Role of Circulating Endothelial Progenitor Cells in Patients with Rheumatoid Arthritis with Coronary Calcification

KAI-HANG YIU, SILUN WANG, MO-YIN MOK, GAIK CHENG OOI, PEK-LAN KHONG, CHU-PAK LAU, WING-HON LAI, LAI-YUNG WONG, KWOK-FAI LAM, CHAK-SING LAU, and HUNG-FAT TSE

ABSTRACT. ***Objective.*** Patients with rheumatoid arthritis (RA) are prone to premature atherosclerosis. We hypothesize that depletion of circulating endothelial progenitor cells (EPC) related to RA can contribute to the development of atherosclerosis.

Methods. We studied coronary calcifications by multidetector computed tomography and their relationship with different subtypes of circulating EPC in 70 patients with RA and 35 age- and sex-matched controls (mean age 54.1 ± 10.2 yrs, 87% were women). The presence of coronary atherosclerosis was defined as an Agatston score ≥ 10. Four subpopulations of EPC were determined by flow cytometry on the basis of surface expression of CD34, CD133, and KDR antigen: CD34+, CD34/KDR+, CD133+, and CD133/KDR+ EPC, respectively.

Results. Among those with RA, 15 patients (21%) had coronary atherosclerosis. The mean Agatston score was higher (61.8 ± 201.7 vs 0.14 ± 0.69; $p = 0.01$) and coronary atherosclerosis was more prevalent (21.4% vs 0%; $p < 0.01$) in patients with RA compared to controls. RA patients with coronary atherosclerosis were older (66.2 ± 6.9 vs 51.5 ± 16.2 yrs; $p < 0.01$), had higher prevalence of hypertension (46.7% vs 14.5%; $p = 0.01$), and had lower CD133/KDR+ (0.45% ± 0.28% vs 0.89% ± 0.81%; $p < 0.01$) and CD133+ EPC levels (0.74% ± 0.39% vs 1.22% ± 0.83%; $p < 0.01$), but similar CD34/KDR+ and CD34+ EPC levels (all $p > 0.05$) compared to those without. Multiple logistic regression revealed that older age (OR 1.25, 95% CI 1.10–1.41, $p < 0.01$) and lower CD133/KDR+ EPC (OR 0.07, 95% CI 0.00–0.97, $p < 0.01$) were independent predictors for coronary atherosclerosis in patients with RA.

Conclusion. Our results demonstrated that RA patients with coronary atherosclerosis have significantly lower levels of CD133/KDR+ and CD133+ EPC than those without. In addition to older age, lower levels of circulating CD133/KDR+ EPC also predicted occurrence of coronary atherosclerosis in RA patients. (J Rheumatol First Release Jan 15 2010; doi:10.3899/jrheum.090782)

Key Indexing Terms:
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Patients with rheumatoid arthritis (RA) have increased cardiovascular mortality compared with the general population[1-5]. Although the mechanisms remain unclear, it is well established that their excess cardiovascular morbidity and mortality is attributed to an increased risk of acute myocardial infarction and coronary heart disease mortality[6-8]. In addition, conventional risk factors cannot fully explain the accelerated atherosclerosis observed in patients with RA[9]. As a result, it has been postulated that the chronic inflammatory state associated with RA may contribute to the accelerated atherosclerosis. Indeed, previous studies have demonstrated that patients with RA have premature atherosclerosis with an increased prevalence and severity of coronary artery calcification[10,11] and greater intima-media thickness (IMT)[12,13] compared with age-matched controls. Our recent studies also showed that patients with RA have premature and diffuse arterial calcification over multiple vascular beds[14].

Experimental studies suggest that bone marrow-derived endothelial progenitor cells (EPC) play an important role in

From the Cardiology Division, Department of Medicine; Department of Diagnostic Radiology; Division of Rheumatology, Department of Medicine; Statistics and Actuarial Science; and Research Centre of Heart, Brain, Hormone and Healthy Aging, Li Ka Shing Faculty of Medicine, the University of Hong Kong, Hong Kong, China.

