[2]	0.391			

Data are expressed as mean ± SD or median (25 percentile, 75 percentile).
Abbreviation: VH-IVUS, virtual histology intravascular ultrasound; MPs, microparticles; CS, carotid stenting;
APDMPs, carotid artery (CA) platelet-derived microparticles; VPDMPs, right internal jugular vein (RIJV) platelet-derived microparticles; AEDMPs, CA endothelium-derived microparticles; VEDMPs, RIJV endothelium-derived microparticles;
†Post/Pre-CS ratio denotes that the level of MPs after CS is divided by those of MPs prior to CS.
1Variables were compared between right CS and left CS groups using independent t test or Mann-Whitney U test.
2Pre-CS and post-CS level of MPs were compared using Wilcoxon sign-rank test.

Consistently, the blood level of EDAc-MPs in both CA and RIJV (AEDMPs and VEDMPs, respectively) expressed an identical pattern of PDAc-MPs in groups 1 and 2.

The level of these four MPs, i.e., PDAc-MPs and EDAc-MPs in CA and RIJV, increased after carotid stenting. Of note, the ratios of post-CS to pre-CS PDAc-MPs level in both CA and RIJV were significantly increased among the study patients. Besides, the ratio of post-CS to pre-CS EDAc-MPs level was significantly increased in CA, but not significantly elevated in RIJV.

■ Comparison of plasma PMs prior to and after CS in both CA and RIJV in 92 study patients

Flow cytometric analysis exhibited that the plasma level of PDAc-MPs in CA was significantly

higher after CS than prior to CS. Additionally, this measurement further displayed that the plasma level of PDAc-MPs in RIJV was also significantly higher in the post-CS than pre-CS procedure. Similarly, the plasma level of EDAc-MPs in CA was also significantly higher after CS than prior to CS. However, this parameter did not differ in RIJV between the time intervals of post CS and prior to CS. These finding suggest that the CS procedure could predominantly activate PDAc-MPs rather than those of EDAc-MPs that may be mainly through activation of platelet activity in circulation **(Figure 1)**

■ Correlation between post/pre-CS ratio of microparticles and percentage of necrotic tissues of stenotic carotid-plague components (Table 3, Figure 2)

To elucidate the correlation between necrotic area of CA stenosis and plasma levels of MPs, Spearman's correlation analysis was utilized. The results showed that Post-CS MPs levels, including PDAc-MPs and EDAc-MPs, in CA were significantly associated with necrotic area in CA stenosis. Additionally, the ratio of post-CS to pre-CS PDAc-MPs level in CA was significantly predictive of an increase in necrotic area of CA stenosis, suggesting that the more component of necrotic lipid core of carotid plague, the higher activation level of PDAc-MP in systemic circulation after carotid stenting.

■ Correlation between plasma MPs levels and necrotic area and combined one-year cumulative clinical events (Table 4)

To determine the correlation between one-year cumulative combined clinical events (defined as transient ischemic attack, acute IS after CS, IS, or death identified during clinical follow-up) and necrotic area of CA and plasma levels of MPs, we performed Spearman's correlation analysis. The results showed no significant correlation between these parameters.

■ Multiple linear regression analysis of predictors for predictive of the ratio of pre-CS to post-CS plasma MPs levels in CA and RIJV (Table 5).

The multiple regression analysis demonstrated that age was significantly predictive of ratio of post-CS/pre-CS PDAc-MPs and EDAc-MPs levels in CA. Male gender was significantly negatively predictive of the ratio of post-CS/pre-CS PDAc-MPs and EDAc-MPs levels in CA and positively predictive of the ratio of post-CS/pre-CS EDAc-MPs level in RIJV. On the other hand, diabetes mellitus was weakly predictive of the ratio of post-CS/pre-CS EDAc-MPs level in RIJV.

