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An Increased Monocyte Count Predicts Coronary Artery Spasm in Patients with Resting Chest Pain and Insignificant Coronary Artery Stenosis

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Background : Coronary atherosclerosis with inflammation gives rise to coronary vasospasm in the patients with coronary vasospastic angina. We have postulated that the peripheral leukocyte count and the differential count are associated with vasospastic angina.

Methods : 144 patients who underwent intracoronary ergonovine provocation testing between January 2002 and December 2004 were divided into two groups: Group I (72 patients with provoked spasm, mean age: 54.8±10.7 years, males: 75%) and Group II (72 without spasm, mean age: 55.3±10.2 years, males: 35%). Blood sampling was done to measure the lipid profiles and inflammatory markers, including the high sensitive C-reactive protein (hsCRP) levels and the monocyte counts. We compared the angiographic findings and laboratory data between the two groups.

Results : There were no significant differences in the levels of serum lipid and hsCRP between the two groups. The white blood cell count and the monocyte count were higher in Group I than with Group II (7496.4±2622.28 vs. 6703.2±1768.37/mm^3, respectively, p=0.035; 627.5±270.70 vs. 426.9±205.76/mm^3, respectively, p<0.001). Gensini's score was higher in Group I than in Group II (2.2±2.88 *vs.* 0.5±1.03, respectively, p<0.001). Multivariate analysis showed that the monocyte count and Gensini's score were independent factors affecting coronary spasm (p=0.047 and p=0.018, respectively). According to a receiver operating characteristics curve analysis, the area under the curve of the monocyte count was 0.738, that of the neutrophil count was 0.577 and that of the WBC count was 0.572. The cut-off value of the monocyte count was 530/mm^3; the sensitivity and specificity of this cut-off value were 64% and 76%, respectively.

Conclusions : The peripheral monocyte count is an independent marker for predicting vasospastic angina in the patients with resting chest pain and insignificant coronary artery stenosis.

Key Words : Coronary disease, Atherosclerosis, Vasospasm, Leukocytes

INTRODUCTION

Coronary artery spasm plays an important role in the pathogenesis of a variety of ischemic heart disease, including not only variant angina, but also unstable angina, myocardial infarction and sudden death[1]. Although it is still unclear, coronary artery spasm seems to be closely related to the

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atherosclerotic change in blood vessels.

A few studies have recently reported that atherosclerotic lesions and elevated levels of biologic markers such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are observed in the patients with coronary vasospasm, and these biologic markers are involved in the early inflammatory responses[2, 3]. Other studies have reported that the peripheral monocyte count and the percentage of activated T-lymphocytes are increased in the patients with variant angina[4, 5].

It also has been widely accepted that the peripheral leukocyte count or the level of high sensitivity C-reactive protein (hsCRP) are indicators for the atherosclerotic change in the early inflammatory responses[6]. In this study, we assessed the feasibility using the peripheral leukocyte count and the differential count for diagnosing the patients with vasospastic angina.

MATERIALS AND METHODS

Study Population

We retrospectively reviewed the medical records of 144 patients who underwent intracoronary ergonovine provocation testing at Wonkwang University Hospital between January 2002 and December 2004. The intracoronary ergonovine test was performed (1) for the patients in which chest pain was noted at rest (2) for those patients whose cardiac attack was relieved by the use of sublingual nitroglycerin and (3) for those patients in whom significant coronary artery diseases (>50% of the luminal diameter of the major coronary arteries) were absent. The exclusion criteria were (1) cases in which acute myocardial infarction was noted within the recent six months (2) those cases in which coronary intervention was performed (3) those cases with other infectious diseases and (4) those cases with hepatic and renal diseases.

Data Collection

Coronary angiography was performed with the patients in a fasting state by the Judkin method following puncture of the femoral artery or via a radial artery approach. No pharmacological therapy except nitrate injection was attempted for at least 72 hour prior to coronary angiography. The severity of coronary atherosclerotic lesions in all the patients was evaluated on at least three projections.

Ergonovine provocation testing was performed for the patients in whom significant coronary stenosis was absent, as previously reported[7, 8]. *First*, the 12 lead electrocardiogram and arterial pressure were monitored after the carbon electrodes (Fukuda Ltd., Japan) were attached; *second*, ergonovine in 0.9% saline solution was injected into the right coronary artery at 10 μg/min for 4 min for a maximal dose of 40 μg, and then the ergonovine was injected into the left coronary artery at 16 μg/min for 4 min for a total dose of 64 μg with at least a 5 min interval between each injection; and *third*, the occurrence of chest pain, the change of the ST segment on the EKG and the development of spasm on coronary angiography were examined. We performed frequent test shots at 30-sec intervals with using contrast media during testing, if possible. Positive results were defined as cases in which more than 99% of the focal spasm was noted on coronary angiography in the presence of typical chest pain or abnormal EKG findings. We assigned the patients with spasm and those without spasm to Groups I and II, respectively.

The coronary arteries were measured each time after the intracoronary administration of nitrate and after the completion of testing. The angiographic characteristics of the coronary atherosclerotic lesions were defined by the Gensini's score[9]. In this scoring system, a greater reduction of the luminal diameter is assigned a higher score and a proximal lesion in the left anterior descending or the circumflex artery is assigned a higher score than a distal lesion. Significant stenosis was defined as a luminal narrowing of 50% or greater.

After overnight fasting, blood sampling was done to measure the total cholesterol, triglyceride, high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol, lipoprotein (a), the erythrocyte sedimentation rate (ESR) and the high-sensitivity C-reactive protein (hsCRP). A complete blood count (CBC) with a differential count was performed within 24 hours before the coronary angiography. We compared the laboratory data between the two groups.

Statistical Analysis

Statistical analysis was done using dBSTAT for Window (dBSTAT Inc, Seoul, Korea). All the data were expressed as means±standard deviation. Intergroup analysis was done using the independent *t*-test and the χ^2 test. Multivariate analysis was done to determine the factors related to vasospastic angina. A receiver operating characteristics (ROC) curve analysis was done to determine whether a CBC with the differential count would be feasible for making the diagnosis. Statistical significance was set at $p<0.05$.

RESULTS

In our series, the mean age of patients was 55.1±10.4 and the male-to-female ratio was 79:65. Overall, 72 patients were positive for the intracoronary ergonovine test with a positive rate of 50%. The mean value of Gensini's score was 1.34±2.33

Table 1. Baseline characteristics

Age (years)	55.1±10.44
Male gender (%)	79 (54.9)
Presence of provoked spasm (%)	72 (50.0)
Systemic hypertension (%)	17 (11.8)
Diabetes mellitus (%)	10 (6.9)
Smokers (%)	60 (41.7)
Previous history of statin therapy (%)	8 (5.6)
Family history of coronary artery disease (%)	23 (15.9)
Ejection fraction (%)	68.2±10.55
Gensinis score	1.34±2.33

(Table 1).

Coronary spasm was more common in the males than in the females (75.0% vs. 34.7%, respectively $p<0.001$); and it was also more common in smokers than in non-smokers (59.7% vs. 23.6%, $p<0.001$). There were no significant differences in the concentrations of serum lipid, hsCRP and ESR between the two groups. The levels of the white blood cell count were 7496.4±2622.28/mm^3 (range: 3,100～16,900/mm^3) in Group I and 6703.2±1768.37/mm^3 (range: 3,600～12,100/mm^3) in Group II, and the levels of the monocyte count were 627.5±270.70/mm^3 (range: 200～1,630/mm^3) in Group I and 426.9±205.76/mm^3 (range: 100～1,000/mm^3) in Group II. The Wwhite blood cell (WBC) and monocyte counts were significantly higher in Group I than in Group II ($p=0.035$ and $p<0.001$, respectively). Gensini's score was also significantly higher in Group I than in Group II (2.2±2.88 vs. 0.5±1.03, respectively, $p<0.001$) (Table 2).

The mMonocyte count was more elevated in the smokers than in the non-smokers (623.3±292.10 vs. 458.6±210.35/mm^3, respectively, $p<0.001$), in the male patients than in the female patients (579.9±271.40 vs. 463.2±231.37/mm^3, respectively, $p=0.007$), and in the patients with diabetes than in the patients without diabetes (683.0±346.03 vs. 515.6±250.09/mm^3, respectively, $p=0.049$), but the monocyte count was not more elevated in the patients with hypertension than in the patients without hypertension (574.1±315.85 vs. 520.9±252.26/mm^3, respectively, $p=0.430$). The monocyte count was positively correlated with Gensini's score ($r=0.192$, $p=0.021$).

Multivariate analysis showed that the peripheral monocyte count and Gensini's score were independent factors for coronary vasospastic angina ($p=0.047$ and $p=0.018$, respectively) (Table 3). To estimate the risk of vasospatic angina, we adjusted the odds ratio of the monocyte count; the greater the risk, the higher the monocyte count. The risk of developing vasospasm was more than ten times higher in the patients whose monocyte count was $\geq$606/mm^3 than those whose monocyte count was $\leq$375/mm^3 (Table 4).

