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Association between the ratio of anti-angiogenic isoform of VEGF-A to total VEGF-A and adverse clinical outcomes in patients after acute myocardial infarction

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ABSTRACT

Background: Vascular endothelial growth factor-A (VEGF-A) promotes neovascularization and is attracting considerable attention as a remarkable risk factor in patients after acute myocardial infarction (AMI). In contrast, the association between VEGF-A_{165}b, which is the main anti-angiogenic isoform of VEGF-A, and adverse clinical outcomes after AMI remains unclear. The present study aimed to investigate the association between serum VEGF-A_{165}b and major adverse cardiac and cerebrovascular events (MACCEs) after percutaneous coronary intervention (PCI) for AMI.

Methods: We evaluated 23 patients with AMI who underwent primary percutaneous coronary intervention. VEGF-A and VEGF-A_{165}b levels were measured on admission (day 1) and at days 3, 7, and 30 after PCI.

Results: The levels of total VEGF-A tended to be lower, while the ratio of VEGF-A_{165}b to total VEGF-A tended to be higher in patients with MACCEs than in those without. The patients with a high ratio of VEGF-A_{165}b to total VEGF-A had a significantly higher risk of MACCEs using the cut-off values for MACCEs at day 30 after PCI (0.87 vs. 0.25, log-rank test, $p = 0.0058$).

Conclusion: The assessment of VEGF-A_{165}b combined with VEGF-A may be a valuable screening tool for predicting MACCEs in clinical practice.

1. Introduction

Vascular endothelial growth factor-A (VEGF-A) has become the focus of intense interest because of its essential role in neovascularization [1]. VEGF-A is up-regulated in hypoxia and can promote angiogenesis after acute myocardial infarction (AMI) [2]. Previous clinical studies on VEGF-A in patients with AMI have demonstrated that low levels of circulating VEGF-A were an independent risk factor for adverse clinical outcomes after AMI [3,4]. VEGF-A produces various isoforms with distinct biological activities through alternative messenger RNA splicing [5]. VEGF-A_{165}b is the main anti-angiogenic isoform of VEGF-A [6,7].

Several reports have demonstrated the remarkable elevation of VEGF-A_{165}b in some diseases characterized by impaired angiogenesis, vascular damage, and hypoxia such as systemic sclerosis [8], and peripheral artery disease (PAD) [9–11]. And it is suggested that VEGF-A_{165}b prevents the physiological consequences of the pro-angiogenic behavior of VEGF-A by several VEGF receptor signals [14]. Recent studies demonstrated VEGF-A_{165}b associated with infarct size in patients with AMI [17] and dysregulated VEGF-A_{165}b in senescent endothelial cells may contribute to the risk of coronary heart disease [18]. Insufficient angiogenesis can inhibit the healing process of the myocardium and endothelial cells after AMI. However, the association between VEGF-A_{165}b and adverse clinical outcomes after AMI has not been elucidated. Therefore, we evaluated the serial changes in circulating serum VEGF-A and VEGF-A_{165}b levels and their association with adverse clinical outcomes after percutaneous coronary intervention (PCI) in patients with AMI.

2. Methods

This study was a prospective, single-center, observational study approved by the ethics committee of the Nagoya University Graduate

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School of Medicine and conducted in accordance with the ethical principles of the Declaration of Helsinki.

From July 2015 to February 2017, we evaluated 66 consecutive patients with AMI who underwent PCI within 24 h of symptom onset at Nagoya University Hospital. Exclusion criteria were as follows: patients with hemodialysis ($n = 4$), active malignancy ($n = 5$), and collagen disease ($n = 2$) because these conditions affect the VEGF-A and VEGF-A_{165}b levels. Finally, 23 patients for whom blood samples could be obtained at all time points (on admission [day 1] and at days 3, 7, and 30 after PCI were enrolled in this study.

AMI was diagnosed based on the third universal definition of myocardial infarction [19]. The primary endpoint was major adverse cardiac and cerebrovascular events (MACCEs), which were defined as the composite of cardiovascular death, recurrent myocardial infarction (MI), coronary revascularization, and stroke after AMI. Coronary revascularization was defined as PCI or coronary artery bypass grafting. PCI performed on a later day for the residual lesions identified during primary PCI was excluded. Stroke was defined as a newly developed neurological deficit and relevant findings on magnetic resonance imaging. The patients were divided into two groups according to the incidence of MACCEs.

Blood samples were obtained from all patients on admission (day 1) and at days 3, 7, and 30 after PCI. Serum samples were stored at −80 °C. Serum VEGF-A levels were determined using an enzyme-linked immunosorbent assay (ELISA) kit (Human VEGF Quantikine ELISA Kit, DVE00, R&D Systems), and the limit of detection was 9 pg/mL (intra-assay and inter-assay coefficients of variation were 4.5% and 7.0%, respectively). VEGF-A ELISA kit does not discriminate between pro- and anti-angiogenic isoforms of VEGF-A. Serum VEGF-A_{165}b levels were determined using the ELISA kit (Human VEGF-$_{165}$b ELISA Kit, MBS720132, MyBiosource), and the limit of detection was 1 pg/mL (intra-assay and inter-assay coefficients of variation were <10% and <10%, respectively) [9]. We obtained creatine kinase (CK) levels on admission, every 3 h until peak values were reached, and 24 h after PCI. We analyzed the area under the concentration versus time curve (AUC) for CK [20]. The left ventricular ejection fraction (LVEF) was measured with echocardiography at an outpatient clinic within 6 months after PCI.

