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Basic Science

Deficiency of Endothelial Progenitor Cells Associates with Graft Thrombosis in Patients Undergoing Endovascular Therapy of Dysfunctional Dialysis Grafts

Tsung-Yan Chen,[1] Lin Lin,[1] Mu-Yang Hsieh,[1,2] Jui-Cheng Kuo,[1] Chia-Ling Wang,[1] Ren-Huei Wang,[1] Chao-Lun Lai,[1,2] Po-Hsun Huang[3] and Chih-Cheng Wu[1,2,3,4]

Background: The deficiency of endothelial progenitor cells has been demonstrated to be associated with cardiovascular events in patients undergoing dialysis. However, their correlation with dialysis graft outcomes remains unknown. The objective of this study was to investigate the relationship between circulating endothelial progenitor cells and dialysis graft outcomes.

Methods: After excluding 14 patients with acute coronary syndrome, decompensated heart failure or graft thrombosis in the prior three months, a total of 120 patients undergoing dialysis who underwent endovascular therapy of dysfunctional dialysis grafts were prospectively enrolled. Blood was sampled from study subjects in the morning of a mid-week non-dialysis day. Surface makers of CD34, KDR, and CD133 were used in combination to determine the number of circulating endothelial progenitor cells. All participants were prospectively followed until June 2013.

Results: The median follow-up duration was 13 months, within which 62 patients experienced at least one episode of graft thrombosis. Patients with graft thrombosis had lower $CD34^+KDR^+$ cell counts compared with patients without graft thrombosis (median 4.5 vs. 8 per 10^5 mononuclear cells, p = 0.02). Kaplan-Meier analysis demonstrated thrombosis-free survival was lower in the low $CD34^+KDR^+$ cell count group (30%) than in the high $CD34^+KDR^+$ cell count group (61%; p = 0.007). Univariate analysis showed diabetes, high sensitive C-reactive protein, lesion length and $CD34^+KDR^+$ cell counts associated with graft thrombosis. Multivariate analyses confirmed an independent association between low $CD34^+KDR^+$ cell counts and graft thrombosis (hazard ratio, 2.52; confidence interval, 1.43-4.44; p = 0.001).

Conclusions: Our study demonstrated an independent association between low circulating endothelial progenitor cell counts and dialysis graft thrombosis.

Key Words: Endothelial progenitor cell • Graft • Hemodialysis • Thrombosis

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[1]Cardiovascular Center, National Taiwan University Hospital, Hsinchu Branch, Hsinchu; [2]College of Medicine, National Taiwan University; [3]School of Medicine, National Yang-Ming University, Taipei; [4]National Tsing-Hua University, Institute of Biomedical Engineering, Hsinchu, Taiwan.
Corresponding author: Dr. Chih-Cheng Wu, Cardiovascular Center, National Taiwan University Hospital, Hsinchu Branch, No. 25, Lane 442, Sec. 1, Jingguo Rd., Hsinchu City 300, Taiwan. Tel: 886-3-532-6151 ext. 2010; Fax: 886-3-532-2140; E-mail: chihchengwumd@gmail.com
* Dr. Tsung-Yan Chen and Dr. Lin Lin contributed equally as the first authors.

INTRODUCTION

Reliably functioning vascular access is critical for patients undergoing hemodialysis. Although a native fistula has been suggested as preferred access, prosthetic grafts are unavoidable in a substantial portion of patients with unfavorable anatomy.[1] Unfortunately, access grafts are prone to thrombotic complications – a serious problem that may result in increased medical costs and

loss of grafts. Despite the application of surveillance programs with pre-emptive angioplasty, thrombosis has remained a serious problem in patients with dialysis grafts.

The most common cause of graft thrombosis is venous stenosis located at the graft-venous anastomosis or outflow veins.[2] However, thrombosis may develop without underlying anatomical abnormalities.[3,4] In patients with end-stage renal disease (ESRD), traditional cardiovascular risk factors cannot explain such high thrombotic events.[5] Physiological and anatomical differences between arteries and veins, hemodynamic stress, repeated puncture, thrombophilia and uremic milieu have been proposed as possible contributors.[2] However, there may be other factors responsible for such a high thrombosis rate of dialysis grafts.

Maintenance of endothelial integrity and function plays a pivotal role in the prevention of thrombosis. A growing body of evidence suggests that bone marrow-derived circulating endothelial progenitor cells (EPCs) can become incorporated in sites of endothelial injury and restore vascular function.[6] Circulating EPCs have been demonstrated to be representative of the repair capacity and vascular function.[7] In patients with ESRD, both the number and function of EPCs are decreased.[8] The deficiency of EPCs has been demonstrated to be associated with cardiovascular events in patients undergoing hemodialysis.[9] However, the relationship between EPCs and outcomes associated with dialysis grafts remain unknown. The aim of the current study was to investigate the relationship between circulating EPCs and outcomes associated with dialysis grafts.

