Original Article

Levels of Circulating Neopterin in Patients with Severe Carotid Artery Stenosis Undergoing Carotid Stenting

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***Aims*: The association between an elevated serum neopterin level and the development of coronary artery complex lesions has been extensively assessed; however, the correlation between the serum neopterin level and the development of carotid artery stenosis has seldom been reported. This study tested whether this biomarker is increased in patients with severe carotid artery stenosis (≥70%) undergoing carotid artery (CA) stenting and investigated independent predictors of an increased circulating neopterin level.**

***Methods*: Fifty patients with severe CA stenosis (CAS) undergoing CA stenting were consecutively enrolled in this study from January 2009 through December 2011. The serum neopterin levels of age- and gender-matched acute ischemic stroke (AIS) patients (n=120) and control subjects (CS) (n=33) were also measured. A blood sample was prospectively collected from each patient in the catheterization room.**

***Results*: The serum levels of neopterin were significantly higher in the CAS patients than in the AIS patients or CS and significantly higher in the AIS patients than in the CS (all $p<0.001$). An analysis of the variables of 170 patients (CAS+AIS) demonstrated that age, a previous history of stroke and severe CAS were significantly correlated with an increased serum level of neopterin (all $p<0.005$). A multivariate binary logistic regression analysis of the severe CAS patients (n=50) demonstrated that age and the creatinine level were independent predictors of a high neopterin level (neopterin level ≥16.52 ng/dL, i.e., according to the median value of neopterin) (all $p<0.05$).**

***Conclusions*: The circulating neopterin levels are significantly higher in patients with severe CAS than in those with AIS. The presence of CAS, age and the creatinine level were significantly correlated with an increased serum neopterin level.**

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Key words: Carotid artery stenosis, Neopterin, Cerebrovascular disease

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Introduction

Numerous studies have shown that inflammatory biomarkers play a pivotal role in the risk stratification and prediction of clinical outcomes in patients with acute coronary syndrome[1-5]. Immunohistochemical staining of atherosclerotic lesions further demonstrates

that complex interactions between the activation of inflammatory and immune cells within vulnerable plaques play a crucial role in the inflammatory process and are associated with atherosclerosis development and plaque vulnerability[1, 2, 4, 6]. These complex interactions ultimately result in atheroembolic plaque disruption and acute atherosclerotic occlusive syndrome[1-8]. Therefore, inflammatory biomarkers of atherosclerotic disease may be not only potential biomarkers of plaque instability[1-8], but also predictors of prognostic clinical outcomes[3, 5, 7, 9-11]. Undoubtedly, coronary artery disease (CAD) and cerebral vascular disease are two sides of the same coin[12]. Therefore, it is reasonable that plaque vulnerability and disruption are not only restricted to the epicardial coronary arteries, but also involve other arterial systems, including the carotid arteries and cerebral arteries[6, 13, 14].

Neopterin is a pteridine derivative and a side product of the guanosine triphosphate-biopterin pathway[15]. It is expressed on monocytes and macrophages and primarily secreted by activated macrophages after stimulation by IFN-γ[16] and has been recommended as a biomarker of immune activation and the macrophage activity[16-18]. Additionally, neopterin has been found to mediate the inflammatory process within vulnerable plaques[15, 16]. Furthermore, neopterin has been revealed to play a key role in atherosclerotic processes and is related to the presence of unstable coronary lesions in patients with coronary artery disease[19-21]. An increased serum level of neopterin has therefore been suggested to be a biomarker of acute widespread atherothrombotic plaque inflammation and CAD activity[19-21].

Astonishingly, despite the association between an elevated serum level of neopterin and acute coronary syndrome and chronic stable angina[15, 16, 19-21], the correlation between the serum neopterin level and carotid artery disease[6, 14, 22-24] and acute ischemic stroke (AIS) has seldom been reported[25]. Therefore, the present study tested the potential of the serum neopterin concentration as a clinical biomarker to reflect the severity of extracranial carotid artery (ECCA) stenosis and further tested whether there is a correlation between an increased circulating level of neopterin and plaque instability.