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K-H. Yiu, MBBS, Cardiology Division, Department of Medicine; S. Wang, MD, MPhil, Department of Diagnostic Radiology; M-Y. Mok, MBBS, Division of Rheumatology, Department of Medicine; G.C. Ooi, MD; P-L. Khong, MD, Department of Diagnostic Radiology; C-P. Lau, MD; W-H. Lai, MPhil; L-Y. Wong, MPhil, Cardiology Division, Department of Medicine; K-F. Lam, PhD, Statistics and Actuarial Science; C-S. Lau, MD, Division of Rheumatology, Department of Medicine; H-F. Tse, MD, PhD, Cardiology Division, Department of Medicine, and Research Centre of Heart, Brain, Hormone and Healthy Aging.

Address correspondence to Dr. H-F. Tse, Cardiology Division, Department of Medicine, The University of Hong Kong, Room 1928, Block K, Queen Mary Hospital, Hong Kong. E-mail: hftse@hkucc.hku.hk

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the maintenance of endothelial integrity and hemostasis[15]. The number of circulating EPC has been shown to be inversely correlated with cardiovascular risk factors and vascular function[16,17] and to predict cardiovascular events[18]. Prior studies showed that RA is associated with depletion of circulating EPC, which correlates with impaired brachial endothelial function[19] and increased carotid IMT[20,21]. However, the relationship between the circulating EPC and subclinical coronary atherosclerosis as determined by coronary calcification remains unclear. Further, the relationship of different subtypes of EPC with coronary atherosclerosis is also unknown. We hypothesized that depletion of different subtypes of circulating EPC is associated with increased coronary atherosclerosis as detected by multidetector computer tomography (MDCT) in patients with RA.

MATERIALS AND METHODS

Study population. From January 2006 to January 2007, 77 consecutive Chinese patients age > 18 years who met American College of Rheumatology (formerly, the American Rheumatism Association) classification criteria for RA were recruited[22]. Patients with a documented history of cardiovascular disease including coronary artery disease (n = 3), myocardial infarction (n = 3), or stroke (n = 1) were excluded. As a result, our study involved 70 RA patients without established cardiovascular diseases. During the study period, 35 age- and sex-matched (1:2 matching) controls were recruited from the health-check program organized by the hospital. For comparison, Chinese controls who did not meet classification criteria for RA or other inflammatory disease were also recruited from a community health screening program. The study was approved by the institutional ethical review board and all subjects gave their written informed consent.

Study protocols. Baseline demographic and clinical characteristics, blood sampling, and MDCT were obtained prospectively on the same day in all study subjects. Cardiovascular risk factors including diabetes mellitus, hypercholesterolemia, and hypertension were assessed. Body height and weight and blood pressure of all subjects were measured as described[23]. Hypertension was defined as either resting systolic or diastolic blood pressure ≥ 140/90 mm Hg on 2 occasions or prescription of antihypertensive medication. Diabetes mellitus was defined as a serum fasting glucose ≥ 7.1 mmol/l or prescription of antihyperglycemic medication. Hypercholesterolemia was defined as a fasting total serum cholesterol level of ≥ 4.9 mmol/l or prescription of statin. For patients with RA, data on rheumatoid factor, inflammatory markers, current use of disease-modifying antirheumatic drugs (DMARD), prednisolone, and nonsteriodal antiinflammatory drugs were retrieved from medical records. In our study, none of the RA patients were treated with biological agents.

Fasting blood samples were obtained from all subjects to determine serum creatinine, glucose, and lipid levels. C-reactive protein level (CRP) was measured using a Hitachi 747 analyzer (Boehringer Mannheim, Mannheim, Germany) and a particle-enhanced immunoturbidimetric assay (Roche Diagnostics, Mannheim, Germany) as described[24].

MDCT imaging. All subjects underwent coronary CT scan using a 64-slice MDCT as described (Lightspeed, VCT, GE Healthcare, Princeton, NJ, USA)[14]. In brief, all scans were performed with subjects in the supine position and included regions from the aortic arch to the fundus of the heart. A prospective electrocardiogram-gated cardiac scan was obtained with the following scan parameters: rotation time 0.35 s; slice thickness 2.5 mm; 120 kV; 250 mA; trigger delay 70% R-R interval. Patients were instructed to hold their breath for 30 s during scanning.

MDCT images were reviewed at the post-processing image workstation (Advantage Windows 4.02, GE Healthcare). Complete data were available from all the scans, without misregistration of slices due to artifacts of motion, respiration, or asynchronous electrocardiographic triggering. To ensure the continuity and consistency of the interpretation of coronary calcium scores (CCS), 2 expert investigators (SW, GCO), who were unaware of the subjects' clinical status, analyzed all the scans. The interobserver and intraobserver variability correlation coefficients of calcium score measurements were 0.92 and 0.91, respectively.

Analysis of MDCT calcium score. Measurement of CCS was performed using commercial "SmartScore" software (GE Healthcare) with the threshold option set for pixels > 130 Hounsfield units and expressed in Agatston units. CCS was calculated as the sum of CCS in the left main coronary artery, left anterior descending artery, left circumflex coronary artery, right coronary artery, and posterior descending artery[14]. A CCS score of ≥ 10 Agatston score was considered as the presence of significant coronary atherosclerosis[23].

Flow cytometry. The circulating EPC were determined by the expression of surface markers CD34+, CD133+, and KDR+ on mononuclear cells, and their numbers were measured by fluorescence-activated cell analysis of peripheral blood sample as described[24]. In brief, 100 μl of peripheral blood was incubated with a phycoerythrin-conjugated monoclonal antibody against human KDR (Sigma, St. Louis, MO, USA), followed by a fluorescein isothiocyanate (FITC)-conjugated CD34 and CD133 antibodies (Beckman Coulter, Fullerton, CA, USA). FITC-labeled anti-human CD45 antibody was used for differential gating during flow analysis. FITC-labeled IgG1a (Beckman Coulter) and phycoerythrin-labeled IgG2b (Becton Dickinson, Franklin Lakes, NJ, USA) served as the isotypic control for color compensation. Analysis was performed with an automated fluorescence-activated cell counter (Elite, Beckman Coulter) in which 1,000,000 events were counted. The percentages of all the measured components defined as the absolute cell counts divided by the lymphocyte counts were calculated. The intraobserver variability testing found an intraclass correlation coefficient of 0.9 ($p < 0.001$).

Statistical analysis. Continuous variables were presented as mean ± standard deviation or median (range, interquartile range) for the Agatston score as the distribution is highly skewed. Categorical data were presented as frequencies and percentages. Continuous variables were tested for normal distribution with the Kolmogorov-Smirnov test. Statistical comparisons were performed by using Student's t test and Wilcoxon rank-sum test for continuous variables with and without valid normality assumption, respectively, and Fishers' exact test for discrete variables. Simple logistic regression analysis was used to evaluate the odds ratios (OR) and confidence intervals (CI) for Agatston score > 10 in patients with RA. Multiple logistic regression analyses were performed. The final model was obtained using a backward elimination method by deleting the most insignificant variable. All statistical analyses were performed using SPSS for Windows (Version 15.0, SPSS, Chicago, IL, USA). All p values are 2-sided, while $p < 0.05$ was considered statistically significant.

RESULTS

Clinical characteristics. Demographic characteristics of the study population are shown in Table 1. The mean age of the overall study population was 54.1 ± 10.2 years, and a majority of patients were female (87%). In patients with RA, the mean duration of RA disease was 12.7 ± 10.7 years, and 49 of them (70%) had positive rheumatoid factor. More than two-thirds of patients were taking DMARD, and 17% prednisolone. There were no significant differences in the clinical and biochemical characteristics between control subjects and patients with RA, except that patients with RA had a higher prevalence of hypertension (Table 1). Further, there were no significant differences in the mean percent-

Table 1. Clinical characteristics in patients with and without coronary atherosclerosis (CA).