METHODS

Study participants and protocols

From January 2010 to December 2012, we prospectively enrolled end-stage renal disease (ESRD) patients undergoing maintenance hemodialysis at our hemodialysis center who required management of vascular accesses in the angiographic unit. Patients were excluded with the following criteria: (1) patients who received regular dialysis for less than 6 months; (2) patients with acute or chronic infectious disease, decompensated heart failure, myocardial infarction, acute limb ischemia or stroke requiring hospitalization in the previous three months; and (3) patients with thrombosis of vascular access in the previous three months.

Clinical data, access characteristics, and details of the angioplasty procedure were obtained from patient medical records, angioplasty reports, and hemodialysis records. All data were reviewed by an investigator blinded to the patients' clinical and analytic data. Cardiovascular disease was defined as coronary, peripheral, or cerebral artery disease. All eligible patients received hemodialysis (4 hours) three times a week using a synthetic dialysis membrane. The dialysate used in all patients was an ionic composition and bicarbonate-based buffer (dialyzers were not reused). The adequacy of dialysis was assessed monthly using a single pool Kt/V of urea nitrogen.

The study complied with those provisions of the Declaration of Helsinki. Written informed consent was obtained from all study participants, and the study was approved by the Institutional Review Board of our hospital.

Laboratory methods

Blood samples were drawn after a 12-hour overnight fast, and all medications were stopped before diagnostic procedures were undertaken. Plasma biochemical parameters measured included cholesterol, calcium, phosphate, and albumin, which were analyzed using standard laboratory procedures. Single-pool Kt/V of urea nitrogen was calculated after study enrollment using the second-generation logarithmic formula of Daugirdas. Assessment of the circulating EPCs by flow cytometry was performed by researchers blinded to the clinical data. As shown in Figure 1, the circulating EPCs were gated with monocytes and defined as $CD34^+$, $CD34^+KDR^+$, and $CD34^+KDR^+CD133^+$, respectively. Briefly, a volume of 1000 μL peripheral blood was incubated for 30 minutes in the dark with allophycocyanin (APC)-conjugated monoclonal antibody against human KDR (R&D System, Minneapolis, MN, USA), phycoerythrin (PE)-conjugated monoclonal antibody against human CD133 (Miltenyi Biotec, Germany), and fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies against human CD34 (Becton, Dickinson Pharmingen, Franklin Lakes, NJ, USA). After incubation, cells were lysed, washed with phosphate-buffered saline (PBS), and fixed

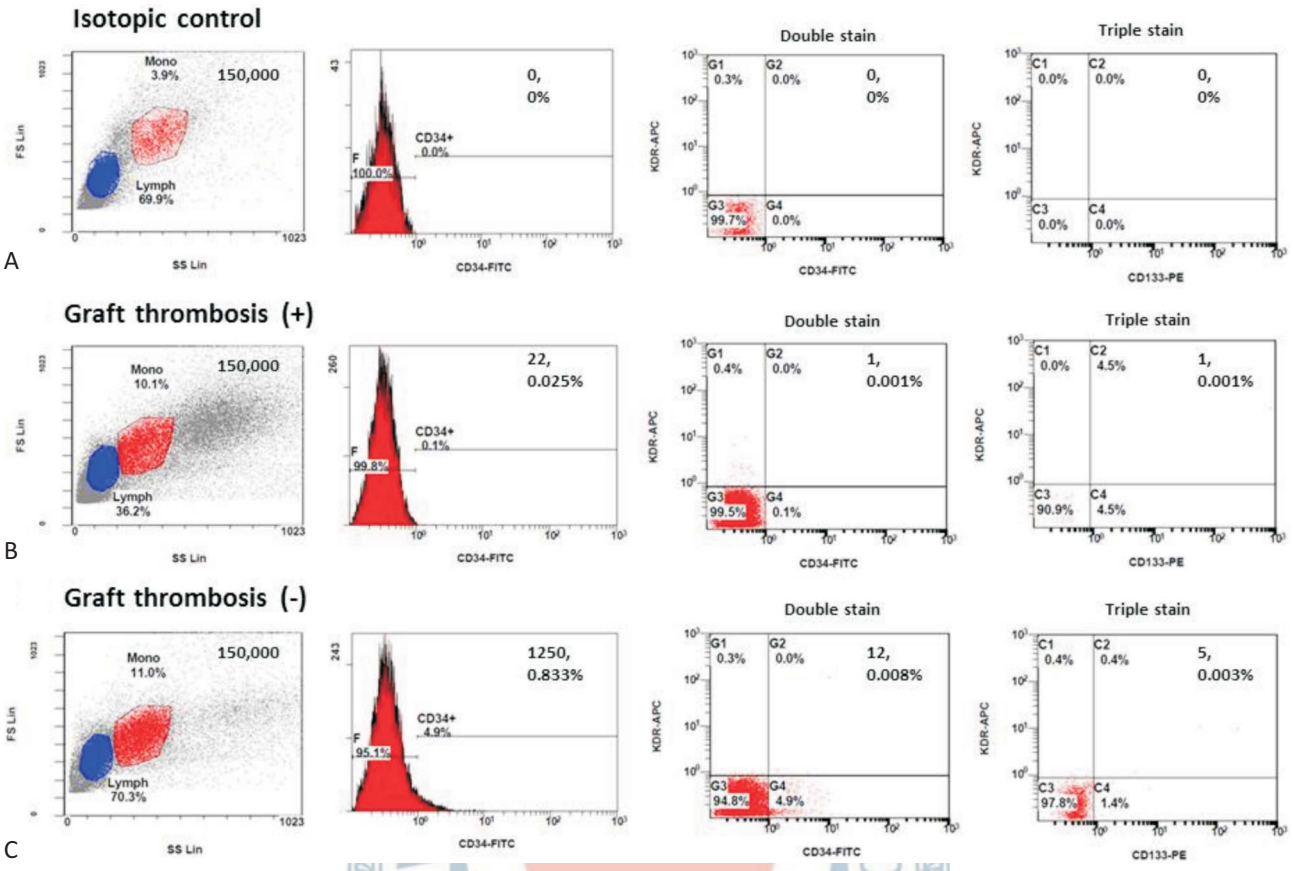

Figure 1. *Representative flow cytometry analysis. Panels show mononuclear cells (MNCs) that were gated by forward/sideward scatter (FSC/SSC) in isotype controls (A), patients with graft thrombosis (B), and patients without graft thrombosis (C). The numbers of circulating endothelial progenitor cells (EPCs) were defined as CD34+, CD34+KDR+, and CD34+KDR+CD133+, respectively.*

in 2% paraformaldehyde before analysis. Each analysis via flow cytometry included 150,000 events. The number of cells was normalized and expressed as a percentage (%) of cells, as well as cells per 1×10^5 mononuclear cells (MNC). To assess the reproducibility of EPC measurements, circulating EPCs were measured from 2 separate blood samples in 10 subjects. Ultimately, a strong correlation between the two measurements was observed ($r = 0.90$, $p < 0.001$).

Follow-up and definition

All study participants were prospectively followed and subsequent monitoring included physical examination and dynamic venous pressure monitoring at each hemodialysis session. Transonic examination of access blood flow rate was evaluated immediately after the intervention, followed by monthly examinations. The referring nephrologists were blinded to their patients' EPC levels. When abnormal clinical or hemodynamic parameters fulfilling the original referral criteria were detected, patients were referred for angiography and angioplasty as appropriate.

Access restenosis was defined as > 50% diameter stenosis anywhere within the outflow vein associated with clinical evidence of dysfunction. Access thrombosis was defined as a sudden cessation of access function rendering hemodialysis impossible, and requiring thrombectomy or placement of another hemodialysis access. Access failure was defined as abandonment or surgical revision of the access.

Statistical analysis

Continuous data are presented as means ± standard deviation (SD) for normal distributed variables and median with inter-quarter range (IQR) for non-normally distributed variables. Categorical data were presented as

percentage and compared using the Chi-square test, with Yates' correction and Fisher's exact test as appropriate. For normally distributed data, means between categories were compared using a t-test. For non-normally distributed data, the Mann-Whitney U test was used for comparison between categories. A cutoff number of circulating $CD34^+KDR^+$ cells was determined by receiver operative characteristic (ROC) analysis to maximize the power in predicting future graft thrombosis. The outcomes of vascular access between EPC groups were compared using a Chi-square test. The thrombosis-free patency of the access was estimated using the Kaplan-Meier method and differences between groups were compared using the log-rank test. Cox regression analysis was used for estimating the relative hazard of baseline variables to predict graft thrombosis events. Subjects in the higher tertile of $CD34^+KDR^+$ cell counts were used as the reference group for patients with low $CD34^+KDR^+$ cell counts. All variables with $p < 0.05$ in the univariate analysis were entered into a multivariate analysis to determine independent predictors. A p value of < 0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS software for Windows (version 20, SPSS Inc., Chicago, IL, USA).

RESULTS

EPC and baseline data

From January 2010 to December 2012, a total of 134 patients were prospectively enrolled. Fourteen patients were excluded due to the presence of acute coronary syndrome, decompensated heart failure, or graft thrombosis in the prior three months. The final study group was comprised of 120 patients with a mean age of 70 years (SD: 12 years) and a median hemodialysis duration of 36 months (IQR: 19-62 months). Baseline characteristics of the study participants and dialysis grafts are provided in Table 1. The number of circulating $CD34^+$ cells ranged from 3-7530 per 10^5 MNC (median: 46 per 10^5 MNC), $CD34^+KDR^+$ cells ranged from 0-52 per 10^5 MNC (median: 7 per 10^5 MNC), and $CD34^+KDR^+CD133^+$ cells ranged from 0-31 per 10^5 MNC (median: 6 per 10^5 MNC) at baseline.

To further clarify the importance of $CD34^+KDR^+$ cells, we then determined a cutoff value. A ROC curve analysis showed 5.5/10^5 MNC to be the value (area under the curve = 0.70) to maximize the power of $CD34^+KDR^+$ cells as a predictor of future graft thrombosis (Figure 2). Patients were stratified into two groups according to the cutoff value of $CD34^+KDR^+$ cell counts at baseline. The low EPC group included 50 patients with circulating $CD34^+KDR^+$ cell counts ≤ 5.5 per 10^5 MNC, and the high EPC group included 70 patients with circulating $CD34^+KDR^+$ cell counts ≥ 5.5 per 10^5 MNC.

Patients in the low EPC group were diagnosed with more cardiovascular disease ($p = 0.009$) compared with the high EPC group. More patients in the high-EPC group were taking renin-angiotensin system blockers ($p = 0.01$). Otherwise, factors such as gender, HD duration, hypertension, diabetes mellitus, dyslipidemia, and active smoking were comparable between the high and low EPC groups. Other common cardiovascular medications, including antiplatelets, β-blockers, calcium channel blocker, and lipid lowering agents did not differ between the two groups. Upper arm access was elevated in the high EPC group, and other access and procedural factors were comparable between high and low EPC groups.

Follow-up

After endovascular therapy, patients were followed until June 2013, with a median follow-up duration of 13 months (IQR: 6-32 months). During the study period, one patient received a kidney transplant and had an access thrombosis event before transplantation. No patient was transferred to peritoneal dialysis. Eighteen patients died before the end of follow-up and twelve had graft thrombosis before death. Eleven patients had graft failure, and four of them had graft thrombosis before access failure.

EPC and outcomes of AV grafts

The outcomes of dialysis grafts stratified by high or low baseline $CD34^+KDR^+$ cell counts are shown in Table 2. Ninety-two patients (77%) received re-interventions for restenosis or thrombosis, and the incidence of re-intervention did not differ between the two groups. Sixty-two patients (52%) had at least one episode of graft thrombosis in the follow-up period. Patients with graft thrombosis had lower $CD34^+KDR^+$ cell counts compared with patients without graft thrombosis (median,

Table 1. Baseline characteristics of study participants

Characteristic	All (N = 120)	Low $CD34^+KDR^+$ cell count (N = 50)	High $CD34^+KDR^+$ cell count (N = 70)	p value
Demographic factors				
Age (year)	70.6 ± 12.5	72.5 ± 13.0	69.3 ± 12.1	0.18
Gender (men/women)	42/78	17/33	25/45	1.00
HD duration (mo)	36 (19, 62)	36 (24, 61)	36 (18, 65)	0.84
Risk factors				
Hypertension, N (%)	69 (57.5%)	27 (54%)	42 (60%)	0.58
Diabetes, N (%)	56 (46.7%)	26 (52%)	30 (42.9%)	0.36
Dyslipidemia, N (%)	16 (13.3%)	8 (16%)	8 (11.4%)	0.59
Current smoker, N (%)	12 (10.0%)	3 (6%)	9 (12.9%)	0.36
CVD, N (%)	52 (43.3%)	29 (58%)	23 (32.9%)	0.009
Laboratory data				
Cholesterol (mg/dl)	159.3 ± 40.4	161.0 ± 36.4	157.9 ± 43.5	0.71
Albumin (g/dl)	3.8 ± 0.49	3.77 ± 0.49	3.80 ± 0.49	0.73
Hemoglobin (g/dl)	10.3 ± 1.3	10.4 ± 1.3	10.3 ± 1.4	0.70
WBC ($10^3/\mu L$)	7.2 ± 3.1	7.3 ± 2.6	7.1 ± 3.4	0.81
N/L ratio	7.6 ± 7.7	7.6 ± 6.8	7.5 ± 8.5	0.94
Calcium (mg/dl)	9.5 ± 0.9	9.4 ± 0.9	9.5 ± 0.9	0.69
Phosphate (mg/dl)	4.7 ± 1.5	4.6 ± 1.3	4.8 ± 1.7	0.46
Ca X P (mg^2/dl^2)	44.6 ± 15.5	43.0 ± 12.9	45.8 ± 17.3	0.38
Kt/V	1.49 ± 0.32	1.45 ± 0.32	1.53 ± 0.32	0.18
HS-CRP (mg/L)	2.4 ± 3.8	2.7 ± 2.3	2.2 ± 2.4	0.51
Medications				
Anti-platelet	49 (40.8%)	22 (44%)	27 (38.6%)	0.58
β-blocker	16 (13.3%)	6 (12%)	10 (14.3%)	0.79
Calcium blocker	26 (21.7%)	7 (14%)	19 (27.1%)	0.12
ACEI/ARB	16 (13.3%)	2 (4%)	14 (20%)	0.01
Lipid-lowering agents	13 (10.8%)	3 (6%)	10 (14.3%)	0.23
Folic acid	120 (100%)	50 (100%)	70 (100%)	0.99
Access, lesion and procedural factors				
Shunt age (month)	34 (19, 59)	36 (24, 59)	32 (15, 51)	0.43
Upper arm access	31 (25.8%)	7 (14%)	24 (34.3%)	0.02
Right arm access	28 (23.3%)	12 (24%)	16 (22.9%)	1.00
Pre-PTA stenosis (%)	75 ± 15	75 ± 15	74 ± 16	0.69
Post-PTA stenosis (%)	6 ± 8	9 ± 7	5 ± 9	0.43
Reference diameter (mm)	8.0 ± 1.8	7.6 ± 1.2	8.2 ± 2.1	0.15
Lesion length (mm)	38 ± 9	36 ± 14	39 ± 15	0.28

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; Ca X P, calcium and phosphate product; CI, confidence interval; CVD, cardiovascular disease history; HD, hemodialysis; HS-CRP, high sensitive C-reactive protein; Kt/V, urea clearance; N/L ratio, neutrophil/lymphocyte ratio; PTA, percutaneous transluminal angioplasty; WBC, white blood cell.

4.5 vs. 8 per 10^5 MNC, p = 0.02). The result was similar when a CD133 surface marker was added to define EPCs (median, 4 vs. 7 per 10^5 MNC, p = 0.03; Figure 3). In the low $CD34^+KDR^+$ cell count group, thrombosis occurred in 35 patients (70%). In the high $CD34^+KDR^+$ cell count group, thrombosis occurred in 27 patients (39%). The Kaplan-Meier plots showed that the thrombosis-free survival of low $CD34^+KDR^+$ cell count group was nearly half of that of high $CD34^+KDR^+$ cell count group (30% vs. 61%; p = 0.007; Figure 4). When surface marker CD133 was added, the Kaplan-Meier analysis also showed lower thrombosis-free survival of the low $CD34^+KDR^+CD133^+$ cell count group than that of the high $CD34^+KDR^+CD133^+$ cell count group (37% vs. 60%; p = 0.04). Additionally,

the incidence of death and vascular access failure was comparable between the two groups.

Cox analyses for predictors of graft thrombosis

The incidence of hemodialysis graft thrombosis was not associated with demographic factors, medications, biochemical profiles, or access factors. However, it was significantly associated with diabetes [hazard ratio (HR), 1.85, 95% confidence interval (CI), 1.11-3.07, p = 0.02], high sensitive C-reactive protein (HS-CRP) (HR, 1.06, CI, 1.00-1.12, p = 0.05), lesion length (HR, 1.04; CI, 1.01-1.08, p = 0.008) and baseline level of circulating $CD34^+KDR^+$ cells (low vs. high, HR, 2.23; CI, 1.34-3.70, p = 0.002; Table 3).

A multivariate Cox regression analysis adjusted for covariates, including diabetes, HS-CRP, lesion length, hemodialysis duration, history of cardiovascular disease, use of calcium channel blocker and shunt age confirmed

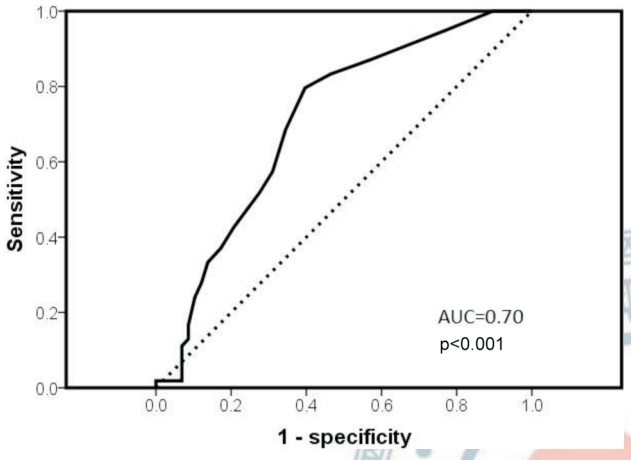

Figure 2. *Receiver operator characteristic curve of baseline $CD34^+KDR^+$ cell counts for graft thrombosis. The area under receiver operator characteristic curve (AUC) was 0.70 for baseline levels of $CD34^+KDR^+$ cell counts, which was significantly different from a random distribution (dash line, p < 0.001).*

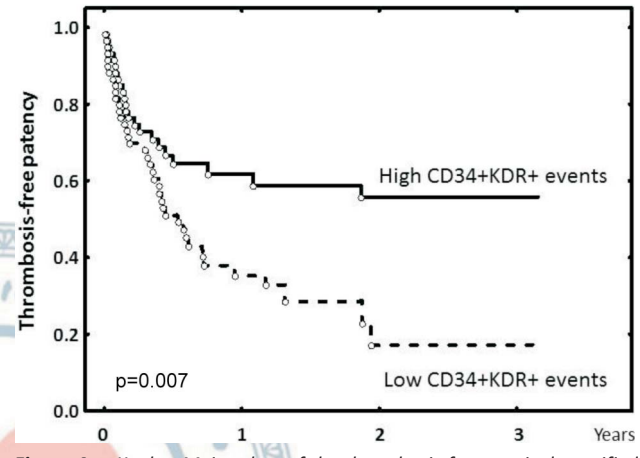

Figure 4. *Kaplan-Meier plots of the thrombosis-free survival stratified by high and low $CD34^+KDR^+$ cell count. The figure demonstrates the proportion of patients without thrombosis of dialysis grafts according to their circulating $CD34^+KDR^+$ cell counts at baseline, stratified by $5.5/10^5$ mononuclear cells into low (dash line) and high (solid line) groups.*

Table 2. Relation between baseline circulating $CD34^+KDR^+$ cell count and the outcomes of dialysis grafts

Outcomes	All (N = 120)	Low $CD34^+KDR^+$ cell count (N = 50)	High $CD34^+KDR^+$ cell count (N = 70)	p value
Re-intervention	92 (77%)	41 (82%)	51 (73%)	0.28
Thrombosis	62 (52%)	35 (70%)	27 (39%)	0.001
Failure	11 (9%)	5 (10%)	6 (9%)	1.00
Death	18 (15%)	9 (18%)	9 (13%)	0.45

p, Chi-square test for low and high $CD34^+KDR^+$ cell count groups.

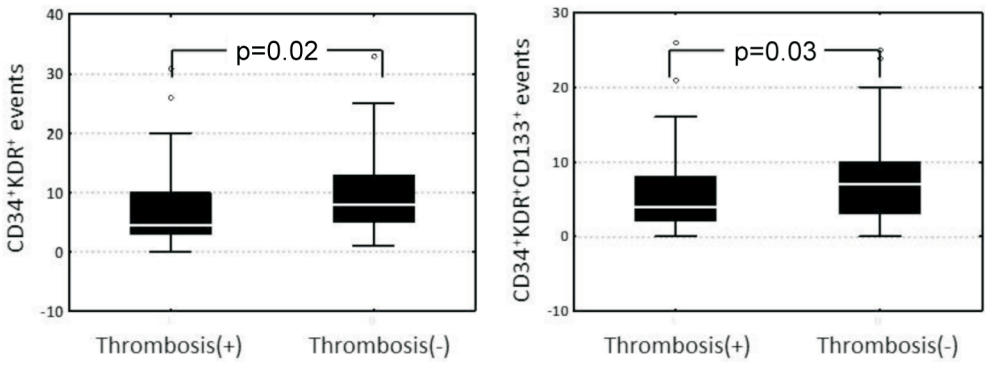

Figure 3. *Comparisons of the number of endothelial progenitor cells between patients with and without thrombosis.*

Table 3. Univariate analysis of predictors for graft thrombosis

Factors	Unit of increase	Hazard ratio	95% CI	p value
Demographic, medication and risk factors				
Age	1 yr	1.01	0.99-1.03	0.46
Female gender	Yes	1.30	0.77-2.22	0.33
HD duration	1 mon	0.99	0.99-1.00	0.07
Diabetes	Yes	1.85	1.11-3.07	0.02
CVD	Yes	1.61	0.97-2.67	0.06
Hypertension	Yes	0.70	0.42-1.15	0.15
Dyslipidemia	Yes	1.11	0.54-2.25	0.78
Antiplatelet	Yes	0.89	0.54-1.49	0.67
β-blocker	Yes	0.69	0.30-1.60	0.39
CCB	Yes	0.52	0.26-1.03	0.06
ACEI/ARB	Yes	0.55	0.23-1.27	0.16
Lipid-lowering agents	Yes	0.84	0.36-1.95	0.69
Laboratory data				
Low $CD34^+KDR_+$ count	Yes	2.23	1.34-3.70	0.002
$CD34^+KDR_+$ count	$1/10^5$ MNC	0.96	0.92-0.99	0.01
Cholesterol	1 mmol/L	0.99	0.99-1.00	0.35
Albumin	1 g/dl	0.65	0.39-1.09	1.00
Hemoglobin	1 g/L	0.88	0.72-1.08	0.21
WBC	1/cumm	1.07	0.98-1.17	0.13
N/L ratio	1 unit	1.04	0.99-1.08	0.06
Calcium	1 mg/dL	0.83	0.62-1.12	0.22
Phosphate	1 mg/dL	1.18	0.99-1.40	0.07
Ca X P Product	$1\ mg^2/dl^2$	1.01	0.99-1.03	0.20
Kt/V	1 unit	0.60	0.27-1.30	0.19
HS-CRP	1 mg/L	1.06	1.00-1.12	0.05
Access, lesion and procedural factors				
Shunt age	1 mon	0.99	0.98-1.00	0.07
Upper arm access	Yes	0.95	0.53-1.67	0.84
Right arm access	Yes	1.21	0.69-2.14	0.51
Pre-PTA stenosis	1%	0.99	0.97-1.01	0.27
Post-PTA stenosis	1%	1.02	0.99-1.05	0.17
Reference diameter	1 mm	0.95	0.81-1.11	0.53
Lesion length	1 mm	1.04	1.01-1.08	0.008

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; Ca X P, calcium and phosphate product; CVD, cardiovascular disease history; HS-CRP, high sensitive C-reactive protein; Kt/V, urea clearance; MNC, mononuclear cell; N/L ratio, neutrophil/lymphocyte ratio; PTA, percutaneous transluminal angioplasty; WBC, white blood cell.

an independent association between $CD34^+KDR^+$ cell count with the occurrence of graft thrombosis, either entered as a categorical variable (low vs. high: HR, 2.52; CI, 1.43-4.44; p = 0.001) or a continuous variable (per 1 cell/10^5 MNC increase: HR, 0.94; CI, 0.90-0.99; p = 0.01). Diabetes and lesion length remained independent predictors of graft thrombosis in the multivariate analysis (Table 4).

DISCUSSION

Main finding

Deficiency of circulating EPCs, defined by either $CD34^+KDR^+$ cells or $CD34^+KDR^+CD133^+$ cells, was associated with thrombosis of hemodialysis grafts. Patients with low levels of circulating $CD34^+KDR^+$ cells had a 2-fold increased risk of dialysis graft thrombosis compared with patients with high $CD34^+KDR^+$ cell counts. In addition, this association was independent of traditional risk factors, biochemical factors and anatomical factors.

Comparison with previous studies

Vascular access complications are major sources of morbidity and medical expenditure for patients undergoing hemodialysis.[10] Among the complications, thrombosis is the most common cause of secondary vascular

Table 4. Multivariate analysis of predictors for graft thrombosis

Factors	Unit of increase	Hazard ratio	95% confidence interval	p value
$CD34^+KDR^+$ cell count as a categorical variable				
Diabetes	Yes	2.01	1.12-3.61	0.02
HS-CRP	1 mg/L	1.02	0.97-1.07	0.42
Lesion length	1 mm	1.04	1.01-1.08	0.01
Low $CD34^+KDR^+$ count	Yes	2.52	1.43-4.44	0.001
$CD34^+KDR^+$ cell count as a continuous variable				
Diabetes	Yes	1.81	1.01-3.23	0.05
HS-CRP	1 mg/L	1.03	0.93-1.02	0.27
Lesion length	1 mm	1.04	1.01-1.08	0.02
$CD34^+KDR^+$ count	$1/10^5$ MNC	0.94	0.90-0.99	0.01

HS-CRP, high sensitive C-reactive protein; Kt/V, urea clearance; MNC, mononuclear cell; N/L ratio, neutrophil/lymphocyte ratio.

access failure.[2] Graft accesses are more prone to acute thrombosis than native fistulas. Although percutaneous thrombectomy is highly successful for salvage, the reported 3-month unassisted patency is poor for grafts, ranging from 30%-40%. Prevention of thrombosis is consequently of great clinical and economic importance.

A major predisposing factor for thrombosis is an anatomical stenosis at outflow veins, which can cause stasis of blood in the vascular access. Adequate monitoring to identify anatomical stenosis is essential to prevent thrombosis. Nonetheless, access thrombosis may occur without outflow venous stenosis. Previous studies have demonstrated thrombophilia and inflammation as possible causes of thrombosis. However, both antiplatelet and anticoagulant agents have showed inconclusive or negative results. The traditional cardiovascular risk factors and uremia-related risk factors did not predict dialysis graft thrombosis. Currently, there is a paucity of strategies to prevent graft thrombosis.

According to Virchow's triad, thrombosis is associated with hypercoagulability, endothelial injury, and stasis of blood flow. The role of endothelial injury in graft thrombosis has not been extensively investigated. Nonetheless, animal models have consistently shown the capacity of circulating EPC to repair endothelium of damaged vessels, providing a mechanical barrier for the thrombogenic subendothelial matrix.[11] In human studies, EPCs were reported to be associated with vascular events, restenosis after coronary interventions and late stent thrombosis.[12-14] In hemodialysis patients, the number and function of EPCs was depressed and $CD34^+$ cells had been demonstrated to be associated with cardiovascular events after a one-year follow-up.[9] However, the role of EPCs on thrombosis regarding dialysis access was not examined. Our study was the first to demonstrate the association between EPC deficiency and thrombosis of dialysis grafts.

Possible mechanisms

There were several possible mechanisms which could explain the association between EPCs and dialysis graft thrombosis. First, patients undergoing hemodialysis are prone to vascular injury due to a high prevalence of atherosclerotic diseases, daily punctures of vascular access, and frequent endovascular procedures. An intact endothelium possesses both anti-platelet and antithrombotic properties. Disruption of endothelium can expose thrombogenic subendothelial matrix, the primary event in the initiation of thrombosis. Experimental studies have consistently demonstrated that EPCs are involved in re-endothelialization of injured vessels, providing a mechanical barrier for the thrombogenic subendothelial tissue.[11] Dialysis graft thrombosis is usually secondary to blood stasis, caused by stenosis or thrombosis at outflow veins.[15] Delayed endothelial repair at outflow veins may initiate thrombotic events at vessel injury sites, followed by blood stasis and graft thrombosis.

Second, in human studies, EPCs have been shown to be associated with endothelial function and have been suggested to be a marker of vascular health.[16] A healthy endothelium possesses not only antiplatelet and antithrombotic function, but also anti-inflammation and anti-proliferative function.[17] A rapid regeneration of the endothelial monolayer may prevent restenosis development. Previous studies have shown that deficiency of EPCs is associated with rapid and frequent restenosis af-

ter angioplasty, both in interventions of coronary arteries and dialysis accesses.[18,19] Consequently, patients with low EPCs are more likely to have thrombotic events secondary to rapid intimal hyperplasia with frequent restenosis.

Third, EPCs may be a better maker of overall vascular function compared with individual cardiovascular risk factors or biomarkers. That is because a cellular maker may summarize the effect of various cardiovascular risk factors and uremia-related risk factors. Vascular risk factors, such as diabetes, dyslipidemia, hypertension, and smoking, affect the number and function of EPCs in non-uremic patients.[7,20] Uremia-related factors, such as asymmetric dimethylarginine (ADMA), indoxyl sulfate, and p-cresol sulfate had been reported to inhibit EPCs.[21-23] Cardio-protective factors, such as statins or exercise, were also reported to elevate EPC levels. Individual risk factors are usually insufficient to predict the outcomes of dialysis vascular access. In contrast, a cellular marker, such as $CD34^{+}KDR^{+}$ cell counts in the present study, may reflect the cumulative effect of various risk factors and hence demonstrate a better association with vascular access events.

Other contributors

Although diabetes is a leading cause of vascular disease, previous studies revealed inconsistent results regarding its role in dialysis access thrombosis. In both univariate and multivariate models, we found that diabetes increased the risk of thrombosis, but not the risk of restenosis. Several mechanisms contribute to a prothrombotic state in diabetic patients, including endothelial dysfunction, coagulation activation, and platelet hyper-reactivity which result from the interaction among hyperglycemia, insulin resistance, inflammation, and oxidative stress on the vessels.

In this study, circulating markers for systemic inflammation, such as HS-CRP and neutrophil/lymphocyte ratio (NLR), were marginally associated with graft thrombosis. In patients with advanced kidney diseases, HS-CRP was associated with thrombosis of fistulas; NLR was associated with the outcome of peripheral artery diseases after percutaneous transluminal angioplasty (PTA).[24,25] Pathological examination had revealed preferential expression of matrix metalloproteinase-9 near the vascular lumen of thrombosed arteriovenous fistulas, which may cause disruption of the neointima with subsequent exposure of the highly procoagulant subendothelial tissues to circulating blood.[26] Long lesion length was associated with graft thrombosis in both univariate and multivariate analyses, consistent with previous data that longer lesions had shorter primary patency after angioplasty.[27,28]

Currently, there is a paucity of effective pharmacotherapy to prevent graft thrombosis. Statin, angiotensin-converting enzyme inhibitors, calcium blockers, folic acid and antiplatelet had been reported to improve the outcome of dialysis grafts in observation studies.[5,29,30] However, the results between different studies were inconsistent and rare randomized control trails were available. Consequently, the use of medications to prevent thrombosis of grafts remained controversial and was not suggested by the evidence-based guidelines.[1] No association between the use of medication and graft outcomes in this study but no conclusion could be made because of our limited sample size and observational design.

Limitation

Our study has several limitations. First, there is no universal definition of EPC. KDR is also widely expressed on blood, endothelial, and cardiac cells and thus may not be sufficient to discriminate among those cells expressing the marker CD34.[31] Although a variety of surface makers helpful to identify EPC had been proposed, they were not available when this study was conducted.[32] Second, the EPC function properties were not assessed in this study. Previous studies have demonstrated an association between EPC circulating counts and function.[13] Third, the direct causal relationship between EPC level and graft thrombosis cannot be validated in the observational clinical studies. The role of EPCs has to be proved in animal models or therapeutic trials to see their effects by modulating the number or function of EPCs.

Clinical implication

The results of the current study provide a novel association between circulating EPCs and hemodialysis graft thrombosis. Based on this finding, methods to modulate the EPC number or function, including physical exercise, pharmacological modulation (statin, G-CSF), infusion of autologous EPCs, capturing EPC to the

denudated endothelium, deserve to be investigated for the prevention of graft thrombosis.[18,33,34] In addition, $CD34^{+}KDR^{+}$ cell count may be a better marker to identify the high-risk patients of graft thrombosis. It will be helpful in therapeutic or surveillance planning, such as aggressive monitoring, EPC-modulating intervention, or early surgical revision.

CONCLUSIONS

For the first time the results of present study demonstrate that low EPC counts are associated with thrombosis of dialysis grafts. This association was independent of clinical, biochemical and access factors. Whether EPCs can be modified to prevent thrombosis of dialysis grafts deserves further investigation.

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DISCLOSURE

The authors state that they have no conflict of interest to declare.

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