Materials and Methods

Ethics and Enrollment and Exclusion Criteria

This study was approved by the Institutional Review Committee on Human Research of Chang Gung Memorial Hospital (No 97-2280B) in 2008 and conducted at Kaohsiung Chang Gung Memorial Hospital within a five-year period.

The enrollment criteria have been described in detail in our previous reports[26-28]. Briefly, the enrollment criteria were as follows: (1) severe ECCA stenosis (≥70%) determined on magnetic resonance imaging (MRI)/MR angiography (MRA) and duplex ultrasound of the carotid arteries performed prior to extracranial and intracranial angiographic studies; (2) a history of stroke (>2 months), transient ischemic attack or dizziness related to significant ECCA stenosis. The exclusion criteria were as follows: (1) a history of acute or recent stroke (<2 months), myocardial infarction or surgery or trauma within the preceding two months; (2) unconsciousness or unwillingness to undergo the procedure. A total of 50 patients with severe ECCA stenosis undergoing CA stenting were consecutively and prospectively enrolled into the study from January 2009 through December 2011.

Procedures and Protocol

Simultaneous coronary and carotid angiographic examinations were carried out in each patient using the routine transradial or transbrachial arterial approach. Additionally, the catheter looping and retrograde engagement technique was used for the carotid angiographic study and CA stenting. The detailed procedures and protocols have been described in our previous studies[26-28].

Acute Ischemic Stroke Patients and Healthy Age- and Gender-Matched Controls

To determine whether the circulating neopterin levels are higher in the severe ECCA stenosis setting than in the AIS setting or vice versa, we enrolled 120 patients with AIS using randomized age- and gender-matched methods to rule out the possible effects of these two variables. The patients consisted of some of the disease-control subjects in another recently reported study[29]. In these patients, AIS was defined as the sudden onset of the loss of the global or focal cerebral function persisting for more than 24 hours. The radiological criteria for a diagnosis of AIS included a new finding of low attenuation density in focal or diffuse brain areas on computed tomography of the brain or the appearance of area(s) of high intensity (bright spots) on diffusion-weighted image (DWI) magnetic resonance imaging (MRI) or low intensity on apparent diffusion coefficient (ADC) value MRI[29][29]. Additionally, the serum levels of neopterin were measured in 33 age- and gender-matched control subjects. Informed consent was obtained from all study subjects.

Table 1. Baseline Characteristics of the Study Patients and Healthy Control Subjects

Variables	CVD ($n=170$)	Normal ($n=33$)	p value
Age (yrs)	65.6 ± 11.5	63.0 ± 5.7	0.057
Male gender (%)	75.3% (128)	66.7% (22)	0.302
Body mass index (kg/m^2)	24.3 ± 4.1	26.8 ± 2.3	0.127
Current smoking (%)	30.0% (51)	0% (0)	< 0.001
Diabetes mellitus (%)	38.8% (66)	0% (0)	< 0.001
Hypertension (%)	70.0% (119)	0% (0)	< 0.001
Dyslipidemia (%)*	47.1% (80)	45.5% (15)	1.000
Neopterin level (ng/dL)	22.7 ± 19.3	7.5 ± 2.7	< 0.001
Creatinine level (mg/dL)	1.1 ± 0.9	0.8 ± 0.2	0.416

The data are expressed as the mean ± SD or % (n) of patients.
CVD = cerebrovascular disease.
* indicates patients with either hypercholesterolemia, hypertriglycemia or both.

Imaging Studies and Laboratory Investigations

In addition to complete clinical assessments, other studies were performed as described in our recent report[29], including examinations of chest X-ray films, routine brain computed tomography, duplex scanning of the carotid arteries and routine cardiac analyses with 12-lead electrocardiograms and echocardiography. Moreover, the white blood cell count and biochemical data were acquired on admission.