A receiver operating characteristics (ROC) curve analysis was done to assess the feasibility of using the total WBC count, including the neutrophil and monocyte counts, in diagnosing patients with coronary vasospastic angina. According to an ROC curve analysis, the area under the curve (AUC) of the monocyte count was 0.738, which was greater than that of the neutrophil count (0.577, $p=0.007$) and the WBC count (0.572, $p=0.003$). This suggests that the monocyte count was a more reliable diagnostic indicator than the neutrophil and WBC counts

Table 2. Clinical and laboratory findings

Characteristics	Group I (n=72)	Group II (n=72)	*p* value
Age (years)	54.8±10.73	55.3±10.20	0.763
Males (%)	54 (75.0)	25 (34.7)	<0.001
Smokers (%)	43 (59.7)	17 (23.6)	<0.001
Hypertension (%)	11 (15.3)	6 (8.3)	0.302
Diabetes mellitus (%)	6 (8.3)	4 (5.6)	0.743
WBC (/mm^3)	7496.4±2622.28	6703.2±1768.37	0.035
Neutrophil (/mm^3)	4385.6±2052.94	3802.6±1480.63	0.055
Lymphocyte (/mm^3)	2170.0±914.72	2185.9±544.57	0.899
Monocyte (/mm^3)	627.5±270.70	426.9±205.76	<0.001
Eosinophil (/mm^3)	307.89±250.50	220.9±242.92	0.143
ESR (mm/hr)	12.1±15.17	10.6±8.64	0.675
hsCRP (mg/L)	3.5±5.72	2.2±2.68	0.187
Total cholesterol (mg/dL)	184.7±32.04	192.4±38.12	0.191
Triglyceride (mg/dL)	183.0±134.09	167.3±82.02	0.398
HDL-cholesterol (mg/dL)	48.4±13.66	49.47±9.36	0.578
LDL-cholesterol (mg/dL)	106.9±30.65	114.95±41.62	0.194
BUN (mg/dL)	15.9±4.64	14.7±4.17	0.108
Creatinine (mg/dL)	1.0±0.30	0.9±0.19	0.005
Uric acid (mg/dL)	4.9±1.30	5.3±1.76	0.623
Gensinis score	2.2±2.88	0.5±1.03	<0.001

WBC, white blood cell; ESR, erythrocyte sedimentation rate; hsCRP, high-sensitivity C-reactive protein; HDL, high density lipoprotein; LDL, low density lipoprotein; BUN, blood urea nitrogen.

Table 3. Predictive factors of coronary vasospastic angina according to multivariate analysis

Variable	Odds ratio	95% Confidence interval	*p* value
Male gender	3.699	0.569-24.061	0.171
Smoker	0.729	0.124-4.309	0.728
WBC count	0.999	0.999-1.001	0.634
Neutrophil count	1.000	0.999-1.001	0.616
Monocyte count	1.004	1.000-2.009	0.047
Total cholesterol	0.995	0.975-1.016	0.632
hsCRP	1.025	0.867-1.212	0.772
Serum creatinine	22.167	0.269-1828.746	0.169
Gensinis score	1.753	1.103-2.785	0.018

WBC, white blood cell; hsCRP, high-sensitivity C-reactive protein.

Table 4. Adjusted odds ratios for coronary vasospastic angina in the quartiles of the monocyte count

Variable	Odds ratio	95% Confidence interval	*p* value
1st ($\leq$375/mm^3)*	1.000		
2nd (376-500/mm^3)	3.423	1.228-9.539	0.019
3rd (501-605/mm^3)	8.285	2.699-25.428	<0.001
4th ($\geq$606/mm^3)	10.774	3.581-32.416	<0.001

*The first quartile was used as a reference group.

(Figure 1). The cut-off value was 530/mm^3, for which the sensitivity and specificity were 64% and 76%, respectively.

DISCUSSION

This clinical study indicates that an increased peripheral monocyte count is an independent factor related to coronary vasospastic angina and it is a predictor of coronary vasospasm in patients with resting angina and insignificant coronary artery stenosis.

Coronary artery spasm has been regarded as an etiologic factor that's involved in the development of various ischemic heart diseases. Nevertheless, the exact mechanism by which coronary spasm develops remains unclear. To date, many authors have postulated that the irregular activity of the autonomic nervous system (ANS) provokes vasospastic angina; that is, once the ANS is activated, it not only stimulates vascular smooth muscle cells to contract, but it also induces platelets to release serotonin, a powerful coronary vasoconstrictor[10]. In association with this, Miwa *et al*. reported that the activation of the sympathetic nervous system might be one of the crucial factors affecting coronary vasospasm[11]. Yoshio et al. and Kim et al. both conducted spectral analysis on the heart rate variability has measured by 24 hours ambulatory Holter monitoring. According to these authors, the power spectral density of the low frequency component was increased before the onset of cardiac attack. This indicates that activation of the sympathetic nervous system might play a crucial role in the development of vasospasm[12, 13].

