Coronary Artery Disease

SYNTAX Score is Associated with Circulating Endothelial Progenitor Cells in Patients with Coronary Artery Disease

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Background: The SYNTAX score (SXscore) is a comprehensive, angiographic tool for grading the severity and complexity of coronary artery disease (CAD). The association between the circulating endothelial progenitor cell (EPC) number and the severity and complexity of CAD determined by SXscore is unclear. The purpose of this study is to test the hypothesis that SXscore is associated with EPC levels in CAD patients.

Methods: Flow cytometry was used to assess circulating EPC numbers by quantification of EPC markers (defined as $CD34^+$, $CD34^+KDR^+$, and $CD34^+KDR^+CD133^+$) in the peripheral blood of patients undergoing coronary angiography.

Results: Eighty-eight patients with CAD confirmed by elective coronary artery angiography and 27 age- and sex-matched controls with normal coronary angiogram were enrolled. The SXscore index was evaluated in all CAD patients and ranged from 4 to 43, with a median of 22. We divided the CAD patients into mild CAD (SXscore: 1-22) and severe CAD (SXscore > 22) groups. Compared to mild CAD patients and controls, patients with severe CAD had a higher incidence of hypertension, previous myocardial infarction, and more severe coronary lesions. Furthermore, severe CAD patients had significantly increased concentrations of high-sensitivity C-reactive protein and decreased circulating EPC levels vs. mild CAD patients and controls. Multivariate logistic regression analysis showed that circulating EPC levels were inversely associated with severe CAD ($CD34^+KDR^+$: odds ratio, 0.93 [0.88-0.98]; p = 0.024).

Conclusion: CAD patients with high SXscore have lower circulating EPC levels and enhanced inflammation in comparison to CAD patients with low SXscore.

Key Words: Coronary artery disease • Endothelial progenitor cells • SYNTAX score

INTRODUCTION

Despite the availability of effective preventive measures, coronary artery disease (CAD) remains a leading cause of morbidity and mortality in most industrialized countries. Several risk scores have been proposed to stratify CAD patients at a high risk of revascularization for multivessel stenosis.[1,2] The SYNTAX (Synergy Between PCI With Taxus and Cardiac Surgery) score (SXscore) was recently developed as a comprehensive angiographic tool for grading the severity and complexity of CAD, in order to assist in patient selection and risk

Received: October 8, 2011 Accepted: January 19, 2012

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stratification of patients who have 3-vessel disease and/or left main CAD who are undergoing revascularization.[3,4] Higher SXscores are indicative of a more complex condition, are likely to represent a bigger therapeutic challenge and to have a potentially worse prognosis in patients undergoing coronary revascularization with percutaneous coronary intervention. Clinical studies have validated the prognosis-predictive value of the SXscore in patients undergoing percutaneous coronary intervention for complex CAD.[5,6]

Convincing evidence indicates that the integrity and functional activity of the endothelial monolayer are important factors in atherogenesis.[7] The traditional view suggests that endothelium integrity is maintained by neighboring mature endothelial cells that migrate and proliferate to restore injured endothelial cells. However, a series of clinical and basic studies prompted by the discovery of bone marrow-derived endothelial progenitor cells (EPCs) has provided new insights into these processes and has demonstrated that the injured endothelial monolayer is regenerated in part by circulating EPCs.[8] The capacity of circulating EPCs to repair vascular injury suggests that they play a pivotal role in maintaining endothelial homeostasis. Altered status of circulating EPCs is a marker of endothelial dysfunction and reflects vascular health; the level of circulating EPCs could be used as a surrogate index of cumulative cardiovascular risk.[9]

The relationship between circulating EPC level and severity of atherosclerotic disease, particularly CAD, remains controversial. Some reports have shown an inverse correlation between circulating EPCs and the presence of significant CAD,[10-12] but others have indicated that the number of circulating EPCs is increased in CAD patients.[13] In addition, studies about chronic inflammation, which could be the possible pathological link between EPC and CAD, are scarce. Therefore, we sought to investigate the relationship between circulating EPC level, inflammation, and the severity and complexity of CAD determined by SXscore.