Blood Sampling and Assessment of the Serum Levels of Neopterin using ELISA

The procedures for blood sampling and the assessment of the serum levels of neopterin using ELISA were reported in detail in our recent study[25]. Briefly, blood samples were obtained in the catheterization room after an arterial sheath was inserted into the artery to assess the serum level of neopterin, WBC count and biochemical data. Blood samples were also obtained in the AIS patients at the bedside[29] and in the control subjects who participated in a health screening program at our health clinic once at 9.00 a.m.

The blood samples were stored at −80℃ until measurement of the neopterin levels was performed in batches at the end of recruitment. The serum neopterin concentrations were assessed using duplicated determination with a commercially available ELISA kit (R&D Systems, Minneapolis, MN). The lower detection limit was 0.7 nmol/L. The intraindividual variability in the neopterin level was assessed in the study patients and control subjects. The mean intra-assay coefficients of variance were all less than 5.0% (3.6%-6.8%). All other biochemical measurements were evaluated by the Clinical-Pathological Analytical Unit of our institution.

Medications

Aspirin was the first choice in acute IS patients unless the patient was allergic or intolerant to aspirin, including having a history of peptic ulcers or upper gastrointestinal tract bleeding during aspirin therapy. Clopidogrel was used in patients who were intolerant to aspirin treatment. Other commonly used drugs included statins, angiotensin-converting enzyme inhibitors, calcium channel blocking agents and beta blockers.

Statistical Analysis

Continuous variables are expressed as the mean ± SD. Categorical data were analyzed using the Chi-square test or Fisher's exact test, and continuous variables were analyzed using the independent t-test. Pearson's test was used to assess the relationships between two quantitative variables. A multiple stepwise logistic regression analysis was used to identify independent predictors of a high neopterin level. The statistical analyses were performed using the SPSS statistical software package for Windows version 13 (SPSS for Windows, version 13; SPSS, IL, U.S.A.). A p value of < 0.05 was considered to be statistically significant.

Results

Baseline Characteristics of the Study Patients and Control Subjects (Table 1)

To test whether the circulating levels of neopterin are higher in the setting of cerebral ischemia (including patients with severe CA stenosis and AIS) than in healthy subjects, the serum level of neopterin in each study subject was measured using the standard ELISA method. Age, gender, body mass index, the presence

Table 2. Comparison of the Relevant Baseline Characteristics, Circulating Biomarkers and Imaging Findings of the 170 Cerebrovascular Disease Patients

Variables	CAS ($n=50$)*	AIS ($n=120$)*	p value
Age (yrs)	67.7 ± 9.4	64.7 ± 12.3	0.081
Male gender (%)	84.0% (42)	71.7% (86)	0.118
Body height (cm)	162.0 ± 7.7	162.7 ± 7.5	0.592
Body weight (kg)	65.4 ± 8.3	63.6 ± 12.4	0.297
Body mass index (kg/m^2)	24.9 ± 2.5	24.0 ± 4.3	0.175
Current smoking (%)	32.0% (16)	29.2% (35)	0.713
Diabetes mellitus (%)	44.0% (22)	36.7% (44)	0.371
Hypertension (%)	76.0% (38)	67.5% (81)	0.359
Dyslipidemia (%)†	58.0% (29)	42.5% (51)	0.091
Previous stroke by history (%)	48.0% (24)	25.0% (30)	0.003
Old stroke by MRI (%)	58.0% (29)	60.8% (73)	0.731
Systolic blood pressure (mmHg)	137.5 ± 16.4	144.1 ± 25.4	0.044
Diastolic blood pressure (mmHg)	78.4 ± 10.1	81.4 ± 13.7	0.111
Neopterin level (ng/dL)	42.3 ± 24.7	14.5 ± 6.8	< 0.001
WBC count (× 10^3/mL)	7.1 ± 2.1	8.1 ± 3.4	0.070
Creatinine level (mg/dL)	1.0 ± 0.4	1.1 ± 1.0	0.617
Significant CAD‡	68% (34)	–	–
Carotid artery stenosis ≥ 70%	100% (50)	0% (0)	< 0.001
Ulcerative plaque in ECCA§	80% (40)	0% (0)	< 0.001

The data are expressed as the mean ± SD or % (n) of patients.
* Carotid artery stenosis (CAS) = high-grade carotid artery stenosis (≥ 70%) patients with carotid artery stenting; AIS = acute ischemic stroke (AIS) patients without significant carotid artery stenosis.
† indicates patients with either hypercholesterolemia, hypertriglycemia or both.
‡ Coronary angiographic study identified coronary artery stenosis ≥ 50%.
§ Indicates that a carotid angiographic study showed the presence of ulcerative atherosclerotic plaque lesions (i.e., unstable plaque lesions).
CAD = coronary artery disease; ECCA = extracranial carotid artery (including either the common carotid or internal carotid artery); WBC = white blood cell.

of dyslipidemia and the creatinine levels did not differ between the cerebral ischemic patients and control subjects (**Table 1**). However, the incidence of risk factors for CAD, including hypertension, current smoking and diabetes mellitus, was significantly higher in the cerebral ischemic patients than in the control subjects. Additionally, the circulating levels of neopterin were significantly higher in the cerebral ischemic patients than in the control subjects. This finding indicates a correlation between brain ischemia and an increase in the circulating level of neopterin.

Comparison of Relevant Baseline Characteristics, Circulating Biomarkers and Imaging Studies in the 170 Cerebrovascular Disease Patients (Table 2)

Age, gender, body height, body weight and body mass index were similar in the patients with severe ECCA stenosis and those with AIS. Additionally, risk factors for CAD and the incidence of old stroke according to MRI findings did not differ between the patients with severe ECCA stenosis and the patients with AIS. Furthermore, diastolic blood pressure was similar between these two groups of study patients. However, the incidence of previous stroke was significantly higher and systolic blood pressure was notably lower in the severe ECCA stenosis patients than in the AIS patients. The creatinine levels and white blood cell counts did not differ between these two groups of patients. However, the circulating levels of neopterin and the incidence of CAD (≥ 50%) were significantly higher in the patients with severe ECCA stenosis than in the patients with AIS (**Table 2**).

Carotid and coronary angiographic studies were simultaneously performed in the severe ECCA stenosis patients; however, these procedures were not performed in the patients with AIS. The angiographic studies showed that 68% of the patients with severe ECCA stenosis had significant CAD (i.e., ≥ 50% stenosis). Additionally, 80% of these patients had vulnerable plaque lesions (i.e., ulcerative plaque lesions) over

Table 3. Correlations between the Serum Neopterin Levels and the Baseline Characteristics of the 170 Study Patients

Variables	*r*	*p* value
Age (yrs)	0.226	0.003
Male gender (%)	−0.082	0.288
Body mass index (kg/m^2)	−0.014	0.854
Current smoking (%)	−0.061	0.433
Diabetes mellitus (%)	0.022	0.771
Hypertension (%)	0.025	0.746
Dyslipidemia (%)*	0.001	0.993
Previous stroke by history (%)	0.218	0.004
Previous stroke by MRI (%)	0.034	0.655
Systolic blood pressure (mmHg)	−0.072	0.353
Diastolic blood pressure (mmHg)	−0.131	0.089
WBC count (×10^3/mL)	−0.066	0.394
Creatinine level (mg/dL)	0.024	0.754
High-grade carotid artery stenosis (≥70%)	0.660	<0.001
Ulcerative plaque†	−0.060	0.679

*indicates patients with either hypercholesterolemia, hypertriglycemia or both.
†Indicates that a carotid angiographic study showed the presence of ulcerative atherosclerotic plaque lesions (i.e., unstable plaque lesions).
MRI = magnetic resonance imaging

Table 4. Multivariate Binary Logistic Regression Analysis of Predictors of a High Neopterin Level among the 170 Cerebrovascular Disease Patients

Variables	Odds Ratio	95% CI	*p* value
High-grade carotid artery stenosis*	59.842	13.546-264.367	<0.001
Age ≥70 yrs old	2.774	1.269-6.064	0.011

CI = confidence interval.
*Severe carotid artery stenosis defined as carotid stenosis ≥70% on carotid duplex echo, magnetic resonance imaging and carotid angiographic studies.

either the internal carotid or common carotid artery documented on carotid angiographic studies.