According to most recent evidence, arterial hypercontractility might be the most common anomaly that causes the atherosclerotic changes. Both animal and clinical studies have supported the presence of cellular events (e.g., endothelial

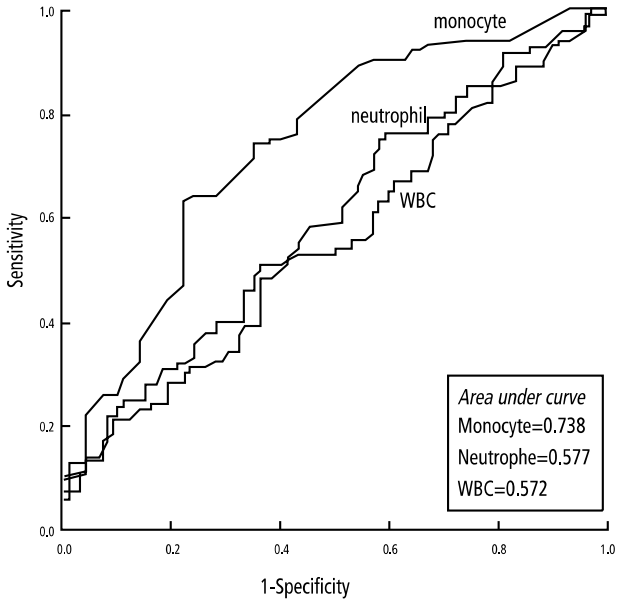

Figure 1. Receiver operating characteristics curve analysis for the diagnosis of coronary vasospastic angina. The area under the curve of the monocyte count was 0.738, which was greater that that of the neutrophil count (0.577, p=0.007) and the WBC count (0.572, p=0.003).

injury) in the atherosclerotic changes. Besides this, adhesion molecules and leukotrienes are released by platelets and macrophages during the cellular events[2, 14, 15]. Patients with coronary spasm exhibit endothelial dysfunction as well as local hyperreactivity of the coronary arteries[16, 17]. According to Miwa et al., the plasma concentrations of soluble E-selectin and intercellular adhesion moleculae-1 were elevated in the patients with variant angina. This is suggestive of an association between the inflammatory responses and coronary spasm[3]. Terashima et al. reported that the chronic activation of T-lymphocytes, and CD8+ T-lymphocytes in particular, was associated with the development of coronary spasm. This implies that the systemic immune and inflammatory responses play a crucial role in the development of coronary spasm[4]. More directly, Hong et al. have maintained that atherosclerotic changes were seen via intravascular ultrasound in all the patients with coronary spasm[18]. The present study has shown that Gensini's score was significantly higher in the patients with spasm, which is similar to the results of these study. Further, intractable spasm can develop in the underlying atherosclerotic lesions[19].

Several clinical studies have shown the total WBC and monocyte counts to be independent risk factors for coronary heart disease due to atherosclerosis[20-24]. Especially, monocytes are present in all stages of atherosclerosis; they potentiate inflammatory responses during early plaque development and initiate breakdown and rupture of the fibrous cap[25]. Based on the above results, it can be inferred that an increased monocyte count is an indicator for the early atherosclerotic changes in the patients who are suspected of having vasospastic angina. Presumably, the various chemokines released from the spastic coronary artery might be related to an increased monocyte count. The present study has shown that the monocyte count was positively correlated with Gensini's score. This leads to the speculation that an increased monocyte count indicates the initiation or progression of atherosclerotic changes in patients with vasospastic angina. Further, the increased monocyte count can be acceptable as an important indicator for vasospastic angina in the patients with normal coronary angiography and who are suspected of having coronary vasospasm, which was demonstrated in the present study.

Our results showed that the CRP was not elevated in our subjects, although CRP has been well documented as a marker for the early inflammatory responses[26]. Accordingly, CRP was not associated with coronary vasospasm, which is in agreement with the previous results[4].

There are several limitations of this study. We retrospectively analyzed the data, and we examined only a small number of patients. Therefore a prospective study that includes a large number of patients may be needed to confirm the results. We suggest here that the monocyte count was a marker for early atherosclerosis; however, we did not compare the monocyte count according to the severity of coronary atherosclerosis. Although our study showed that the monocyte count was positively correlated with Gensini's score, another study will be needed to confirm our hypothesis.

In conclusion, vasospastic angina is closely related to early atherosclerotic change, and the peripheral monocyte count is a more reliable predictor for vasospastic angina than the other inflammatory markers such as hsCRP. Our results indicate that the peripheral monocyte count is a clinically feasible marker for predicting vasospastic angina in the patients with resting chest pain and angiographycally insignificant coronary artery stenosis. However, a prospective clinical study should be conducted to confirm our findings.

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