	Controls, n = 35	RA Patients, n = 70	p	Without CA†, n = 55	With CA††, n = 15	p
Age, yrs	53.5 ± 8.7	54.7 ± 11.4	0.58	51.5 ± 16.2	66.2 ± 6.9	< 0.01**
Male sex, n (%)	5 (14.2)	12 (17.1)	0.78	8 (14.5)	4 (26.7)	0.27
Duration of disease, yrs	—	12.7 ± 10.7	—	11.7 ± 10.7	16.5 ± 10.1	0.12
Hypertension, n (%)	2 (5.7)	15 (21.4)	0.05	8 (14.5)	7 (46.7)	0.01*
Diabetes, n (%)	0 (0)	2 (2.9)	1.0	1 (1.8)	1 (6.7)	0.39
Hyperlipidemia, n (%)	0 (0)	2 (2.9)	1.0	2 (3.6)	0 (0)	1.0
Smoker, n (%)	4 (11.4)	9 (12.9)	1.0	5 (9.1)	4 (26.7)	0.09
SBP, mm Hg	120.5 ± 15.6	126.9 ± 19.3	0.82	122.5 ± 18.1	143.3 ± 14.2	< 0.01**
DBP, mm Hg	74.0 ± 13.2	75.0 ± 11.1	0.76	73.4 ± 10.7	80.8 ± 10.9	0.03*
CRP, mg/dl	1.0 ± 0.8	1.3 ± 1.9	0.21	1.1 ± 1.6	1.8 ± 2.7	0.33
Triglyceride, mmol/l	1.1 ± 0.5	1.3 ± 0.7	0.15	1.2 ± 0.7	1.4 ± 0.5	0.36
Total cholesterol, mmol/l	5.1 ± 0.7	5.0 ± 1.2	0.31	4.8 ± 1.1	5.4 ± 1.3	0.15
HDL, mmol/l	1.7 ± 0.4	1.6 ± 0.4	0.06	1.5 ± 0.4	1.7 ± 0.3	0.11
LDL, mmol/l	1.6 ± 0.4	2.8 ± 0.9	0.68	2.8 ± 0.8	3.1 ± 1.1	0.26
Serum creatinine, μg/l	60.2 ± 15.2	66.3 ± 16.7	0.57	64.6 ± 29.4	68.2 ± 17.2	0.12
Rheumatoid factor, n (%)	—	49 (70)	—	39 (70.9)	10 (66.7)	0.76
Taking NSAID, n (%)	—	11 (15.7)	—	10 (18.2)	1 (6.7)	0.44
Taking Prednisolone, n (%)	—	12 (17.1)	—	8 (14.5)	4 (26.7)	0.27
Taking DMARD, n (%)	—	47 (67.1)	—	37 (67.3)	10 (66.7)	1.00
Hydroxychloroquine, n (%)	—	28 (40)	—	23 (41.8)	5 (33.3)	0.76
Methotrexate, n (%)	—	31 (44.3)	—	25 (45.4)	6 (40)	0.78
Sulfasalazine, n (%)	—	14 (20)	—	9 (16.4)	5 (33.3)	0.16
Leflunomide, n (%)	—	2 (2.9)	—	2 (3.6)	0 (0)	1.00
Endothelial progenitor cells (EPC)						p (a) p (b)
CD34+ EPC, %	4.95 ± 3.31	6.28 ± 4.75	0.10	6.02 ± 3.95	7.21 ± 7.06	0.53 0.25
CD34/KDR+ EPC, %	2.54 ± 2.78	2.73 ± 3.16	0.76	2.83 ± 3.35	2.35 ± 2.38	0.54 0.80
CD133+ EPC, %	1.20 ± 1.12	1.12 ± 0.78	0.47	1.22 ± 0.83	0.74 ± 0.39	< 0.01** < 0.01**
CD133/KDR+ EPC, %	0.91 ± 0.37	0.79 ± 0.75	0.28	0.89 ± 0.81	0.45 ± 0.28	< 0.01** < 0.01**

* p , 0.05, ** p < 0.01. † Coronary calcium score (CCS) negative: Agatston score < 10; †† CCS positive: Agatston score ≥ 10; CA: coronary calcium score positive:Agatston score > 10. p (a): RA patients with or without CA; p (b): control vs RA patients with CA. SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL: high density lipoprotein; LDL: low density lipoprotein; CRP: C-reactive protein; NSAID: nonsteroidal antiinflammatory drug; DMARD: disease modifying antirheumatoid drug.

ages of circulating CD34+, CD34/KDR+, CD133+, and CD133/KDR+ EPC between control subjects and patients with RA (Table 1).

Compared with controls, patients with RA had higher mean Agatston score (61.8 ± 201.7 vs 0.14 ± 0.69; p = 0.01). Among those with RA, 15 patients (21%) had coronary atherosclerosis (defined by CCS scores ≥ 10) compared to no controls (0%; p < 0.01). As shown in Table 1, patients with RA who had coronary atherosclerosis were older (66.2 ± 6.9 vs 51.5 ± 16.2 years; p < 0.01) and had higher prevalence of hypertension (46.7% vs 14.5%; p = 0.01), systolic blood pressure (143.3 ± 14.2 vs 122.5 ± 18.1 mm Hg; p < 0.01), and diastolic blood pressure (80.8 ± 10.9 vs 73.4 ± 10.7 mm Hg; p = 0.03). However, there were no significant differences in the prevalence of other cardiovascular risk factors, medications, CRP, and presence of rheumatoid factor between RA patients with and without coronary atherosclerosis.

EPC and coronary atherosclerosis. As shown in Table 1, there were no significant differences in the percentages of circulating CD34/KDR+ EPC and CD34+ EPC between RA patients with or without coronary atherosclerosis and control subjects. However, the percentages of circulating CD133+ EPC and CD133/KDR+ EPC were significantly lower in RA patients with coronary atherosclerosis compared with those without coronary atherosclerosis and control subjects (Table 1; all p < 0.01). Nevertheless, there were no significant correlations between CCS and different subgroups of circulating EPC in patients with RA (CD34/KDR+ EPC: r = 0.09, p = 0.45; CD34+ EPC: r = 0.96, p = 0.43; CD133/KDR+ EPC: r = –0.14, p = 0.25; CD133+ EPC: r = –0.12, p = 0.33).

EPC and clinical characteristics. As shown in Table 2, there were no significant correlations between different subgroups of circulating EPC with age, duration of RA, systolic and diastolic blood pressure, serum total cholesterol, triglyceride, and high or low density lipoprotein. However, there was a significant but only modest inverse relationship between CRP and CD133/KDR+ EPC (r = –0.27, p = 0.02).

Further, there were no significant differences in the mean percentages of circulating CD34+ (6.30% ± 4.46% vs 6.25%

Table 2. Correlations of endothelial progenitor cell (EPC) subgroups and clinical variables.

Clinical Variables	CD34+ EPC %		CD34/KDR+ EPC %		CD133+ EPC %		CD133KDR+ EPC%	
	r	p	r	p	r	p	r	p
Age	–0.11	0.36	–0.09	0.42	–0.21	0.09	–0.15	0.2
Duration of disease	–0.94	0.44	–0.1	0.4	–0.01	0.92	–0.09	0.44
SBP	–0.08	0.49	–0.01	0.98	–0.13	0.27	–0.16	0.19
DBP	–0.13	0.27	–0.17	0.17	–0.18	0.14	–0.23	0.06
Triglyceride	0.2	0.1	0.11	0.35	–0.65	0.59	–0.02	0.86
Total cholesterol	0.04	0.72	0.17	0.17	–0.05	0.67	0.05	0.69
HDL	–0.26	0.08	–0.09	0.44	–0.02	0.87	0.12	0.32
LDL	–0.02	0.86	0.11	0.37	–0.03	0.81	–0.03	0.83
C-reactive protein	0.11	0.38	0.01	0.99	–0.18	0.13	–0.27	0.02*
Creatinine	0.35	0.25	0.02	0.85	–0.25	0.35	–0.58	0.68

* $p < 0.05$. SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL: high density lipoproteins; LDL: low density lipoproteins.

± 5.40%; p = 0.75), CD34/KDR+ (2.81% ± 3.29% vs 2.56% ± 2.94%; p = 0.97), CD133+ (1.19% ± 0.77% vs 0.98% ± 0.80%; p = 0.30), and CD133/KDR+ EPC (0.81% ± 0.74% vs 0.76% ± 0.78%; p = 0.80) between RA patients with and those without DMARD treatment.