Correlation between the Serum Neopterin Levels and Relevant Variables among the 170 Study Patients (Table 3)

Next, a Pearson correlation analysis was performed to elucidate which baseline variables among the 170 patients (patients with severe ECCA stenosis and patients with AIS) were correlated with the serum level of neopterin. Age, a history of previous stroke and the presence of severe ECCA stenosis were significantly correlated with an increased serum level of neopterin.

Independent Predictors of a High Circulating Level of Neopterin (Table 4)

To identify independent predictors of an increased serum level of neopterin among the 170 cerebrovascular disease patients, a multivariate binary logistic regression analysis was performed. Severe ECCA stenosis was the strongest independent predictor of an increased serum level of neopterin (**Table 4**). Additionally, an age ≥70 years was also a significant independent predictor of an increased serum level of neopterin.

Correlation between the Serum Neopterin Level and the Severity of ECCA Stenosis and Relevant Variables among the 50 Severe ECCA Stenosis Patients (Table 5)

Additionally, a Pearson correlation analysis was

Table 5. Correlations between the Neopterin Levels and the Baseline Variables in the 50 High-Grade Carotid Artery Stenosis Patients

Variables	*r*	*p* value
Age (yrs)	0.345	0.014
Male gender (%)	0.050	0.732
Body mass index (kg/m^2)	−0.129	0.386
Current smoking (%)	−0.089	0.539
Diabetes mellitus (%)	−0.006	0.969
Hypertension (%)	−0.057	0.693
Dyslipidemia (%)	−0.171	0.235
Previous stroke (%)	0.158	0.274
Previous stroke by MRI (%)	0.046	0.749
Systolic blood pressure (mmHg)	−0.009	0.950
Diastolic blood pressure (mmHg)	−0.253	0.077
White blood cell count (×10^3/mL)	0.050	0.732
Creatinine level (mg/dL)	0.364	0.009
Significant CAD	−0.159	0.272
Ulcerative plaque	−0.060	0.679

MRI = magnetic resonance imaging; CAD = coronary artery disease, defined as coronary angiographic findings of coronary artery obstruction ≥ 50%

performed to determine the correlation between the baseline variables and the circulating levels of neopterin in the 50 severe ECCA stenosis patients. The results demonstrated that age and the creatinine level were the two relevant variables strongly and significantly associated with an increased circulating level of neopterin (**Table 5**). However, there were no statistically significant correlations between the circulating neopterin level and the severity of ECCA stenosis (r = 0.099 p = 0.493). On the other hand, when we compared the ECCA stenting patients with the 20 AIS patients who had received carotid Doppler/MRA to evaluate carotid artery stenosis, we found that there was a statistically significant correlation between the circulating neopterin level and the severity of carotid artery stenosis (r = 0.514, p < 0.0001).

A receiver operating characteristics (ROC) curve of the cutoff value of neopterin for predicting significant ECCA stenosis (≥ 70%) was calculated (**Fig. 1**). The results showed that a circulating level of neopterin of ≥ 21.9 ng/dL was the most powerful predictor of ECCA stenosis of ≥ 70%, with a sensitivity of 92.0% and a specificity of 92.2%. The positive predictive value was 79.7% and the negative predictive value was 97.2%. The area under the curve (AUC) for a cutoff value of neopterin of ≥ 21.9 was 0.942 [95% confidence interval (CI), (0.895, 0.988), p < 0.001].