MATERIALS AND METHODS

Study participants

The study included 204 consecutive patients who were admitted to Taipei Veterans General Hospital between November 2010 and January 2011, designated to undergo coronary angiography because of suspected CAD. Subjects were excluded if they fulfilled the following criteria: (1) presence of acute coronary syndrome within 2 months; (2) presence of malignant diseases, dilated cardiomyopathy, significant valvular disease, and chronic lung disease; (3) history of undergoing percutaneous coronary intervention or coronary artery bypass surgery. The presence of CAD was documented by coronary angiography, which was performed using 5-F to 7-F diagnostic catheters at a standard 30 frames/second and stored on digital media. CAD was defined as having ≥ 50% stenosis of ≥ 1 of the major coronary arteries, and receiving consistent medication for more than 3 months. Finally, 88 patients with CAD and 27 age- and sex-matched controls with normal coronary angiogram were enrolled. Medical history, including hypertension, diabetes mellitus, peripheral artery disease, chronic kidney disease, hyperlipidemia, smoking history, previous myocardial infarction, previous cerebrovascular disease, heart failure, atrial fibrillation, and current drug treatment was obtained during a personal interview and from medical files. All patients gave informed consent, and the study was approved by the local research ethics committee.

SYNTAX score calculation

The total SXscore was derived from the summation of the individual scorings for each lesion (defined as ≥ 50% stenosis in vessel ≥ 1.5 mm). A calculation of the SXscore was based on an algorithm for the sequential morphological evaluation of dominance; number of lesions, trifurcation, bifurcation, aorto-ostial lesion, severe tortuosity, long lesion (> 20 mm), heavy calcification, thrombus, and diffuse/small vessels. A full description of the SXscore calculation was reported elsewhere.[3] The SXscore was applied to all 88 patients with CAD. All angiographic variables pertinent to SXscore calculation were computed by 2 of 5 experienced cardiologists who were blinded to clinical information and laboratory data obtained before the procedure. To decrease interobserver variation, the scores measured by individual angiographers were randomly monitored and reviewed by a senior angiographer. In case of disagreement, the opinion of the third observer was obtained, and the final decision was made by consensus.

Laboratory investigations

Blood pressure was recorded and based on the average of 3 different measurements taken after 15 minute resting periods. Venous blood was drawn in the morning after an overnight fast. Plasma liver function tests and other biochemical blood measurements including fasting blood glucose, creatinine, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol, and triglyceride levels were determined by standard laboratory procedures. Plasma high-sensitive C-reactive protein (hsCRP) level was assessed using latex-enhanced immunonephelometric assay (Dade Behring, Marburg, Germany) as previously described.[14]

Assay of circulating EPCs

Assessment of circulating EPCs was performed by researchers who were masked to the clinical data. We incubated 1000 μL peripheral blood for 30 minutes in the dark with monoclonal antibodies against human KDR (R&D, Minneapolis, MN, USA), followed by incubation with allophycocyanin (APC)-conjugated secondary antibody, with fluorescein isothiocyanate (FITC)-labeled monoclonal antibodies against human CD45 (Becton Dickinson, Franklin Lakes, NJ, USA), with PE-conjugated monoclonal antibody against human CD133 (Miltenyi Biotec, Germany), and with FITC-conjugated monoclonal antibodies against human CD34 (Becton Dickinson Pharmingen, USA). After incubation, cells were lysed, washed with phosphate-buffered saline (PBS), and fixed in 2% paraformaldehyde before analysis. Each analysis included 100,000 events. The numbers of circulating EPCs were gated with monocytes and defined as $CD34^{+}CD45^{low}$, $CD34^{+}KDR^{+}CD45^{low}$, and $CD34^{+}KDR^{+}CD133^{+}CD45^{low}$. To assess the reproducibility of EPC measurements, circulating EPCs were measured from 2 separate blood samples in 10 subjects; there was a strong correlation between the 2 measurements ($r = 0.90$, $p < 0.001$).