Predictors of coronary atherosclerosis in RA. Univariate analysis demonstrated that RA patients with coronary atherosclerosis were associated with older age, a higher prevalence of hypertension, and a lower circulating percentage of CD133+ EPC (Table 3; $p < 0.05$). However, multiple logistic regression analysis revealed that older age (OR 1.25, 95% CI 1.10–1.41; $p < 0.01$) and lower level of CD133/KDR+ EPC (OR 0.07, 95% CI 0.00–0.97; $p < 0.01$) were independent predictors for coronary atherosclerosis in patients with RA (Table 3).

DISCUSSION

In our study, different subgroups of circulating EPC were measured in patients with RA, and their relationships with the occurrence of subclinical coronary atherosclerosis were

Table 3. Predictors for coronary atherosclerosis by univariate and multivariate multiple logistic regression.

	Presence of Coronary Atherosclerosis†					
	Univariate Analysis			Multiple Logistic Regression		
	OR	95% CI	p	OR	95% CI	p
Age	1.21	1.09–1.34	< 0.01**	1.25	1.10–1.41	< 0.01**
Male sex	2.13	0.54–8.40	0.50			
Duration of disease	1.04	0.99–1.09	0.13			
Triglyceride	1.37	0.62–3.01	0.43			
Total cholesterol	1.49	0.92–2.42	0.10			
HDL	2.24	0.63–7.88	0.21			
LDL	1.54	0.83–2.88	0.17			
C-reactive protein	1.19	0.91–1.55	0.21			
Creatinine	2.53	0.82–3.25	0.18			
Hypertension	5.14	1.46–18.1	0.01*			
Diabetes	3.86	0.23–65.6	0.35			
Hyperlipidemia	NA††	NA††	—			
Smoking	3.64	0.84–15.9	0.09			
Rheumatoid factor	0.82	0.24–2.78	0.75			
NSAID	0.32	0.04–2.74	0.30			
Prednisolone	2.14	0.54–8.39	0.28			
DMARD	0.97	0.29–3.27	0.97			
CD34+ EPC %	1.05	0.94–1.17	0.40			
CD34/KDR+ EPC%	0.95	0.77–1.16	0.60			
CD133+ EPC %	0.23	0.05–0.99	0.05*			
CD133/KDR+ EPC %	0.18	0.29–1.15	0.07	0.07	0.00–0.97	0.01**

† Coronary calcium score > 10. †† OR cannot be estimated due to the complete separation of the data, so that we have no case from one of the groups. * $p < 0.05$. ** $p < 0.01$. EPC: endothelial progenitor cells; DMARD: disease modifying antirheumatic drugs; HDL: high density lipoproteins; LDL: low density lipoproteins; NSAID: nonsteroidal antiinflammatory drugs.

examined. Patients with RA had significantly higher mean CCS scores as well as prevalence of coronary atherosclerosis versus age- and sex-matched controls. In RA patients with coronary atherosclerosis, percentages of circulating CD133+ and CD133/KDR+ EPC were significantly lower than in those without coronary atherosclerosis. Further, the circulating level of CD133/KDR+ EPC was inversely correlated with CRP level. As well, multiple logistic regression analysis revealed that older age and lower level of CD133/KDR+ EPC were independent predictors of coronary atherosclerosis in patients with RA.

In concordance with prior studies[25,26], our results confirm that in patients with RA the presence of premature atherosclerosis as detected by coronary calcification was not associated with the majority of conventional cardiovascular risk factors, except for age. Previous clinical studies showed that duration and severity of RA might account for development of premature atherosclerosis as measured by carotid IMT[25-27]. These findings[25-27] suggest the potentially pivotal role of chronic inflammation in the pathogenesis of premature atherosclerosis. In our study, there was no significant correlation between CRP levels and severity of coronary atherosclerosis. In line with our results, Chung, *et al* also did not observe any relationship between CRP level and CCS in patients with RA. As RA is a chronic inflammatory condition, a single measurement of CRP should not be able to reflect the inflammatory burden in patients with RA.