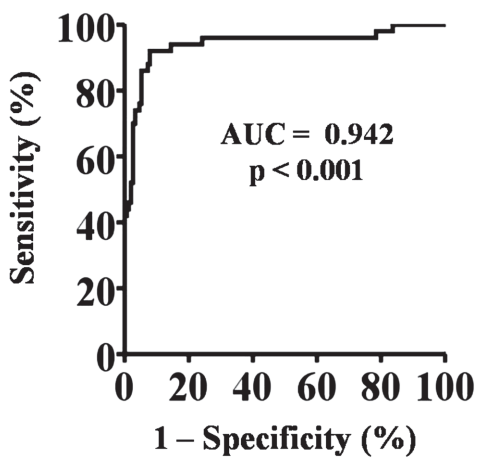

Fig. 1. The receiver operating characteristics (ROC) curve analysis revealed that the a circulating level of neopterin of ≥ 21.9 ng/dL was the most powerful predictor of ECCA stenosis of ≥ 70%, with a sensitivity of 92.0% and a specificity of 92.2%, p < 0.0001. AUC = area under the curve.

Comparison of the Baseline Characteristics, Laboratory and Imaging Findings and Angiographic Results in the 50 Severe ECCA Stenosis Patients Divided into High and Low Neopterin Groups (Table 6)

To investigate the associations between the relevant variables and high and low levels of circulating neopterin, the patients with a neopterin level of < 34.6

Table 6. Comparison of Baseline Characteristics between the High and Low Neopterin Groups among the 50 High-Grade CAS Patients with Carotid Stenting

Variables	High neopterin* Group 1 ($n=25$)	Low neopterin* Group 2 ($n=25$)	p value
Age (yrs)	70.6 ± 9.1	64.8 ± 8.8	0.028
Male gender (%)	76.0% (19)	92.0% (23)	0.247
Body mass index (kg/m^2)	24.6 ± 1.5	25.2 ± 3.0	0.636
Current smoking (%)	20.0% (5)	44.0% (11)	0.069
Diabetes mellitus (%)	44.0% (11)	44.0% (11)	1.000
Hypertension (%)	80.0% (20)	72.0% (18)	0.508
Dyslipidemia (%)	48.0% (12)	68.0% (17)	0.152
Previous stroke by history (%)	52.0% (13)	44.0% (11)	0.571
Previous stroke by MRI (%)	64.0% (16)	52.0% (13)	0.390
Neopterin level (ng/dL)	59.3 ± 24.5	25.4 ± 7.0	< 0.001
White blood cell count (× 10^3/mL)	7.1 ± 1.9	7.1 ± 2.4	0.892
Creatinine level (mg/dL)	1.2 ± 0.5	0.9 ± 0.2	0.016

The data are expressed as the mean ± SD or % (n) of patients.
*High neopterin group defined as patients with ≥ the median level (34.6 ng/dL) of neopterin and low neopterin group defined as patients with < the median level (34.6 ng/dL) of neopterin.
MRI = magnetic resonance imaging.

Table 7. Comparison of the Incidence of Coronary Artery Disease and Carotid Artery Obstruction Based on Angiographic Results between the High and Low Neopterin Groups Among the 50 High-Grade CAS Patients

Variables	High neopterin* Group 1 ($n=25$)	Low neopterin* Group 2 ($n=25$)	p value
Coronary angiographic findings of CAD†			
Left main (%)	4% (1)	8% (2)	1.000
Left anterior descending artery (%)	44% (11)	44% (11)	1.000
Left circumflex artery (%)	40% (10)	56 (14)	0.258
Right coronary artery (%)	32% (8)	36% (9)	0.765
Incidence of ischemic heart disease	56% (14)	80% (20)	0.128
Carotid angiographic findings			
Right common carotid artery (%)	8% (2)	8% (2)	1.000
Right internal carotid artery (%)	56% (14)	64% (16)	0.564
Left common carotid artery (%)	4% (1)	4% (1)	1.000
Left internal carotid artery (%)	60% (15)	76% (19)	0.225
Carotid stenosis (%)	79.8 ± 9.7	81.7 ± 11.1	0.518
MLD of culprit lesion (mm)	1.16 ± 0.56	1.28 ± 0.75	0.507
RVD of culprit lesion (mm)	5.43 ± 0.98	4.98 ± 1.23	0.155
Lesion length (mm)	18.9 ± 4.9	19.2 ± 5.5	0.851

The data are expressed as the mean ± SD or % (n) of patients.
*Higher neopterin group defined as patients with > the median level (34.62 ng/dL) of total neopterin and lower neopterin group defined as patients with < the median level (34.62 ng/dL) of neopterin. BMI = body mass index; WBC = white blood cell count.
† CAD = coronary artery disease, defined as coronary angiographic findings of coronary artery obstruction ≥ 50%

ng/dL were classified into a low neopterin group (group 2), while those with a neopterin level of ≥ 34.6 ng/dL were classified into a high neopterin group (group 1) (**Tables 6** and **7**).