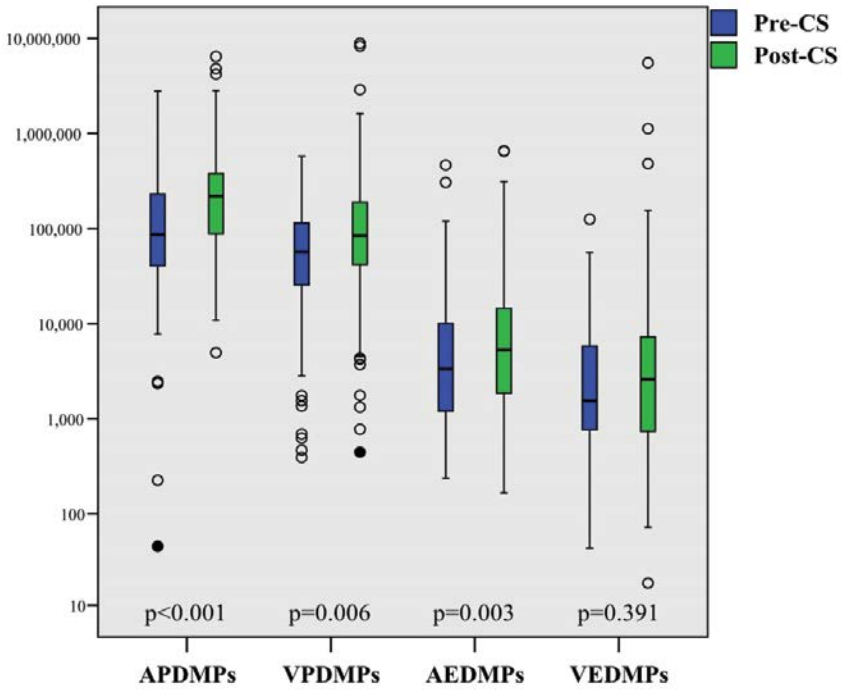

Figure 1: Comparison of the difference of plasma microparticles prior to vs. post carotid stenting (CS) in carotid artery and right internal jugular vein (RIJV).

(1) For platelet-derived activated microparticles in carotid artery (APDMPs), pre-CS vs. post CS, p<0.001. (2) For platelet-derived microparticles in RIJV (VPDMPs), p = 0.006. (3) For endothelial-derived activated microparticles in carotid artery (AEDMPs), p = 0.003. (4) For endothelial-derived activated microparticles in RIJV (VEDMPs), p = 0.391.

Table 3: Correlation of ratio of post/pre-CS MPs, NLR and PLR to the percentage of necrotic area of stenotic carotid plagues.

Variables	R	P-value
APDMPs-Pre	-0.070	0.594
APDMPs-Post	0.395	<0.001
Post/Pre-CS ratio of APDMPs	0.465	<0.001
VPDMPs-Pre	0.111	0.359
VPDMPs-Post	0.047	0.697
Post/Pre-CS ratio of VPDMPs	0.022	0.858
AEDMPs-Pre	0.062	0.603
AEDMPs-Post	0.312	0.010
Post/Pre-CS ratio of AEDMPs	0.133	0.287
VEDMPs-Pre	0.236	0.053
VEDMPs-Post	0.107	0.370
Post/Pre-CS ratio of VEDMPs	-0.134	0.277
NLR	0.185	0.112
PLR	0.171	0.141

Abbreviation: CS, carotid stenting; MPs, microparticles; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; R, Spearman's correlation coefficient; APDMPs, carotid artery (CA) platelet-derived microparticles; VPDMPs, right internal jugular vein (RIJV) platelet-derived microparticles; AEDMPs, CA endothelium-derived microparticles; VEDMPs, RIJV endothelium-derived microparticles;
Statistics was done with Spearman's rank correlation.