Several studies have shown that depletion of circulating EPC is observed in patients with RA compared with controls[19-21]. However, there were no significant differences in different subgroups of EPC between RA patients and controls in our study. This may be related to the overall low disease activity in our cohort of RA patients as reflected by a similar CRP level compared to controls. Indeed, Grisar, *et al* demonstrated that RA patients with low disease activity score had comparable EPC levels versus control subjects[19]. Nevertheless, our results showed that RA patients with coronary atherosclerosis had significantly lower levels of CD133/KDR+ EPC than controls and those RA patients without coronary atherosclerosis. Further, RA patients without coronary atherosclerosis had EPC levels similar to those of controls. These findings suggest a potential link between depletion of CD133/KDR+ EPC and coronary atherosclerosis in patients with RA.

A comprehensive role of EPC in patients with RA after direct atherosclerotic assessment has not been fully validated. Recent studies demonstrated that measurement of coronary calcification is a reliable way to detect early atherosclerosis in asymptomatic patients and is a powerful surrogate marker for future cardiovascular events[27,28]. Studies[10,11] have shown a higher incidence of coronary calcification in patients with RA compared with control subjects. In addition, our recent study[14] highlighted the occurrence of premature and extensive calcification over multiple vascular beds in patients with RA. In our study, we hypothesized that chronic inflammation is associated with the depletion of circulating EPC, which subsequently contributes to the development of coronary atherosclerosis. Accordingly, measurement of circulating EPC levels may be more accurate in predicting development of coronary atherosclerosis. Further, the possible relationships of coronary atherosclerosis with different subgroups of EPC were also studied.

Since the initial report by Asahara, *et al*[29], EPC have been defined by surface expression of CD34 and KDR. As a result, the majority of subsequent studies have focused on the relationship between CD34/KDR+ EPC and development of atherosclerosis[30,31]. After mobilization from the bone marrow into the peripheral circulation, more mature EPC are negative for CD133 but are positive for CD34 and KDR[32,33]. Therefore, CD133+ or CD133/KDR+ EPC and CD34+ or CD34/KDR+ EPC represent subgroups of immature and mature EPC in the circulation, respectively.

Further, the co-expression of KDR with CD133 or CD34 is also a more specific marker for EPC than the presence of either CD133 or CD34 alone. In addition, the expression of CD133/CD34/KDR may represent an intermediate stage of EPC maturation. However, the presence of CD133/CD34/KDR EPC was not determined in the present study. Our results showed that RA patients with coronary atherosclerosis had significantly lower levels of CD133+ and CD133/KDR+ immature EPC, but not CD34+ and CD34/KDR+ mature EPC, compared to those without coronary atherosclerosis. Further, the circulating level of CD133/KDR+ EPC also only inversely correlated with the CRP level, but not with the other cardiovascular risk factors, suggesting that active inflammation might contribute to a lower level of EPC in patients with RA. Although the exact mechanism remains unclear, alteration of chemokine expression in RA patients related to chronic inflammation might have a greater influence on immature EPC than mature EPC[34].

Multiple logistic regression analysis demonstrated that in addition to older age, low level of CD133/KDR+ EPC was an independent predictor for coronary atherosclerosis in patients with RA. Prior studies have shown the potential association between depletion of EPC and atherosclerosis as measured by carotid IMT[19]. Our findings not only confirmed this result but further demonstrated that depletion of the population of immature EPC was associated with the development of coronary atherosclerosis in patients with RA.

Study limitations. First, this was a cross-sectional observational study and the causal relationship between EPC and arterial atherosclerosis in patients with RA could not be assessed. Second, the functional and proliferative activities of EPC were not determined; these require further studies to

identify their role in development of premature atherosclerosis. Third, clinical disease activity, using instruments such as the Disease Activity Score-28, was not measured in our cohort. Nevertheless, measurement of CRP in this study is probably more representative of inflammatory activities as these disease scores include certain assessments that could be subjective and not necessarily inflammatory.

Our study demonstrated that RA patients with coronary atherosclerosis as determined by CCS was associated with depletion of primordial CD133+ and CD133+KDR+ EPC but not mature CD34+ and CD34+KDR+ EPC. Further, circulating levels of CD133/KDR+ EPC independently predicted occurrence of coronary atherosclerosis. These findings suggest that depletion of EPC observed in RA patients could at least partly explain the occurrence of premature coronary atherosclerosis in patients with RA.

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