As shown in **Table 6**, the mean level of neopterin was significantly higher in group 1 than in group 2 ($p < 0.001$). The two groups were similar in terms of the body mass index, gender, cardiovascular risk factors, history of stroke, magnetic resonance images and white blood cell count. However, age and the creati-

Table 8. Multivariate Binary Logistic Regression Analysis of Predictors of a High Neopterin Level in the 50 High-Grade Carotid Artery Stenosis Patients

Variables	Odds Ratio	95% CI	*p* value
Creatinine*	12.047	1.223-118.650	0.033
Age*	1.081	1.005-1.163	0.037

CI = confidence interval.
* indicates that a continuity value was used in the analysis.

nine levels were significantly increased in group 1 in comparison with those observed in group 2.

There were no significant correlations between the circulating level of neopterin and the carotid or coronary angiographic findings (**Table 7**).

Independent Predictors of a High Circulating Level of Neopterin in the 50 Patients with Severe ECCA Stenosis (Table 8)

To determine which variables were independent predictors of a high neopterin level in the 50 severe ECCA stenosis patients, a multivariate binary logistic regression analysis was performed. The results showed that age and the creatinine level were the only independent predictors of a high level of circulating neopterin.

Discussion

This study investigated the circulating levels of neopterin in patients with severe ECCA stenosis undergoing CA stenting. The results have several clinical implications. First, the circulating levels of neopterin were significantly increased in the AIS patients and more markedly increased in the severe ECCA stenosis patients than in the control subjects. Second, the circulating levels of neopterin did not differ between the severe ECCA stenosis patients with or without angiographic findings of plaque disruption. Third, when the AIS patients with insignificant ECCA stenosis were pooled together with the AIS patients with severe ECCA stenosis, a statistically significant correlation between the circulating neopterin level and the severity of ECCA stenosis was found in the current study. Finally, age, the creatinine level and the presence of severe ECCA stenosis were each significantly positively correlated with the circulating level of neopterin.

The Levels of Circulating Neopterin in the Patients with Cerebrovascular Diseases

The serum neopterin levels were remarkably increased in the patients with ECCA stenosis and AIS in comparison with those observed in the control subjects. Previous studies have reported that the neopterin levels are notably higher in patients with carotid artery disease than in healthy subjects[6, 14, 22]. Recently, we also showed that the circulating neopterin level is remarkably increased after AIS[25]. Additionally, an increased circulating level of neopterin has been suggested to be a useful biomarker for predicting acute coronary syndrome[19, 20]. In a review of acute arterial occlusive syndrome, which is two sides of the same coin of acute coronary syndrome and AIS, it is not surprising that the data from our recent report[25], the present study and previous studies are comparable[19, 20]. Therefore, the findings of the current study reinforce the findings of previous studies[6, 14, 22, 25].

Increased inflammation has been reported in patients with carotid artery disease and AIS[14, 22, 30]. Additionally, the neopterin level has been reported to be a useful biomarker of immune activation and inflammatory reactions[16-18]. The present study found that the circulating levels of neopterin were markedly increased in both patients with severe ECCA stenosis and those with AIS, suggesting the progression of inflammation in these patients. Interestingly, our other recent study demonstrated that the serum level of high-sensitivity C-reactive protein (hs-CRP), an indicator of inflammation, is remarkably increased during both the acute and convalescent phases of ischemic stroke and is independently predictive of 90-day major adverse neurological events in patients with ischemic stroke[31]. Therefore, we suggest that these two inflammatory biomarkers (i.e., the hs-CRP and neopterin levels) are potentially mutual factors predictive of prognostic outcomes in patients with AIS. In addition to strengthening the findings of previous studies[16-18], this study further supports the link between cerebrovascular diseases and persistent inflammatory reactions. These findings thus encourage the use of immunomodulatory drugs and medications that can attenuate the inflammatory process.