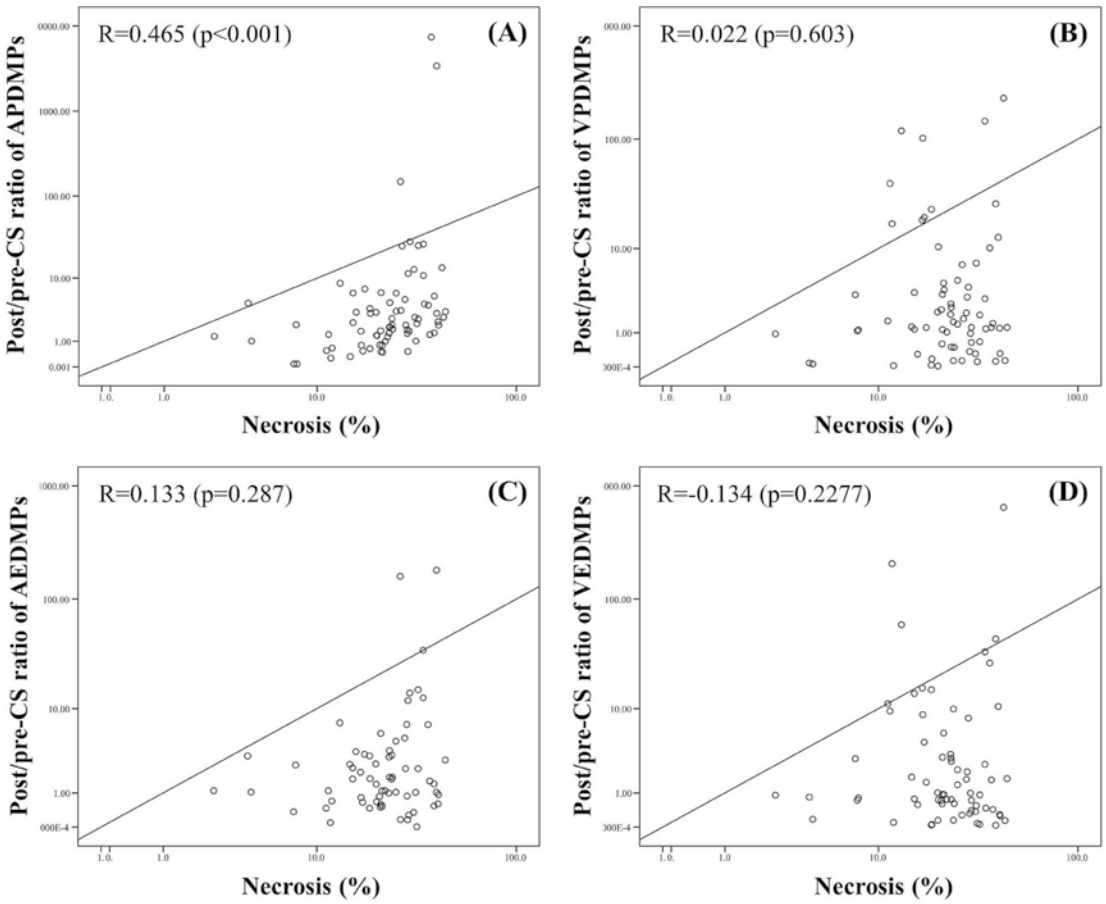

Figure 2: Correlation between the ratio of post-carotid stenting (CS) to pre-CS microparticles (MPs) in carotid artery (CA) and right internal jugular vein (RIJV) and necrosis of carotid plague.

A) Moderate correlation between the ratio of post-CS to pre-CS APDMPs and necrosis area (expressed as %), $p < 0.001$. APDMPs = platelet-derived activated microparticles in CA. B) No correlation between the ratio of post-CS to pre-CS VPDMPs and necrosis area, $p = 0.603$. VPDMPs = platelet-derived activated microparticles in RIJV. C) No correlation between the ratio of post-CS to pre-CS AEDMPs and necrosis area, $p = 0.287$. AEDMPs = endothelial-derived activated microparticles in CA. D) No correlation between ratio of post-CS to pre-CS VEDMPs and necrosis area, $p = 0.228$. VEDMPs = endothelial-derived activated microparticles in RIJV.

Table 4: Correlation of necrosis, post/pre-CS ratio of MPs, PLR and NLR to cumulative one-year clinical events

Variables	R	P-value
Necrosis	-0.146	0.207
Post/Pre-CS ratio of APDMPs	-0.098	0.359
Post/Pre-CS ratio of VPDMPs	0.037	0.736
Post/Pre-CS ratio of AEDMPs	0.072	0.522
Post/Pre-CS ratio of VEDMPs	0.020	0.855

Abbreviation: CS, carotid stenting; MPs, microparticles; R, Spearman's correlation coefficient; APDMPs, carotid artery (CA) platelet-derived microparticles; VPDMPs, right internal jugular vein (RIJV) platelet-derived microparticles; AEDMPs, CA endothelium-derived microparticles; VEDMPs, RIJV endothelium-derived microparticles; Statistics was done with Spearman's rank correlation.

Additionally, prescription of ACEI/ARB was significantly but negatively predictive of ratio of post-CS/pre-CS EDAc-MPs level in CA. Furthermore, bilateral CA stenosis was significantly negatively predictive of post-CS/pre-CS PDAc-MPs and EDAc-MPs level in RIJV. Moreover, the necrotic tissue was the most strongly significantly predictive of the ratio of PDAc-MPs and EDAc-MPs levels in CA.