Statistical analysis

Data were expressed as the mean ± standard deviation for numeric variables and as the number (percent) for categorical variables. Comparisons of continuous variables between groups were performed by student's *t*-test and one-way ANOVA. Subgroup comparisons of categorical variables were assessed by a chi-square or Fisher's exact test. Correlations between EPC level, SXscore and hsCRP level were analyzed by Pearson correlation. Linear regression was done to confirm the relationship between SXscore and EPC levels. To examine the effects of various factors on severe CAD (SXscore > 22), the following factors were considered simultaneously as variables for univariate logistic regression analysis: EPC levels, age, gender, hypertension, diabetes, hyperlipidemia, peripheral artery disease, chronic kidney disease, heart failure, atrial fibrillation, previous myocardial infarction, previous cerebrovascular disease, previous percutaneous coronary intervention, and hsCRP levels. Multivariate analysis was also done to confirm the association of EPC marker ($CD34^{+}KDR^{+}$) and severe CAD. Data were analyzed using SPSS software (version 17, SPSS, Chicago, Illinois, USA). A p-value of < 0.05 was utilized to indicate statistical significance.

RESULTS

Clinical, laboratory, and procedural data

The mean age of 115 study patients (71 male, 62%) was 69 ± 9 years. The SXscore, applied to all CAD patients, ranged from 4 to 43, with a median of 22 (interquartile range, 13-27) and a mean of 21 ± 11. Based on the presence of CAD and the angiographic characteristics of the CAD patients determined by SXscore, we stratified subjects as no CAD (controls), mild CAD (SXscore, 1-22), and severe CAD (SXscore > 22). The baseline characteristics of the 3 groups are presented in Table 1. No significant differences were noted in age, gender, diabetes mellitus, peripheral artery disease, hyperlipidemia, smoking, previous cerebrovascular disease, heart failure, and atrial arrhythmia. However, patients with CAD had a significantly higher incidence of hypertension ($p = 0.043$) and previous myocardial infarction ($p = 0.002$) than those without CAD. Table 1 also shows metabolic profiles, medication use, angiographic characteristics, and procedural characteristics in the study subjects. There was no significant difference in the metabolic profiles and current medication use of the 3 groups, although CAD patients were more frequently prescribed antiplatelet agents. Compared to mild CAD patients, severe CAD patients had more left main disease

Table 1. Baseline characteristics, metabolic profiles, medications, angiographic and procedural characteristics of study subjects

	No CAD	Mild CAD (SXscore 1-22)	Severe CAD (SXscore > 22)	p value
Numbers	n = 27	n = 45	n = 43	
Age (years)	68 ± 11	69 ± 9	70 ± 8	0.263
Men, n (%)	21 (77.8%)	40 (88.9%)	31 (72.1%)	0.139
Hypertension, n (%)	18 (66.7%)	39 (87.7%)	38 (88.3%)	0.043
Diabetes mellitus, n (%)	9 (33.3%)	21 (46.7%)	24 (55.8%)	0.097
Peripheral artery disease, n (%)	4 (14.8%)	14 (31.1%)	16 (37.2%)	0.132
Chronic kidney disease, n (%)	10 (37.0%)	18 (40.0%)	16 (37.2%)	0.955
Hyperlipidemia, n (%)	11 (40.7%)	31 (67.4%)	27 (62.7%)	0.055
Current smoker, n (%)	8 (29.6%)	10 (22.2%)	14 (32.6%)	0.548
Previous myocardial infarction, n (%)	0	10 (22.2%)	15 (34.9%)	0.002
Previous cerebrovascular disease, n (%)	2 (7.4%)	5 (11.1%)	8 (18.6%)	0.360
Heart failure, n (%)	9 (33.3%)	17 (37.8%)	17 (39.5%)	0.874
Atrial fibrillation, n (%)	4 (14.8%)	10 (22.2%)	7 (16.3%)	0.676
Laboratory data				
Cholesterol (mg/dL)	162 ± 29	171 ± 37	173 ± 24	0.312
LDL-C (mg/dL)	101 ± 33	109 ± 31	109 ± 28	0.545
HDL-C (mg/dL)	41 ± 12	39 ± 12	39 ± 10	0.779
Triglyceride (mg/dL)	117 ± 42	129 ± 68	131 ± 52	0.572
Creatinine (mg/dL)	1.0 ± 0.2	1.4 ± 1.1	1.3 ± 0.8	0.067
ALT (U/L)	21 ± 10	25 ± 27	29 ± 24	0.373
Fasting glucose (mg/dL)	112 ± 43	144 ± 75	149 ± 69	0.059
Medication				
Aspirin, n (%)	4 (14.8%)	38 (82.6%)	36 (83.7%)	< 0.001
Clopidogrel, n (%)	2 (7.4%)	26 (61.9%)	28 (65.1%)	< 0.001
ACEI, n (%)	6 (22.2%)	9 (20.0%)	6 (14.0%)	0.641
ARB, n (%)	7 (25.9%)	17 (37.8%)	12 (27.9%)	0.486
CCB, n (%)	8 (29.6%)	19 (42.2%)	17 (39.5%)	0.561
Beta blocker, n (%)	11 (40.7%)	18 (40.0%)	16 (37.2%)	0.948
Diuretics, n (%)	8 (29.6%)	14 (31.1%)	10 (23.2%)	0.699
PPAR-γ agonists, n (%)	4 (14.8%)	8 (17.8%)	12 (27.9%)	0.362
Statins, n (%)	11 (40.7%)	25 (55.6%)	20 (46.5%)	0.453
Nitrates, n (%)	11 (40.7%)	24 (53.3%)	23 (53.5%)	0.522
Angiographic characteristics				
SYNTAX score	-	12.4 ± 5.6	29.7 ± 6.5	< 0.001*
CAD with left main stenosis	-	2 (4.4%)	13 (34.6%)	0.002*
Treated coronary artery				
Left anterior descending	-	17 (37.8%)	20 (46.5%)	0.270*
Left circumflex	-	5 (11.1%)	8 (18.6%)	0.246*
Right coronary	-	9 (20.0%)	12 (27.9%)	0.268*
Complexity of CAD				
Single vessel disease	-	20 (44.4%)	5 (11.6%)	0.001*
2-vessel disease	-	19 (42.2%)	14 (32.6%)	0.237*
3-vessel disease	-	6 (13.3%)	24 (55.8%)	< 0.001*
Bifurcation lesion	-	4 (8.9%)	11 (25.6%)	0.035*
Chronic total occlusion	-	2 (4.4%)	8 (18.6%)	0.038*
Procedural characteristics				
Number of treated segments per patient	-	0.9 ± 1.1	1.7 ± 1.7	0.017*
Number of stent deployments per patient	-	0.7 ± 0.9	1.4 ± 1.7	0.016*
Deployment of BMS	-	11 (24.4%)	9 (20.9%)	0.445*
Deployment of DES	-	15 (33.3%)	19 (44.2%)	0.204*

Values are mean ± standard deviation (SD) or number (%). *Data of mild CAD patients compared to those of severe CAD patients. ACEI, angiotensin-converting enzyme inhibitor; ALT, alanine aminotransferase; ARB, angiotensin receptor blocker; CAD, coronary artery disease; CCB, calcium channel blocker; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PCI, percutaneous coronary intervention; PPAR-γ agonists, peroxisome proliferator-activated receptor gamma agonists; γGT, gamma-glutamyl-transferase.

(p = 0.002), 3-vessel disease (p < 0.001), bifurcation lesion (p = 0.035), and chronic total occlusion (p = 0.038). Not surprisingly, severe CAD patients had higher numbers of treated segments (p = 0.017) and received more stent deployments (p = 0.016) per patient than mild CAD patients.

Circulating EPC levels

As shown in Table 2 and Figure 1, patients with severe CAD had reduced circulating EPC levels than mild CAD patients and controls (No CAD vs. mild CAD vs. severe CAD: $CD34^+$, 0.057 ± 0.039 vs. 0.052 ± 0.060 vs. 0.024 ± 0.029%, p = 0.003; $CD34^+KDR^+$, 0.014 ± 0.011 vs. 0.013 ± 0.011 vs. 0.007 ± 0.007%, p = 0.002; $CD34^+KDR^+CD133^+$, 0.014 ± 0.016 vs. 0.011 ± 0.011 vs. 0.005 ± 0.006%, p = 0.006). Moreover, the plasma concentrations of hsCRP were significantly higher in patients with severe CAD than in controls (No CAD vs. mild CAD vs. severe CAD: 0.80 ± 0.59 vs. 1.24 ± 1.19 vs. 1.37 ± 0.83 mg/L, p = 0.047). Although severe CAD patients had insignificantly higher hsCRP levels than those of mild CAD patients, the hsCRP was significantly positively correlated with SXscore by Pearson correlation in the current study (Pearson correlation coefficient [r] = 0.305, p = 0.001). Furthermore, circulating EPC levels were negatively correlated with the SXscore ($CD34^+KDR^+$ [%]: r = -0.403, p < 0.001; Figure 2) and inversely associated with plasma hsCRP levels ($CD34^+KDR^+$ [%]: r = -0.237, p = 0.010) in patients with CAD.

Independent correlates of severe CAD (SYNTAX score > 22)

In order to identify the independent predictors of severe CAD, univariate and multivariate logistic regression analysis were performed as presented in Table 3. Univariate logistic regression showed that the circulating EPC levels ($CD34^+$, $CD34^+KDR^+$, and $CD34^+KDR^+CD133^+$) were inversely associated with severe CAD (all p < 0.05). After adjustment for gender (male), diabetes, and previous myocardial infarction, circulating EPC level (all 3 markers) was still an independently negative predictor of severe CAD ($CD34^+/KDR^+$: odds ratio, 0.93, 95% confidence interval [CI], 0.88-0.98; p = 0.024).

Because the number of severe CAD subjects in the current study was relatively small (43 patients), we could not adjust for more than 4 covariates including EPC number to avoid over-fitting. However, we tested the effects on age, heart failure and statins use on the relationship between EPC levels and severe CAD. EPC

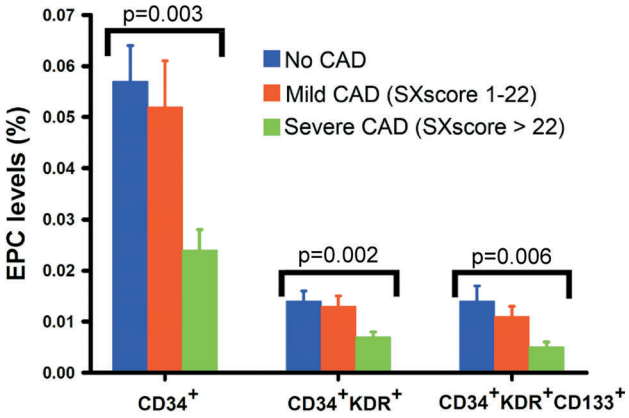

Figure 1. *Association between EPC levels (%) and coronary artery disease (CAD) severity, categorized as controls (no CAD), mild CAD (SYNTAX score [SXscore], 1-22), and severe CAD (SXscore > 22).*

Table 2. Circulating endothelial progenitor cell (EPC) levels and inflammatory markers

Markers	No CAD n = 27	Mild CAD (SXscore 1-22) n = 45	Severe CAD (SXscore > 22) n = 43	p value
EPC levels (%)				
$CD34^+$	0.057 ± 0.039	0.052 ± 0.060	0.024 ± 0.029	0.003
$CD34^+KDR^+$	0.014 ± 0.011	0.013 ± 0.011	0.007 ± 0.007	0.002
$CD34^+KDR^+CD133^+$	0.014 ± 0.016	0.011 ± 0.011	0.005 ± 0.006	0.006
EPC levels (cells/μL)				
$CD34^+$	82.9 ± 56.2	74.3 ± 85.1	31.9 ± 27.0	0.001
$CD34^+KDR^+$	20.4 ± 15.6	16.7 ± 11.6	10.7 ± 11.3	0.006
$CD34^+KDR^+CD133^+$	16.8 ± 14.9	14.3 ± 10.8	8.6 ± 9.6	0.009
hsCRP (mg/L)	0.80 ± 0.59	1.24 ± 1.19	1.37 ± 0.83	0.047

Values are mean ± SD. hsCRP, high-sensitivity C-reactive protein.

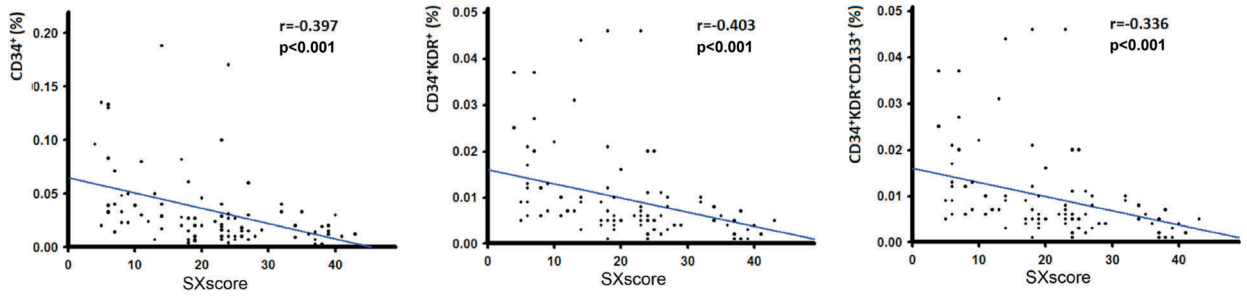

Figure 2. *Correlation between EPC levels (%) and SYNTAX score (SXscore) in patients with coronary artery disease. SXscore is significantly inversely correlated with EPC levels (all 3 markers, p < 0.001). r: Pearson correlation coefficient.*

number ($CD34^+KDR^+$) is an independent negative predictor of severe CAD (SXscore > 22) after adjusting for age (odd ratio: 0.93, 95% CI, 0.89-0.98, p = 0.006), for statin use (odd ratio: 0.94, 95% CI, 0.89-0.99, p = 0.020), and for heart failure (odd ratio: 0.93, 95% CI, 0.88-0.98, p = 0.004). After adjusting for age, statin use, and heart failure, EPC number ($CD34^+KDR^+$) is still a negative predictor of severe CAD (odd ratio: 0.94, 95% CI, 0.89-0.99, p = 0.013) (Table 3).

Table 3. Simple correlation and multivariate analysis of the factors associated with severe CAD (SXscore > 22)

	OR (95% CI)	p value
Univariate analysis		
EPCs (cells/μL)		
$CD34^+$	0.98 (0.96-0.99)	0.003
$CD34^+KDR^+$	0.94 (0.90-0.99)	0.027
$CD34^+KDR^+CD133^+$	0.93 (0.87-0.99)	0.022
Age	1.21 (0.97-1.08)	0.408
Male	3.10 (0.99-9.72)	0.053
Hypertension	1.17 (0.33-4.16)	0.809
Diabetes	1.67(0.74-3.77)	0.215
Hyperlipidemia	1.31 (0.54-3.18)	0.547
Chronic kidney disease	1.13 (0.48-2.66)	0.788
Peripheral artery disease	1.74 (0.57-5.33)	0.334
Heart failure	1.08 (0.46-2.54)	0.866
Atrial fibrillation	0.68 (0.23-1.99)	0.482
Previous myocardial infarction	1.88 (0.73-4.81)	0.191
Previous cerebrovascular disease	2.12 (0.65-6.93)	0.215
Current smoking	1.69 (0.65-4.37)	0.279
hsCRP (mg/L)	1.13 (0.75-1.71)	0.559
Current use of aspirin	0.95 (0.20-2.97)	0.926
Current use of clopidogrel	1.36 (0.58-3.23)	0.480
*Multivariate analysis		
EPCs (cells/μL) $CD34^+KDR^+$		
Model 1	0.93 (0.88-0.98)	0.024
Model 2	0.94 (0.89-0.99)	0.013

CAD, coronary artery disease; CI, confidence interval; OR, odds ratio; PCI, percutaneous coronary intervention.
*Multivariate analysis: Model 1: adjusted for gender (male), diabetes, and previous myocardial infarction; Model 2: adjusted for age, heart failure, and statin use.

DISCUSSION

To the best of our knowledge, this is the first study to show that CAD patients with high SXscore have decreased circulating EPC levels and enhanced inflammation, in comparison to CAD patients with low SXscore and controls. Additionally, circulating EPC levels were inversely correlated with angiographic SXscore and plasma hsCRP levels in patients with CAD. These findings suggest that high SXscore, probably indicative of attenuated endothelial repair capacity and increased systemic inflammation, may contribute to an increased risk of cardiovascular events in CAD patients undergoing coronary revascularization with percutaneous coronary intervention.

The SXscore was recently proposed as a practical angiographic classification to grade the coronary anatomy with respect to the number of lesions and their functional impact, location, and complexity.[3] A higher SXscore indicates more complex CAD lesions and represent a bigger therapeutic challenge, with a potentially worse prognosis in patients undergoing percutaneous coronary intervention. The SXscore has been tested for its ability to predict procedural outcomes and has been used to select the optimal treatment strategy for patients with complex CAD, i.e., percutaneous coronary intervention or coronary artery bypass graft.[4,6] The predictive value of the SXscore was validated in a series of patients undergoing percutaneous coronary intervention for

3-vessel or left main CAD.[5,6] Compared to the angiographic classification of the American College of Cardiology/American Heart Association, the SXscore could better predict the initial and long-term risks of major cardiac and cerebrovascular events.[5] Previous studies have demonstrated that SXscore, which represents lesion complexity, correlated with the prognosis among patients who were undergoing coronary revascularization,[1] and independently predicted major cardiovascular and cerebrovascular event outcomes in patients who were undergoing percutaneous coronary intervention (PCI).[6] In the current study, most of our CAD patients underwent PCI after coronary angiography. The SXscore may also be a useful tool to risk-stratify clinical outcomes and prognosis in the current study.

Convincing evidence indicates that the integrity and functional activity of the endothelial monolayer are critical factors in atherogenesis.[15] Intact endothelium with normal functional activities regulates vascular tone, maintains vascular homeostasis, and prevents the development of atherosclerosis. In humans, extensive endothelial cell damage by cardiovascular risk factors can result in endothelial cell apoptosis with subsequent loss of integrity of the endothelium. The traditional view suggests that endothelium integrity is maintained by neighboring mature endothelial cells that migrate and proliferate to restore injured endothelial cells. Recently, a series of basic and clinical studies were prompted by the discovery that bone marrow-derived EPCs play a pivotal role in endothelium regeneration.[16] Circulating EPCs are derived from bone marrow; their mobilization is triggered endogenously by tissue ischemia, or exogenously by cytokine stimulation. Once in the peripheral blood, EPCs constitute a pool of cells that can actively repair the endothelial layer by forming a patch at sites of intimal damage. Clinical studies demonstrated that the levels of circulating EPCs are associated with vascular endothelial function and cardiovascular risk factors and help to identify patients at increased cardiovascular risk.[17] Reduced levels of circulating EPCs independently predict the progression of atherosclerotic disease and development of cardiovascular events,[11,18] thus supporting an important role for endogenous vascular repair of EPCs with respect to modulating the clinical course of atherosclerotic diseases.

Although the critical role of circulating EPCs in the pathogenesis of atherosclerotic diseases is substantiated by several observations, the relationship between circulating EPCs and CAD remains a subject of debate. Some previous studies have examined the association between circulating EPCs and CAD, or risk factors predisposing to CAD. Vasa et al. reported that the circulating EPC levels in patients with CAD were significantly lower than those in patients without CAD.[9] Wang and coworkers indicated that a decrease in EPC numbers and activity were observed in patients with stable CAD, and EPC levels were negatively correlated with the severity of coronary stenosis assessed by the Gensini score.[12] We decided to evaluate the SXscore in the current study because the Gensini score is based predominantly on plaque burden, without scoring other factors associated with the complexity of CAD. Fadini et al. also reported that EPCs were significantly reduced in subjects with increased intima-media thickness,[19] implying that depletion of EPCs may be an independent predictor of subclinical atherosclerosis. However, Güven and colleagues showed that increased EPC levels were associated with the presence of significant CAD, and EPC numbers correlated with maximum angiographic stenosis severity.[13] These apparent conflicting results may have many explanations, including fundamental differences in the methodologies used to identify circulating EPCs, heterogeneity of patient populations, and the effect of disease stage on the biological properties of circulating EPC levels. There are two major differences between Güven's study and our study. First, they defined significant CAD as having at least one coronary artery with stenosis > 70%. They may not be able to differentiate the severity of CAD in patients with single vessel disease, double vessel disease, or triple vessel disease. However, in the current study, we used the SXscore to classify the severity of CAD, taking into consideration the complexity of all coronary lesions. These included those coronary lesions in the 3 major coronary arteries and all the main branches, and the factors that indicate difficulty in performing percutaneous coronary intervention such as bifurcation, trifurcation, aorto-ostial lesion, severe tortuosity, long lesion, heavy calcification, thrombus, and diffuse/small vessels.[20] Second, they studied and counted EPCs by cell culture. In contrast, we computed EPCs number by flow cytometry. Currently, the available literature sustains the belief that the $CD34^{+}KDR^{+}$ phenotype

by flow cytomery represents the best compromise in terms of detection accuracy, biological meaning, and clinical usefulness. It is also the best choice when EPC count is conceived as a surrogate biomarker of cardiovascular risk.[21]

On the basis of the angiographic classifications by SXscore, we observed that severe CAD patients had fewer circulating EPCs than mild CAD patients and subjects with normal angiographic results. Moreover, circulating EPC levels were negatively correlated with the SXscore in patients with angiographic evidence of CAD. These findings are partially consistent with a recent study showing that lower levels of circulating EPCs predict CAD progression,[7] indicating a critical role for EPCs in the pathogenesis of CAD.

The hsCRP, a vascular inflammatory biomarker, reflects chronic low-grade vascular inflammation and plays a direct role in atherosclerotic plaque rupture and thrombosis with ensuing clinical complications.[22] Verma and coworkers demonstrated that recombinant human CRP directly inhibits EPC differentiation, survival, and function at concentrations known to predict adverse vascular outcomes.[23,24] Therefore, the more notable inflammation observed in CAD patients with high SXscores may suppress EPC levels in the blood, attenuating the repair capacity of the vasculature. We concluded that decreased circulating EPC levels and increased vascular inflammation in CAD patients with higher SXscore reflects impaired vascular repair capacity, which may indicate a poor prognosis for percutaneous coronary intervention. However, we can't exclude the possibility that EPC dysfunction may first occur before the endothelium layer loses its protective function regarding anti-inflammation, with following elevated hsCRP levels. Therefore, enhancement of the regenerative capacity of the injured endothelium by increasing circulating EPC levels may be one way to reduce the incidence of adverse cardiovascular events in CAD patients.

Guidelines for triaging patients for cardiac catheterization recommend risk assessment and non-invasive testing. However, the effectiveness of current non-invasive tests is still insufficient, and the diagnostic yield of elective cardiac catheterization was less than 40%.[25] Furthermore, non-invasive testing which can provide information on lesion complexity is important for decision making by cardiologists. The suspicion of the prevalence of complex coronary lesions encourages active investigation for generalized atherosclerotic diseases. Therefore, it is important to show that EPC level may be a useful tool to predict the severity and complexity of CAD.

STUDY LIMITATIONS

Some limitations of this study should be mentioned. First, the study population is relatively small and further studies are needed to verify our results. Second, the SXscore was mainly created to grade the complexity of coronary anatomy disease in patients with 3-vessel disease. Therefore, evidence of its generalization and utility in patients with mild CAD is insufficient. Additionally, previous studies that have assessed the role of the SXscore have been limited by small sample sizes, variations in follow-up time, or selected patient populations. Third, we did not study the underlying mechanisms to explain why circulating EPCs are reduced in CAD patients with high SXscore. These possible mechanisms include decreased half-life of EPCs, potential impairments in cell production, or continuous exhaustion of circulating EPCs in patients with severe CAD, and should be investigated in future studies. Fourth, we did not evaluate EPC function or clinical endothelial functions, such as adhesion, proliferation, and migratory ability, and flow-mediated dilatation. However, in other studies, EPC and endothelial functions exhibited changes in a similar pattern with respect to EPC number.[26,27] Finally, because this is a cross-sectional clinical investigation, we acknowledge the potential for selection bias for some patients.

CONCLUSION

We demonstrated that patients with severe CAD (SXscore > 22) have decreased circulating EPC numbers and increased inflammation, in comparison to those without CAD or with mild CAD (SXscore 1-22). In addition, circulating EPC numbers were negatively correlated with angiographic SXscore and plasma hsCRP levels in patients with CAD. Therapeutic strategies that enhance EPC levels should be tested in order to assess their utility in reducing disease progression and events at follow-up.

ACKNOWLEDGMENTS

This study was supported in part by research grants from the NSC 97-2314-B-075-039, NSC 98-2314-B-075-035, and UST-UCSD International Center of Excellence in Advanced Bio-engineering NSC-99-2911-I-009-101 from the National Science Council; VGH-V98B1-003 and VGH-V100E2-002 from Taipei Veterans General Hospital, and a grant from the Ministry of Education "Aim for the Top University" Plan.

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