The most important finding of the present study is that the circulating levels of neopterin were substantially higher in the severe ECCA stenosis setting than

in the AIS setting. This finding suggests first that an increased circulating level of neopterin is more strongly related to the chronic persistent rather than acute inflammatory phase and second that the plaque burden is a more useful factor than plaque location for predicting an increase in the circulating levels of neopterin in cerebrovascular disease patients. Third, of distinctive importance, is that in situations of severe ECCA stenosis or AIS, circulating monocytes are activated and can excrete neopterin into the circulation. This may be a better contributing factor for explanation of the increase in the plasma neopterin levels in the setting of severe ECCA stenosis and AIS. Finally, we suggest that serial monitoring of the circulating level of neopterin is important and useful for stratifying patients into low-risk and high-risk subgroups to provide different management strategies for patients with AIS. Our recent data demonstrated that measuring the serum level of neopterin once at 48 hours after AIS effectively predicts 90-day unfavorable clinical outcomes in patients with acute IS[25].

Predictors of an Increased Circulating Level of Neopterin

A previous study clearly showed a preponderance of inflammatory cells in carotid artery plaque[14]. Additionally, another previous study reported that an elevated level of neopterin is associated with carotid plaque with a complex morphology in patients with stable angina pectoris[6]. We therefore hypothesized that an increased circulating level of neopterin is strongly correlated with the inflammatory process and plaque instability. Unexpectedly, we did not find this correlation. A recent study also showed that the level of neopterin is consistently related to the presence of atherosclerotic disease in the carotid artery but not carotid plaque vulnerability[22]. The findings from both our previous study and another recent[22] study may perhaps be due to a time-interval gap, i.e., the half-life of the acute elevated neopterin level after acute plaque rupture is relatively short and the angiographic findings of ulcerative plaques (vulnerable plaques) in the carotid artery are delayed.

Severe ECCA stenosis was the factor most strongly correlated with an increased circulating level of neopterin in the 170 cerebrovascular disease patients. This finding suggests that the neopterin level is a useful accessory biomarker for assessing the significance of ECCA stenosis in daily clinical practice. Additionally, age, a previous history of stroke and the creatinine level were also significantly correlated with an increased circulating level of neopterin. Abundant data have exhibited associations between inflammation, age and chronic kidney disease[32, 33]. Furthermore, circulating inflammatory biomarkers are usually found in patients with ischemic stroke[34]. Our findings are therefore considered to be compatible with the previous findings[32-34].

Study Limitations

This study is associated with several limitations. First, the severe ECCA stenosis sample size was relatively small. This may be one reason why no correlations between the circulating neopterin level and plaque vulnerability were found. Additionally, the relatively small sample size and the fact that all of the carotid stenting patients had high-grade (≥70.0%) ECCA stenosis (i.e., an inhomogeneous distribution of the severity of ECCA stenosis) may explain why there were no statistically significant correlations between the circulating neopterin level and the severity of ECCA stenosis when only the severe ECCA stenosis (≥70.0%) patients were taken into consideration. Second, we did not measure the circulating levels of neopterin after carotid artery stenting. Therefore, we could not measure the impact of carotid stenting on the regulation of the circulating levels of neopterin.

In conclusion, the findings of the present study demonstrated that the circulating neopterin levels are remarkably higher in severe ECCA patients than in AIS patients. When mild to severe carotid artery obstruction in both the severe CAS and AIS patients was taken into account in the statistical analysis, an increased level of neopterin in the circulation was significantly predictive of severe (i.e., ≥70.0%) ECCA stenosis. This implies that the serum neopterin level is correlated with the atherosclerotic plaque burden rather than plaque vulnerability.

Conflicts of Interest

None.

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