Discussion

This study investigated the correlation between the percentage of necrotic area in CA stenosis and blood plasma level of MPs yielded several striking implications. First, the plasma level of PDAc-MPs in CA and RIJV was significantly higher in post-CS than in pre-CS time point, highlighting that the CS procedure would augment the generation of PDAc-MPs. Second, a moderate correlation existed between ratio of post-CAS to pre-CAS PDAc-MPs levels in CA and necrotic area of CA stenosis. Third, multiple linear regression demonstrated that the necrotic

Table 5: Identification of the predictors for post/pre-CS ratio of MPs with multiple regression analysis*

Post/pre-CS ratio†	ADPMPs		VPDMPs		AEDMPs		VEDMPs	
	Coefficient 95% CI	P-value	Coefficient 95% CI	P-value	Coefficient 95% CI	P-value	Coefficient 95% CI	P-value
Age	0.04 (0.01, 0.06)	0.006	-0.13	0.347	0.03 (0.006, 0.06)	0.017	-0.15	0.224
Male gender	-1.23 (-2.06, -0.4)	0.004	0.23	0.084	-1.48 (-2.34, -0.63)	0.001	1.73 (0.19, 3.28)	0.029
Body mass index	-0.06	0.626	-0.08	0.529	-0.11	0.365	-0.08	0.537
Smoking history	-0.13	0.249	0.17	0.203	-0.13	0.284	0.05	0.723
Hypertension	-0.04	0.756	-0.25	0.058	-0.11	0.371	-0.05	0.729
Diabetes mellitus	0.02	0.876	-0.19	0.138	0.16	0.184	-1.02 (-1.99, -0.04)	0.042
Dyslipidemia	0.02	0.838	0.11	0.395	0.11	0.371	0.08	0.536
PAOD	0.08	0.530	-0.05	0.717	0.01	0.969	0.15	0.271
Atrial fibrillation	0.06	0.625	-0.01	0.939	0.07	0.571	0.06	0.645
Old stroke	0.08	0.506	0.08	0.939	-0.01	0.928	-0.03	0.829
NPC or H/N tumor	0.04	0.732	-0.08	0.544	0.08	0.541	-0.003	0.983
ACEI/ARB	-0.13	0.248	-0.18	0.168	-0.70 (-1.24, -0.17)	0.011	-0.11	0.376
Statin	-0.16	0.186	-0.12	0.377	-0.02	0.866	0.05	0.728
NLR	-0.06	0.610	-0.21	0.100	0.06	0.631	-0.11	0.376
PLR	-0.18	0.121	-0.23	0.078	-0.17	0.146	-0.12	0.337
Serum Creatinine	-0.19	0.101	-0.03	0.798	-0.18	0.139	-0.06	0.633
CAD	-0.03	0.782	-0.07	0.611	0.10	0.400	-0.11	0.404
Multi-vessel CAD‡	-0.06	0.624	-0.16	0.216	0.03	0.801	-0.18	0.146
Bilateral CAS	0.02	0.891	-1.29 (-2.38, -0.21)	0.021	-0.17	0.158	-1.52 (-2.52, -0.52)	0.004
Necrosis	0.05 (0.03, 0.08)	<0.001	-0.03	0.803	0.04 (0.01, 0.06)	0.011	-0.13	0.299

Data are expressed as coefficient (95% CI was only shown if statistically significant).
Abbreviation: CS, carotid stenting; MP, microparticles; CI, confidence interval; PAOD, peripheral arterial occlusive disease; NPC, nasopharyngeal cancer; H/N, head and neck; ARB/ACEI, angiotensin II type I receptor blocker/angiotensin converting enzyme inhibitor; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; CAD, coronary artery disease; CAS, carotid artery stenosis.
APDMPs, carotid artery (CA) platelet-derived microparticles; VPDMPs, right internal jugular vein (RIJV) platelet-derived microparticles; AEDMPs, CA endothelium-derived microparticles; VEDMPs, RIJV endothelium-derived microparticles;
*Stepwise multivariate linear regression analysis was done with adjusting for age, gender, comorbidities, medication, and lesion characteristics.
†Post/Pre-CS ratio denotes that the level of MPs after CS is divided by those of MPs prior to CS.
‡Multi-vessel CAD indicated two-vessel and triple-vessel CAD.

area was significantly predictive of the ratio of post-CAS to pre-CAS PDAc-MPs and EDAc-MPs level in CA.