2.1. Statistical analysis

Date is presented as mean ± standard deviation, median (interquartile range [IQR]) or numbers (percentages). The differences in normally distributed values were assessed using the Student *t*-test, while the asymmetrically distributed values were assessed using the Mann Whitney *U* test. The differences in categorical variables were assessed using the Kruskal-Wallis test or chi-square test. Kaplan-Meier analysis was performed to evaluate the cumulative incidence of MACCEs, and comparisons were assessed using the log-rank test. The cut-off values for MACCEs were established using receiver operating characteristic (ROC) curve analysis. A p-value <0.05 was considered statistically significant.

3. Results

The clinical characteristics are summarized in Table 1. During the median follow-up of 187 (IQR: 73–402) days, 9 (39.1%) MACCEs occurred.

The levels of total VEGF-A increased after PCI and reached their peak at day 7, whereas the levels of VEGF-A_{165}b maintained the similar values at each time point (Fig. 1). We also assessed the serial changes in the ratio of VEGF-A_{165}b to total VEGF-A in order to evaluate the total VEGF-A and VEGF-A_{165}b levels simultaneously. The ratio of VEGF-A_{165}b to total VEGF-A was at its lowest at day 7 after PCI, and it increased again at day 30 after AMI (Fig. 1).

Table 1
Clinical characteristics.

Variables		MACCE		*p*
	All ($n = 23$)	No ($n = 14$)	Yes ($n = 9$)	
Age (years)	67.0 ± 10.8	64.3 ± 11.3	71.2 ± 9.0	0.14
Male, n (%)	17 (73.9)	11 (78.5)	6 (66.7)	0.44
Body mass index (kg/m^2)	22.9 ± 2.9	23.2 ± 3.0	22.4 ± 3.0	0.54
Current smoking, n (%)	7 (30.4)	4 (28.6)	3 (33.3)	0.58
Hypertension, n (%)	10 (43.5)	6 (42.9)	4 (44.4)	0.64
Diabetes mellitus, n (%)	7 (30.4)	5 (35.7)	2 (22.2)	0.42
Dyslipidemia, n (%)	16 (69.6)	10 (71.4)	6 (66.7)	0.58
eGFR (mL/min/1.73 m^2)	60.9 (57.0–71.8)	62.1 (58.8–74.1)	59.5 (54.2–68.4)	0.40
LDL cholesterol (mg/dL)	133 ± 28.7	137 ± 29.6	128 ± 28.1	0.52
HDL cholesterol (mg/dL)	50.1 ± 11.4	49.0 ± 10.2	51.9 ± 13.4	0.56
Triglycerides (mg/dL)	182 ± 103	187 ± 93.2	175 ± 121	0.78
Hemoglobin A1c (%)	5.8 (5.7–6.8)	5.9 (5.8–6.9)	5.8 (5.6–6.6)	0.64
C-reactive protein (mg/L)	1.0 (0.7–2.5)	1.0 (0.7–4.8)	0.8 (0.7–3.0)	0.56
Creatine kinase (AUC) (IU/L × h)	43,142 (29788–88,934)	51,164 (31,922–92,034)	40,748 (18,932–78,411)	0.69
Peripheral artery disease, n (%)	2 (8.7)	1 (7.1)	1 (11.1)	0.66
Time to reperfusion (min)	90.9 ± 31.9	85.6 ± 24.9	98.4 ± 40.4	0.37
Culprit lesion (LAD, LCX, RCA)	56.5%, 13.0%, 30.4%	21.4%, 57.1%, 21.4%	44.4%, 55.6%, 0%	0.10
Killip class (I, II, III, IV)	70%, 4.3%, 8.7%, 17%	79%, 0%, 0%, 21%	56%, 11%, 22%, 11%	0.40
TIMI flow grade before PCI (0, 1, 2, 3)	74%, 4.3%, 13%, 8.7%	57%, 7.1%, 21%, 14%	100%, 0%, 0%, 0%	0.03
TIMI flow grade after PCI (0, 1, 2, 3)	0%, 4.3%, 4.3%, 91%	0%, 0%, 7.1%, 93%	0%, 11%, 0%, 89%	0.39
LVEF after PCI (%)	53.3 ± 10.9	55.6 ± 8.3	49.7 ± 13.8	0.21
Medication at discharge				
Antiplatelet agents, n (%)	23 (100)	14 (100)	9 (100)	
ACE-I or ARB, n (%)	21 (91.3)	13 (92.9)	8 (88.9)	0.64
Beta-blocker, n (%)	14 (60.9)	10 (71.4)	4 (44.4)	0.20
Calcium channel blocker, n (%)	3 (13.0)	2 (14.3)	1 (11.1)	0.67
Statin, n (%)	22 (95.7)	13 (92.9)	9 (100)	0.61

Data are indicated as means ± SD or median (interquartile range) or number (percentages). MACCE, major adverse cardiac and cerebrovascular events; eGFR, estimated glomerular filtration rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein; AUC, area under the concentration versus time curve; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; RCA, right coronary artery; TIMI, thrombolysis in myocardial infarction; LVEF, Left ventricular ejection fraction; PCI, percutaneous coronary intervention; ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker.