Acta Cardiol Sin 2015;31:24–32
Original Article
doi: 10.6515/ACS20140310E

Basic Science

Mobilization of Endothelial Progenitor Cells Following Creation of Arteriovenous Access in Patients with End-Stage Renal Disease

Jun-Neng Roan,[1,2,3] Chwan-Yau Luo,[2] Mang-Da Tsai,[2] I-Shuan Wu,[3] Shih-Wei Chang,[4] Chien-Chi Huang,[4] Yau-Sheng Tsai[1] and Chen-Fuh Lam[4,5]

Background: A patent arteriovenous (AV) fistula induces activation of regional vascular endothelium and vascular shear force. Shear stress is an important physiological force in mobilizing endothelial progenitor cells (EPCs). This study aimed to explore the perioperative changes of circulating EPC levels for patients who require hemodialysis and underwent radiocephalic fistula operation.

Methods: This prospective cohort study included patients who received a radiocephalic fistula surgery when they were between 25 and 65 years of age. The subjects were followed for 90 days postoperatively for any stenotic events or immaturity of the fistula. Blood samples were obtained on the day before surgery and at postoperation day (POD) 3 and 30. CD133+/KDR+ cells, defined as EPCs, were analyzed using flow cytometry. Blood flow of the fistula was followed on POD 3 and 30.

Results: A total of 30 patients were enrolled in the study from July 2009 to December 2011. One patient dropped out of the study and seven patients developed a stenotic (or immature) AV fistula (7/29, 24.1%). There were positive linear relationships between EPC numbers and shear rate postoperatively, which were more significant on POD 30. In addition, postoperative mobilization of EPCs was significantly higher in patients who developed a stenotic fistula than those without.

Conclusions: The mobilization of circulating EPCs correlated with a compromised arteriovenous fistula. The biological significance of increased EPC numbers need to be determined in future studies.

Key Words: Arteriovenous fistula • Endothelial progenitor cells

INTRODUCTION

Arteriovenous (AV) fistula is a most common vascular access for hemodialysis in patients with end-stage renal disease (ESRD). Creation of an AV fistula involves a direct anastomosis of the radial/brachial artery and cephalic vein. The establishment of AV fistula exposes the vascular endothelial cells constantly to shear stress.[1,2] Shear stress is a critical modulator of vascular structure and function, including the regulation of vascular tone and diameter, remodeling of vessel wall, platelet aggregation, white blood cell adhesion and other inflammatory responses.[1,3,4] Therefore, maintaining the patency and adequate blood flow in the AV fistula is critically dependent on the fistula shear force and activation of regional vascular endothelium. The discovery of bone marrow-derived endothelial progenitor cells (EPCs) dur-

Received: September 19, 2013 Accepted: March 10, 2014
[1]Institute of Clinical Medicine; [2]Division of Cardiovascular Surgery, Department of Surgery, National Cheng Kung University College of Medicine and Hospital; [3]Department of Surgery, Tainan Municipal Hospital; [4]Department of Anesthesiology, National Cheng Kung University College of Medicine and Hospital, Tainan; [5]Department of Anesthesiology, Buddhist Tzu-Chi General Hospital and Tzu-Chi University School of Medicine, Hualien, Taiwan.
Address correspondence and reprint requests to: Dr. Chen-Fuh Lam, Department of Anesthesiology, Buddhist Tzu-Chi General Hospital and University School of Medicine, No. 707, Chung-Yang Road, Hualien 970, Taiwan. Tel: 886-38-561825; Fax: 886-38-577161; E-mail: lamcf@mail.tzuchi.edu.tw

ing the past decade has improved our understanding of postnatal vasculogenesis and activation of vascular endothelium.[5] Mobilization of EPCs into systemic circulation contributes significantly in the angiogenesis during regeneration process,[6] and reduced numbers of circulating EPCs are sensitive biomarkers for worse clinical outcomes in cardiovascular events.[7,8] Since fluid shear stress is an important physiological force in the regulation of activation and mobilization of EPCs,[9,10] it is therefore essential to study levels of circulating EPCs in patients with ESRD before and after creation of AV fistula, and further correlates with the maturation of fistulas. On the other hand, improved maturation of new fistulas can also enhance the quality of life in ESRD patients. The identification of patients who are at risk of developing an immature fistula may help surgeons in modifying the surgical planning and taking early action for salvage of the malfunctioned fistula during the postoperative follow-up period. This study aimed to explore the perioperative dynamic changes of circulating EPC levels in ESRD patients, who undertook an AV fistula surgery and establish circulating EPC numbers as biological markers for prediction of a successful fistula during the follow-up period.

MATERIALS AND METHODS

Institution and patient recruitment

All surgical procedures for creation of AV fistula were undertaken in the operating rooms of the Tainan Municipal Hospital, Taiwan. The study protocol was approved by the Institutional Review Board of Show Chwan Memorial Hospital (SCMH 981103), and written informed consent was obtained from the patient or patients' legal surrogates. Successive patients of both genders within the ages of 25 to 65 years who were admitted for creation of AV fistula were eligible. All patient demographic data were recorded, including age, sex, co-morbidity diseases, concurrent medication and body mass index, smoking, and blood biochemistry tests such as serum creatinine, albumin, calcium, phosphate, hematocrit and hemoglobin levels. Patients with advanced liver disease or chronic alcoholism, severe congestive heart failure (Fc III/IV), evidence of symptomatic coronary artery disease, peripheral arterial disease (PAD), carotid artery disease, malignancy, pregnancy, hematological disorders and patients who required general anesthesia were excluded from the study. Patients with conditions which might involve neovascularization such as neoplasm, wounds, or significant retinopathy, were also excluded.[11] All patients' regular medications including statins, glucose-controlling drugs, anti-coagulation drugs, platelet aggregation inhibitors, and antihypertensive drugs were continued throughout the study period. Administration of erythropoietin before operation was also recorded. Blood samples were also collected from the healthy volunteers, who were free from any systemic disease according to medical records. The ESRD patients were followed for 90 days after creation of AV fistula. The ESRD patients were regularly followed up in the nephrologist outpatient clinics or local dialysis centers, where a nurse specialist traced the patients' fistula conditions. A mature AV fistula was defined as a fistula that maintained sustainable function for hemodialysis throughout the study period, and an efferent vessel of diameter greater than 5 mm and located less than 6 mm underneath the skin by ultrasonic echo-Doppler examination.[12] The primary end-point was to survey the failure of fistula that was stenotic or immature. An immature fistula was defined as one that could not sustain more than one course of dialysis after a maturation period of at least 6 weeks after surgery. Stenosis of fistula was defined as one that functioned well through at least one course of dialysis but eventually became dysfunctional during the study period (< 90 days postoperatively).[12]

Creation of AV fistula

The standard Brescia-Cimino arteriovenous fistula was performed on the forearm of ESRD patients scheduled for permanent hemodialysis. End-to-side anastomosis of the cephalic vein and the radial artery was created under local anesthesia. Patients who received surgical anastomosis on locations other than wrist area were excluded from the study. Conditions of fistula were assessed by duplex ultrasound measurement of blood flow for at postoperation day (POD) 3 and 30 and by physical examination at POD 90.

Measurement of blood peak volume flow in the AV fistula

Blood flow in the venous limb of the AV fistula was insonated with a 5 or 10 MHz scanning probe (38 mm,

SonoSite, Inc., Bothell, WA, USA) on POD 3 and 30. The blood flow was measured at the segment of fistula 10 cm distal from the anastomosis using the time-averaged velocity (TAV) during the Doppler scanning, and applied to the formula as: volume flow (mL/sec) = TAV (cm/s) × cross-section (cm^2).[13]

The shear rate of the fistula were calculated as peak flow velocity (V_{peak}, cm/s) divided by diameter of the fistula (D, cm).[14]

Collection of blood sample and analysis of circulating EPCs

The baseline blood samples were drawn from the cephalic vein during operation. The other blood samples were collected from the counter-lateral cephalic vein at different study time-points. A total of 5 ml of peripheral blood were obtained at each sampling point. Blood samples were determined for analysis of $CD133^+/KDR^+$ cells, defined as EPCs, using a flow cytometry.[15] Mononuclear cells (MNCs) were isolated by the standard Ficoll assay and plated onto fibronectin-coated culture disc. Colony-forming units (CFUs) of EPCs were recorded at 7 days after plating.[16]

Statistical analysis

Results are presented as mean ± standard error of the mean. Continuous variables were evaluated by non-parametric Mann-Whitney test, Wilcoxon signed rank test or RM ANOVA, as appropriate. Categorical variables were evaluated by Fischer's exact test. Correlations between variables were assessed by Pearson correlation and simple linear correlation analysis. The receiver operating characteristic (ROC) curve was used to define a cut-off value of EPC levels for predicting stenosis of a fistula within the postoperative three months. Statistical significance was accepted at a level of $p < 0.05$.

RESULTS

Patient characteristics

A total of 30 patients were enrolled in the present study from July 2009 to December 2011, and the patient enrollment flow chart is summarized in Figure 1. One patient refused phlebotomy and further clinical follow-up after POD 3; eight patients refused phlebotomy for EPC study but adhered to clinical follow-up until POD 90. No surgical mortality or morbidity was recorded during the follow-up period, and the rate of complete follow-up was 96.7%. Seven patients (7/29, 24.1%) developed a stenotic AV fistula from POD 60 to 90 and secondary intervention was undertaken. Five of the seven patients received either a new fistula or AV shunt surgery, and the other two patients underwent percutaneous transluminal angioplasty to repair the stenotic fistula. All the patients were classified to the patent or stenotic groups according to the study's primary end-points (Table 1). The mean age of the patients was 51.9 ± 8.5 years and consisted of 22 male and 7 female patients. The other patient characteristics were not different between the patent and stenosis groups (Table 1). The use of perioperative medications was also similar between the patent and stenotic AV fistula patients (Table 1).

Follow-up of AV fistula peak blood volume flow rate and shear rate

Overall, the mean blood flow rate in the AV fistula increased with time from 8.24 ± 1.11 mL/sec on POD 3

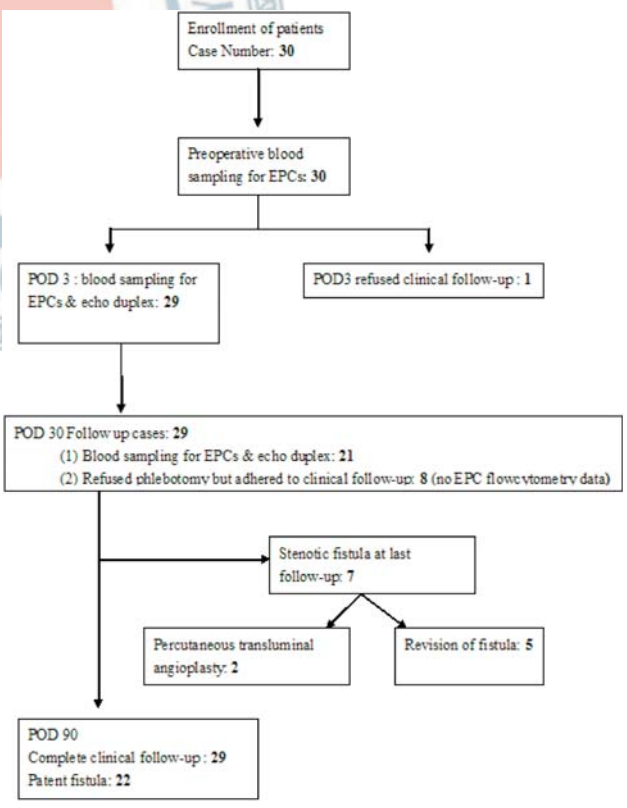


Figure 1. *Flow chart of patient enrollment.*

Table 1. Perioperative patient characteristics

Variables	Patent n = 22 (%)	Stenosis n = 7 (%)	p value
Age	50.1 ± 2.0	56.1 ± 1.8	0.05
Gender (M:F)	17:5	5:2	1.00
BMI	25.3 ± 1.0	26.3 ± 1.6	0.64
Diabetes	12 (54.5)	3 (42.9)	1.00
Hypertension	12 (54.5)	4 (57.1)	1.00
Dyslipidemia	8 (36.4)	1 (14.3)	0.38
Smoking	12 (54.5)	4 (57.1)	1.00
Preoperation hemodialysis	9 (40.9)	3 (42.9)	0.67
% lymphomonocytes (ie. EPCs) at baseline	0.14 ± 0.03	0.18 ± 0.03	0.41
Pre-operative blood tests			
Creatinine	9.01 ± 0.87	12.00 ± 1.00	0.19
Albumin	3.05 ± 0.16	3.27 ± 0.15	0.49
Calcium	7.94 ± 0.29	7.59 ± 0.23	0.44
Phosphate	6.43 ± 0.60	7.81 ± 0.47	0.18
Hematocrit	25.69 ± 2.07	27.01 ± 1.00	0.54
Hemoglobin	8.57 ± 0.75	8.96 ± 0.30	0.58
Pre-operative medications			
Erythropoietin	11 (50.0)	4 (57.1)	1.00
ACE-Is	2 (9.1)	1 (14.3)	1.00
Statins	8 (36.4)	1 (14.3)	0.38
Anti-platelets	8 (36.4)	1 (14.3)	0.38
Calcium channel blocker	15 (68.2)	6 (85.7)	0.64
β-blocker	12 (54.5)	4 (57.1)	1.00
POD 3 fistula duplex			
Diameter (mm)	5.14 ± 0.33	4.82 ± 0.56	0.70
V_{peak} (cm/s)	36.08 ± 2.46	43.83 ± 5.26	0.21
Shear rate	75.32 ± 6.44	91.76 ± 12.00	0.23
POD 30 fistula duplex			
Diameter (mm)	5.90 ± 0.44	4.28 ± 0.36	0.04
V_{peak} (cm/s)	44.98 ± 4.22	39.56 ± 3.75	0.45
Shear rate (1/sec)	81.63 ± 8.34	100.15 ± 16.10	0.11

Data showed as mean ± standard error of the mean.
ACE-Is, angiotensin converting enzyme inhibitors; BMI, body mass index; EPCs, endothelial progenitor cells; POD, postoperation days; Vpeak, peak flow velocity. Shear rate = V_{peak} (cm/s)/Diameter (cm).

to 11.57 ± 1.95 mL/sec on POD 30 (p = 0.50, Table 2-1). The fistula diameters were 5.06 ± 0.28 mm and 5.4 ± 0.38 mm on POD 3 and POD 30 respectively, where p = 0.83. The changes in peak flow rate and shear rate from POD 3 to POD 30 were also similar (Table 2-1). On the other hand, the blood flow rate in the patent AV fistula increased from 8.0 ± 6.0 mL/sec on POD 3 to 14.2 ± 9.8 mL/sec on POD 30 (p = 0.02, Figure 2). The fistula flow rate was significantly higher in the patent group compared to the stenotic fistula group (14.2 ± 9.8 vs. 5.6 ± 1.8 mL/sec, respective; p = 0.02, Figure 2). In comparison with the patent fistula, the diameter of fistula on POD 30 was smaller in the stenosis group (4.28 ± 0.36 mm vs. 5.90 ± 0.44 mm, respectively; p = 0.04, Table 1). The shear rates in the fistula of the two

Table 2-1. The overall changes of blood flow, diameter, peak flow velocity and shear rate of arteriovenous fistula on postoperation day 3 and 30

	POD 3 (n = 29)	POD 30 (n = 21)	p value
Fistula blood flow (ml/min)	8.24 ± 1.11	11.57 ± 1.95	0.50
Fistula diameter (mm)	5.06 ± 0.28	5.43 ± 0.38	0.83
Peak flow velocity (cm/sec)	37.71 ± 2.27	43.40 ± 3.40	0.52
Shear rate (1/sec)	79.29 ± 5.73	82.67 ± 7.59	0.51

Data showed as mean ± standard error of the mean.
POD, postoperation days.

groups both decreased From POD 3 to POD 30 (Table 1).

Flow cytometry of EPCs

In general, the number of circulating EPCs increased from the baseline levels (0.15 ± 0.02% of lymphomonocytes) to 0.26 ± 0.08 and 0.23 ± 0.05% of lymphomonocytes on POD 3 and POD 30, respectively (p = 0.59, Table 2-2). The mobilization of EPCs in patent-fistula group was not significant during the study period, but was significantly increased in the patients who eventually developed an immature fistula or stenosis, where p = 0.02 (Figure 3 and Supplement Figure 1). A cut-off value of 0.12% (number of EPCs in total lymphomonocytes) was determined from the ROC curve to predict a failure AV fistula within 3 months after operation, with a sensitivity of 90% and specificity of 43% (p = 0.06).

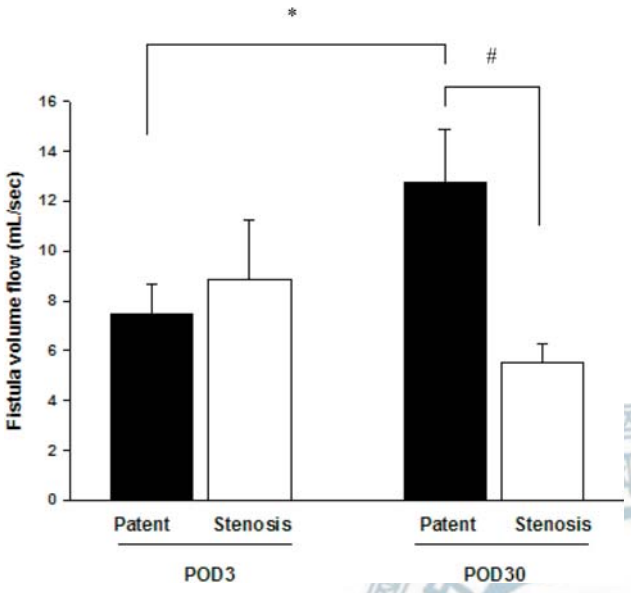


Figure 2. *Peak volume flow of arteriovenous (AV) fistula on POD 3 and 30. The flow rate increased significantly among patients with patent AV fistula (* 7.46 ± 1.20 mL/sec on POD 3 vs. 12.78 ± 2.14 mL/sec on POD 30, p = 0.02). The flow rate increased in patients with a stenotic fistula (8.87 ± 2.40 mL/sec vs. 5.56 ± 0.69 mL/sec, p = 0.32). The average peak volume flow on POD 30 was also higher in patients with a patent AV fistula than in the stenotic group (# 12.78 ± 2.14 mL/sec vs. 5.56 ± 0.69 mL/sec, p = 0.02). POD, postoperation days. Data showed as mean ± standard error of the mean.*

Functional analysis of circulating EPCs

Although EPC numbers increased after creation of AV fistula in ESRD patients, the isolated MNCs yielded a very limited amount of outgrowth colonies, regardless of the time points after operation or maturation

Table 2-2. Overall changes of circulating endothelial progenitor cells (EPCs) before (baseline) and after fistula operation

	Base line (n = 29)	POD 3 (n = 29)	POD 30 (n = 21)
% lymphomonocytes	0.15 ± 0.02	0.26 ± 0.08	0.23 ± 0.05

Data showed as mean ± standard error of the mean.
POD, postoperation days. The mobilization of EPCs was not statistically significant between different time points, p = 0.59.

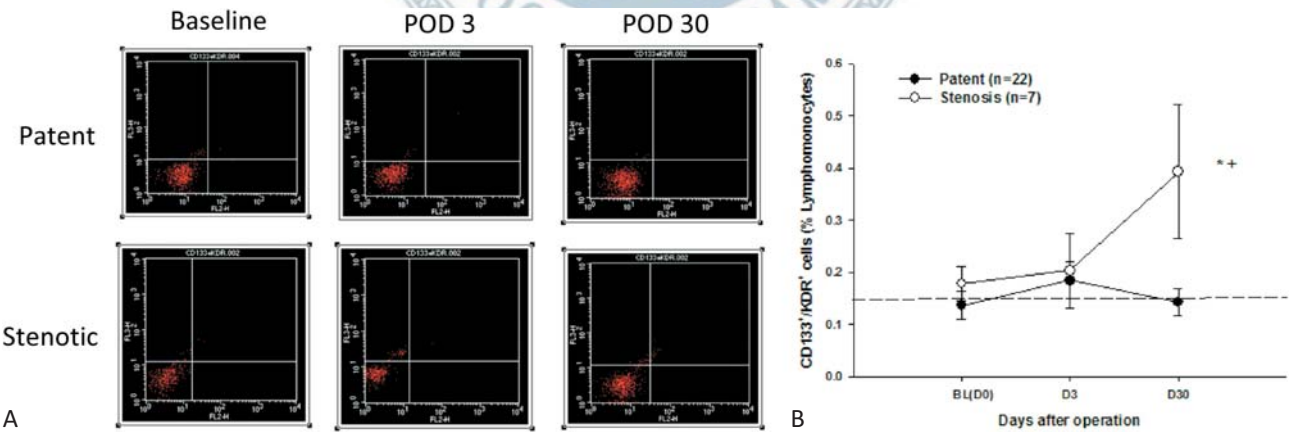


Figure 3. *Circulating endothelial progenitor cells (EPCs) were identified by flow cytometry with the expression of cell surface antigens, CD133 (horizontal axis) and VEGF-R2 (KDR, vertical axis), in the right upper quadrat of each diagram. (A) Representative flow cytometry analysis of blood samples from one patient with a patent fistula and the other patient having a stenotic fistula. (B) There was a significantly higher number of circulating EPCs in patients with a stenotic arteriovenous fistula (stenotic vs. patent, p = 0.02). The EPC numbers were also significant from baseline to POD 30 in stenotic group (0.18 ± 0.03% vs. 0.39 ± 0.13% lymphomonocyte, p = 0.01). *,+: p < 0.05; BL (D0): preoperative baseline. Data showed as mean ± standard error of the mean. Dashed line indicates data from healthy volunteers (n = 7).*

of AV fistula (data not shown).

Correlation of EPC numbers, peak AV fistula blood volume flow and shear rate

There was a weak correlation between EPC numbers and blood flow rates after AV fistula surgery on POD 3, where r = 0.30, p = 0. 14 (Figure 4A). However, there was a significant inverse relationship between the circulating EPC number and AV fistula blood flow rate on POD 30, where r = -0.48, p = 0.04 (Figure 4B). The positive linear relationships between EPC numbers and shear rate were more significant on POD 30 (r = 0.50, p = 0.03; Figure 4D).

DISCUSSION

Our present study demonstrated that creation of AV fistula marginally increased EPC numbers in the systemic circulation of patients with ESRD. Increased circulating EPCs on POD 30 were associated with significantly higher rate of fistula stenosis or immaturity, which was positively correlated with the increased shear stress in the narrowing lumen of the fistula. Multiple stenosis or failure of venous limb remodeling was the major cause of an immature AV fistula during re-interventional observation. Occlusion at the anastomosis or arterial limb was not identified by the echo-duplex examination, suggesting that failure of AV fistula to mature or development of stenosis was not caused by incompetent surgical techniques.[17,18] The direct causal relationship between levels of circulating EPCs and stenosis of forearm AV fistula in ESRD patients was not established in the present observation cohort study. The development of an immature AV fistula is mainly attributed to the formation of neointimal hyperplasia in venous limb, in which progenitor cells have

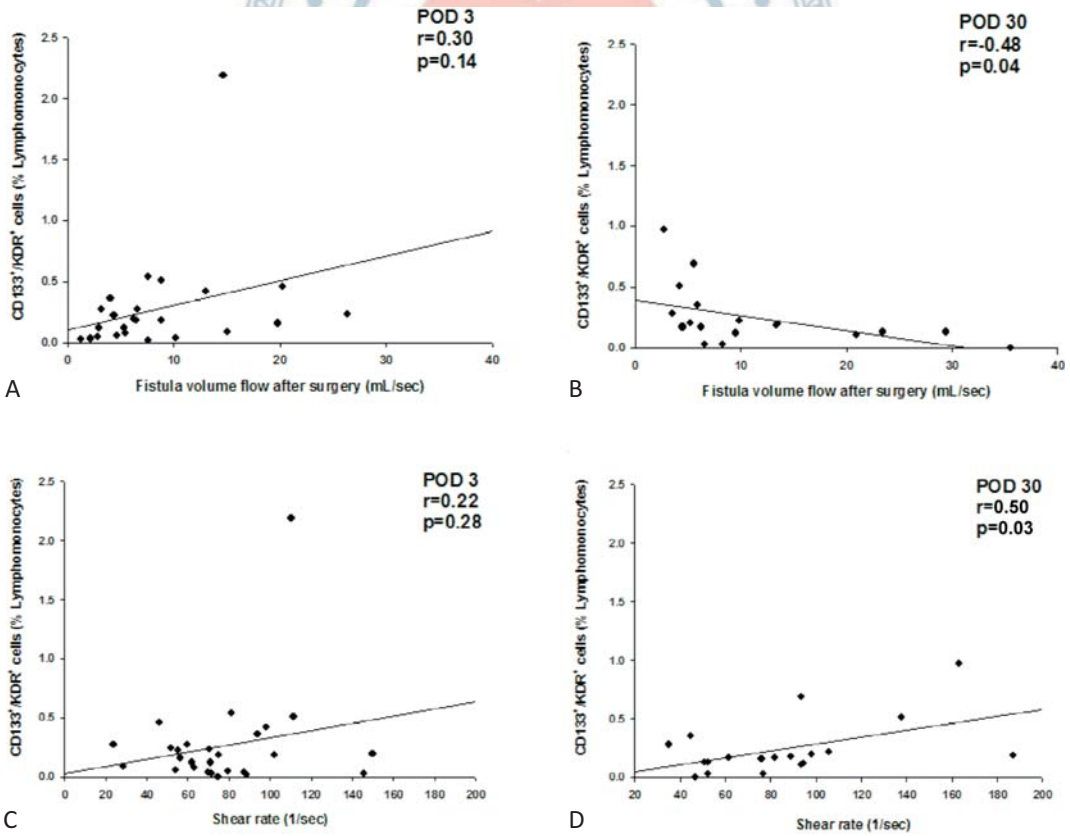


Figure 4. *Correlation of circulating EPC numbers, shear rate and peak volume flow rate of AV fistula measured on POD 3 and 30. (A) A weak but direct relationship between circulating EPC numbers and fistula flow on POD 3 is demonstrated (r = 0.30, p = 0.14). (B) There was a significantly reverse relationship between EPC numbers and fistula flow on POD 30 (r = -0.48, p = 0.04). (C) The linear relationship of EPC numbers and shear rate on POD 3, r = 0.22; p = 0.28. (D) The linear relationship of EPC numbers and shear rate on POD 30, r = 0.50 with p = 0.03. EPC, endothelial progenitor cell; POD, postoperation days.*

variable contribution in this phenomenon.[19,20] Therefore, the causal relationship of increased mobilization of circulating EPCs in the formation of a failure AV fistula could not be established in our current study design. We also speculated that predicting the development of a stenotic or immature fistula with circulating EPC numbers was above 0.12% for lymphomonocyte; the predictive cut-off value reached a sensitivity of 90% and a specificity of 43%. Due to the small size of the data set, the parameter did not reach a statistical significance.

Bone marrow-derived vascular progenitor cells (CD 34^+ cells) are mobilized in response to changes in hemodynamic blood flow with an intact endothelial layer in the aortic wall.[9] However, CFUs of circulating EPCs, considered as a functional assessment for activation of EPCs,[21] did not increase following creation of an AV fistula or enhanced shear stress on the regional vessel walls. This phenomenon indicates a limited effect of increased regional dynamic shear stress on the activation of EPCs in the systemic circulation among ESRD patients with a patent fistula. In addition, uremic toxins such as β_2-microglobulin and indole-3 acetic acid have been reported to reduce the function and levels of EPCs.[22] Since severity of renal diseases and other patient characteristics were similar between the patent and stenotic AV fistula groups, more significantly increased EPC mobilization in the systemic circulation of ESRD patients could serve as a valuable biological prognostic factor for primary failure of an AV fistula. Our study also supported the general concept that the biological function (colony formation capability) of EPCs is impaired in ESRD subjects, despite the quantitative increase in these endothelial progenitors.

The overall primary failure rate of AV fistula in the present study was 24.1%, which fell into the range from 20 to 50% in the literature.[23,24] The commonly reported risk factors for failure of an AV fistula are female gender, diabetes mellitus and creation of fistula in the wrist.[25] In the present study, we provide additional evidence suggesting that the numbers of circulating EPCs could be a valuable prognostic biomarker for the short-term patency of an AV fistula. A reference level of circulating EPCs on the POD 30 might predict primary failure of a fistula, but this is yet to be determined due to the limited patient number in this study.

Intravenous injection of endothelial progenitor cells had been reported in an animal study to reduce neointimal hyperplasia in an injured carotid artery.[26] However, this treatment approach was intermittent and the observation was cross-sectional rather than longitudinal. On the other hand, restenosis after coronary stenting was found during intra-coronary injection of autologous peripheral blood stem-cells in human subjects.[27] Transformation of stem cells is almost impossible to be controlled following in vivo application. There is also convincing data suggesting that bone marrow-derived cells and endothelial cells might transform into fibroblasts or myofibroblasts and indirectly contribute to neointimal formation in the venous limb of a fistula.[3,19]

However, there are a number of limitations in the interpretation of our present study. First, this was a prospective observational study design and recruited a relatively small sample size of patients. The small patient number might provide inadequate statistical power to detect the potential difference in the research objectives. Second, the direct causal relationship between the numbers of circulating EPCs and early failure of an AV fistula in ESRD patients was unable to be established in our current study. Third, the measuring time points of EPCs were relatively insufficient and might have impacted the ability to detect the subtle differences of cell numbers during the study period. Nevertheless, this pilot study presents important clinical insight into the mobilization of EPCs following creation of AV fistula, and provides fundamental evidence for conducting large scale studies in the relevant fields. Although previous studies demonstrated that administration of EPCs reduced neointimal hyperplasia in experimental models of vascular injury,[26] restenosis of coronary artery was reported in patients receiving intracoronary administration of autologous circulating stem cells during coronary stenting.[27] Therefore, the contribution of EPCs in vascular remodeling of an AV fistula remains undetermined. In fact, circulating EPCs may transdifferentiate to fibroblasts or myofibroblasts in certain pathological conditions.[28]

CONCLUSIONS

In summary, circulating EPC numbers increased marginally in ESRD patients who received surgery for creation of an AV fistula in the wrist POD 30. The mobilization of EPCs was statistically more significant in pa-

tients who developed a stenotic or immature fistula during the short-term follow-up. However, the numbers of EPCs did not well correlate with increased blood flow in the radiocephalic fistula early after operation, and became inversely related on POD 30. The mechanism and biological significance of increased circulating EPC numbers following creation of a radiocephalic fistula need to be determined in future studies.

ACKNOWLEDGEMENT

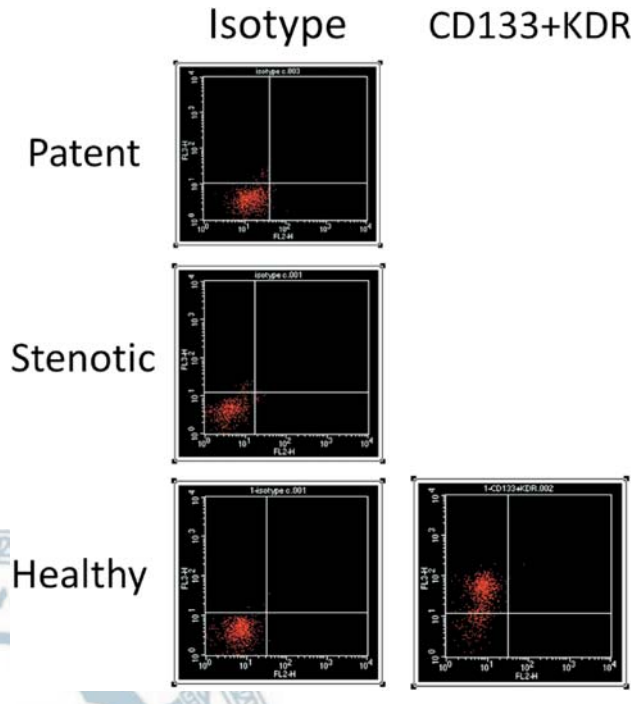
A grant was provided for this work by a landmark project to promote innovation and competitiveness of clinical trials by the clinical trial and research center in National Cheng Kung University Hospital (MOHW103-TDU-B-211-113002 to JNR), the National Science Council of Taiwan (98-2314-B-006-055-MY2 to CFL) and the Tainan Municipal Hospital (98-005 to JNR).

REFERENCES

1. Rajabi-Jagahrgh E, Krishnamoorthy MK, Wang Y, et al. Influence of temporal variation in wall shear stress on intima-media thickening in arteriovenous fistulae. *Semin Dial* 2013;26:511-9.
2. Ene-Iordache B, Mosconi L, Antiga L, et al. Radial artery remodeling in response to shear stress increase within arteriovenous fistula for hemodialysis access. *Endothelium* 2003;10: 95-102.
3. Arciniegas E, Frid MG, Douglas IS, Stenmark KR. Perspectives on endothelial-to-mesenchymal transition: potential contribution to vascular remodeling in chronic pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol* 2007;293:L1-8.
4. Lehoux S, Tedgui A. Cellular mechanics and gene expression in blood vessels. *Journal of Biomechanics* 2003;36:631-43.
5. Asahara T, Murohara T. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997;275:964.
6. Hristov M, Weber C. Endothelial progenitor cells: characterization, pathophysiology, and possible clinical relevance. *J Cell Mol Med* 2004;8:498-508.
7. Maruyama S, Taguchi A, Iwashima S, et al. Low circulating CD34+ cell count is associated with poor prognosis in chronic hemodialysis patients. *Kidney Int* 2008;74:1603-9.
8. Fadini GP, de Kreutzenberg S, Agostini C, et al. Low CD34+ cell count and metabolic syndrome synergistically increase the risk of adverse outcomes. *Atherosclerosis* 2009;207:213-9.
9. Sho E, Sho M, Nanjo H, et al. Hemodynamic regulation of CD34+ cell localization and differentiation in experimental aneurysms. *Arterioscler Thromb Vasc Biol* 2004;24:1916-21.
10. Mazzolai L, Bouzourene K, Hayoz D, et al. Characterization of human late outgrowth endothelial progenitor-derived cells under various flow conditions. *J Vasc Res* 2011;48:443-51.
11. Gill M, Dias S, Hattori K, et al. Vascular trauma induces rapid but transient mobilization of VEGFR2+AC133+ endothelial precursor cells. *Circ Res* 2001;88:167-74.
12. Besarab A, JackWork, Brouwer D, Konner K. Clinical practice guidelines for vascular access. *Am J Kidney Dis* 2006;48:S176-247.
13. Bourquelot P, Rawa M, Van Laere O, Franco G. Long-term results of femoral vein transposition for autogenous arteriovenous hemodialysis access. *Journal of Vascular Surgery* 2012;56:440-5.
14. Pyke K, Green DJ, Weisbrod C, et al. Nitric oxide is not obligatory for radial artery flow-mediated dilation following release of 5 or 10 min distal occlusion. *Am J Physiol Heart Circ Physiol* 2010; 298:H119-26.
15. Roan JN, Fang SY, Chang SW, et al. Rosuvastatin improves vascular function of arteriovenous fistula in a diabetic rat model. *J Vasc Surg* 2012;56:1381-9.
16. Lam CF, Roan JN, Lee CH, et al. Transplantation of endothelial progenitor cells improves pulmonary endothelial function and gas exchange in rabbits with endotoxin-induced acute lung injury. *Anesth Analg* 2011;112:620-7.
17. Voormolen EHJ, Jahrome AK, Bartels LW, et al. Nonmaturation of arm arteriovenous fistulas for hemodialysis access: a systematic review of risk factors and results of early treatment. *J Vasc Surg* 2009;49:1325-36.
18. Bharat A, Jaenicke M, Shenoy S. A novel technique of vascular anastomosis to prevent juxta-anastomotic stenosis following arteriovenous fistula creation. *J Vasc Surg* 2012;55:274-80.
19. Skartsis N, Manning E, Wei Y, et al. Origin of neointimal cells in arteriovenous fistulae: bone marrow, artery, or the vein itself? *Semin Dial* 2011;24:242-8.
20. Castier Y, Lehoux S, Hu Y, et al. Characterization of neointima lesions associated with arteriovenous fistulas in a mouse model. *Kidney Int* 2006;70:315-20.
21. Fadini GP, Losordo D, Dimmeler S. Critical reevaluation of endothelial progenitor cell phenotypes for therapeutic and diagnostic use. *Circ Res* 2012;110:624-37.
22. Brunet P, Gondouin B, Duval-Sabatier A, et al. Does uremia cause vascular dysfunction? *Kidney Blood Press Res* 2011;34:284-90.
23. Allon M, Robbin ML. Increasing arteriovenous fistulas in hemodialysis patients: problems and solutions. *Kidney Int* 2002;62: 1109-24.
24. Huijbregts HJ, Bots ML, Moll FL, Blankestijn PJ. Hospital specific aspects predominantly determine primary failure of hemodialysis arteriovenous fistulas. *J Vasc Surg* 2007;45:962-7.
25. Saucy F, Haesler E, Haller C, et al. Is intra-operative blood flow predictive for early failure of radiocephalic arteriovenous fistula? *Nephrol Dial Transplant* 2010;25:862-7.
26. Werner N, Junk S, Laufs U, et al. Intravenous transfusion of endothelial progenitor cells reduces neointima formation after vascular injury. *Circ Res* 2003;93:e17-24.
27. Kang HJ, Kim HS, Zhang SY, et al. Effects of intracoronary infusion

of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. *Lancet* 2004;363:751-6.
28. Bautch VL. Stem cells and the vasculature. *Nat Med* 2011;17: 1437-43.

SUPPLEMENT



Supplement Figure 1. *Representative flow cytometry analysis for isotype from dialysis patients and a healthy volunteer. Circulating endothelial progenitor cells (EPCs) in the blood sample from a healthy volunteer were identified by flow cytometry with the expression of cell surface antigens, CD133 (horizontal axis) and KDR (VEGF-R2, vertical axis), in right upper quadrant of the diagram.*