Our previous studies have shown the frequency of significant CAD in patients with high-grade CA stenosis was more than 75% [40,41,43]. One important finding in the present study was that the frequency of significant CAD in CS patients was more than 74%. Additionally, the rate of significant stenosis of LM trunk was 10%. Our findings, consistent with our previous studies [40,41,43], imply that CAD and CA stenosis are the two sides of the same coin, resulting from endothelial dysfunction, plaque formation, and arterial obstructive syndrome. Importantly, our findings strongly recommend that carotid and coronary arterial angiographic studies should be simultaneously examined in one stage for categorizing the CA stenosis patients into the high-risk and low-risk subgroups.

Platelet reactivity has been established as a fundamental role in the pathogenesis of atherosclerosis and thromboembolic events [44-46]. Our previous study has further demonstrated that the platelet activity in RIJV (i.e., cerebral circulation) and femoral vein (i.e., systemic circulation) was significantly increased in high-grade CA stenosis patients than in those of at risk control subjects [47]. Additionally, as compared with prior CS, the platelet activity was still persistently or more activated in patients after CS [47]. Intriguingly, our other previous studies have also displayed that the circulating level of soluble CD40 ligand (sCD40L), an index of platelet activation, was significantly higher in patients with coronary arterial

obstructive syndrome undergoing coronary stenting [48-50]. Additionally, sCD40L was predictive of angiographic morphologic features of high-burden thrombus formation [50] that is well known to play a crucial role for procedural failure during coronary intervention and unfavorable prognostic outcome. A principal finding in the present study was that PDAc-MPs was remarkably higher in the time point of post-CS than pre-CS procedure. Additionally, this phenomenon was not only identified in systemic circulation but also in the cerebral circulation, highlighting that the CS procedure would frequently induce an upregulation of PDAc-MPs even undergoing the loading dose of double anti-platelet agents (i.e., aspirin and clopidogrel). Our findings, comparable with our previous studies [47-50], encourage the use of circulating MPs as an alternatively accessory modality for assessing the platelet activity in patients with arterial obstructive syndrome.

It is well recognized that vulnerable plaque usually contains high lipid-core content which is crucial for no-reflow phenomenon during coronary intervention. The acute no-/slow-flow phenomenon always results from the dislodged lipid-core content/debris to plug the microcirculation and results in an unfavorable prognostic outcome. Interestingly, previous studies have shown that leukocyte-derived and endothelial-derived MPs are the promising biomarkers associated with plaque vulnerability in patients with significant CA stenosis [39,51]. In the present study, we found that post-CS PDAc-MPs, EDAc-MPs and ratio of post-CS/pre-CS EDAc-MPs levels **(Table 3 and Figure 2)** were strongly and significantly correlated to necrotic area of CA stenosis. In this way, our finding, in addition to the findings of previous studies [39,51], imply that circulating MPs could be useful non-invasive derived biomarker for predicting the vulnerable plaque not only in patients with CA stenosis but also in those of patients with other arterial obstructive syndromes.

The aforementioned aims of the study in introduction section were (1) to find whether the cerebral/systemic circulation of MPs could be a useful biomarker for predictive of vulnerable plaque in CS stenosis and (2) to assess whether the cerebral/systemic circulation of MPs would be useful biomarker for predictive of embolic IS in patients after receiving CS. The result of 2nd study aim might be disappointing because there was no significant correlation between ratios of post/pre-CS MPs in both CA and RIJV **(Table 4)**. Intriguingly, previous study has found that leukocyte-derived MPs was strongly associated with at risk of thromboembolic and neurologic events in patients with asymptomatic CA stenosis undergoing the endarterectomy [39]. In this way, the results of our and previous study [39] is inconsistent. Perhaps, several reasons could be explained the discrepancy between our results and findings of previous study [39]. First, the carotid intervention procedure was different between these two studies, i.e., CS vs. carotid endarterectomy. Second, filter protection was used for preventing distal embolization in our study but not in the previous study [39]. Third, the measured type of MPs differed between our and previous studies [39].

Study Limitations

This study has limitations. Frist, the patient sample size was still relatively small and the standard deviation of MPs level was extremely high. These phenomena may distort the statistical significance. Second, no correlation between one-year cumulatively untoward clinical outcomes and necrotic area and circulating MPs level could be perhaps due to the extremely highly standard deviation of MPs level.

Conclusion

In conclusion, plasma levels of PDAc-MPs and EDAc-MPs were remarkably increased in the post-CS than pre-CS procedure. The results of the present study displayed that post-CS PDAc-MPs/EDAc-MPs levels and ratio of post/pre-CS PDAc-MPs were significantly correlated to necrotic area of CA stenosis. However, the results of the present study did not show circulating MPs or necrotic area is a useful predictor for one-year cumulative combined clinical events.

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