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Original Article

Endothelial Progenitor Cell Homing Decreases Postoperative Complications in Patients Undergoing Cardiac Surgery with Cardiopulmonary Bypass

Lei Du[1]*, Wen-Tong Meng[2]*, Yu-Jing Zhang[1], Jie Zhang[3], Jing Zhou[4], Jerry Yu[5], Li-Na Gong[1], Ke Lin[6], Lin Bo[7], Liang Zhao[1], and Jin Liu[1]

ABSTRACT

Background: Endothelial progenitor cells (EPCs) play an important role in tissue repair, however, their role in reducing complications of cardiac surgery is unclear. This study evaluated the effect of EPCs on cardiac surgery outcomes.

Methods: The prospective, cohort, single central study was conducted in 127 rheumatic heart patients who received valve replacement with cardiopulmonary bypass from June 2009 to June 2011. Circulating EPCs were evaluated perioperatively. Composite surgical outcome was examined during hospital stays.

Results: EPC counts decreased from 42 cells/ml before surgery to 12 cells/ml 4 hours after surgery, although granulocyte colony-stimulating factor significantly increased. The incidence of death, acute respiratory distress syndrome (ARDS), acute renal dysfunction, heart failure occurred less in high EPC homing group (0%, 47.6%, 0%, 0%) than in medium (2.38%, 50%, 2.38%, 2.38%) and low (2.33%, 69.8%, 6.98%, 6.98%) groups. Composite outcome events were also lower in high EPC homing group (P=0.000). Multivariate analysis showed the occurrence of composite outcome events and ARDS was still significantly different among the 3 groups (P=0.007 and P=0.042 respectively). Similarly, the higher pre-surgery EPC was associated with a lower incidence of composite outcome events (P=0.004).

Conclusions: EPC homing occurs during early stages after cardiac surgery, and may help to reduce complications.

From the [1]Department of Anesthesiology and Translational Neuroscience Center, [2]Laboratory of Stem Cell Biology, State Key Laboratory of Biotherapy, [3]Key Laboratory of Transplant Engineering and Immunology, [4]Department of Lab Medicine, West China Hospital, Sichuan University, Chengdu, China; [5]Respiratory, Critical Care and Sleep Disorders Medicine, Department of Medicine, University of Louisville, Louisville, USA; [6]Department of Cardiothoracic Surgery, [7]Regenerative Medicine Research Center, West China Hospital, Sichuan University, Chengdu, China.

*Contributed equally to the paper.

Correspondence to Dr. Jin Liu at scujinliu@foxmail.com.

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Cardiac surgery under cardiopulmonary bypass has significant complications. Within 30 days after the surgery, 2.3- 2.8% of patients died (1, 2); 7.5% suffered from respiratory failure or infection (1); 32.1% had acute kidney injury, in which 7.4% and 2.6% with AKIN stage 2 and 3 respectively (1), and 0.2% needed renal-replacement therapy (2). This could be worse for elderly patients (3.1%) (3). Since there is no effective treatment for such complications, prevention becomes even more important to improve the outcome of cardiac surgery. Endothelial progenitor cells (EPCs), first identified as the precursor of endothelial cells in 1997 (4), are important in tissue repair after trauma (5). Early evidence showed that EPCs home to the site of vascular injury,

incorporate into the foci of neovascularization and differentiate into matured endothelial cells (6). Recently, however, evidence suggests EPC do not contribute to vascular endothelium (7). Although the role of EPCs in the repair of injured tissue is still unclear, EPC homing is a key step for repair. Accumulating evidence shows that EPCs play an important role in respiratory diseases [asthma (8), pulmonary hypertension (9) and acute lung injury (10)] and in acute kidney injury (11, 12). Delivering EPCs via the coronary artery helps improve cardiac function after acute myocardial infarction (13). Hence, EPC therapy may help reduce complications due to cardiac surgery. Therefore, we tested the hypothesis that EPC homing would improve outcomes from cardiac surgery.

METHODS

Trial Design

From June 1, 2009 to June 30, 2011, patients suffering from rheumatic heart disease and scheduled for valve replacement under cardiopulmonary bypass were eligible for this study. Only the cases of the first day were included, because EPCs must be processed within 2 hours after harvest. Three hundred and twenty-one patients were screened for the Endothelial Progenitor Cell and Prognosis after Cardiac Surgery study (EPCPCS). One hundred and eighty-three patients were excluded because of pulmonary insufficiency (N=47), severe pulmonary artery hypertension (N=21), chronic obstructive pulmonary disease (N=14), asthma (N=4), New York Heart Association (NYHA) classification of IV (N=38), renal insufficiency (N=12), hepatic insufficiency (N=6), repeat surgery for valve replacement (N=5), or refusal to sign the consent (N=36). Therefore, 138 patients were included in the study. All the patients signed informed consents before their participation. This Cohort study was approved by the Ethics Committee of Sichuan University, and registered with the Chinese Clinical Trial Registry (No. ChiCTR-OCS-09000398; www.chictr.org/).

Anesthesia, Cardiopulmonary Bypass and Surgery

All patients received anesthesia and cardiopulmonary bypass according to the clinical practice in West China Hospital (14). Simply, anesthesia was achieved with fentanyl, midazolam and non-depolarizing muscle relaxants, and maintained with propofol and/or inhalation agents. CPB was conducted with a roller pump (Sarns 8000, 3M) and membrane oxygenator (Medtronic Inc, Minneapolis, MN) primed with 500 ml multiple electrolytes injection (Baxter), 1000 ml hydroxyethyl starch (Fresenius Kabi) and 3750 U heparin (porcine; Tianjin Biochem Pharmaceutical Co, Ltd). During surgery, hemodynamic and fluid management were performed according to routine clinical practice in our hospital, based on central venous pressure (CVP), blood pressure and clinical evaluation of the attending anesthesiologist. After termination of CPB, residual pump blood was collected in a bag containing sodium citrate, neutralized by protamine, and returned to the patient. In Cardiac Intensive Care Unit (ICU), patients received analgesia based on the clinical evaluation of the attending doctor in ICU.

Preparation of Blood Samples

For flow cytometry and plasma factors determination, blood samples were drawn before 4 and 20 hours after surgery from a central venous catheter, anti-coagulated with heparin, and centrifuged for 15 min at 1000 g and 4 ℃. The plasma was stored at -80 ℃ until analysis. Both G-CSF and Vascular Endothelial Growth Factor (VEGF) levels were measured by Enzyme Linked Immunoadsorbent Assay kits (R&D Systemics, Inc, Minneapolis, MN and Bender MedSystems GmbH, Vienna Austria), according to the manufacturers' instruction. For the EPC counts, central venous blood was collected into a Vacutainer tube (Becton Dickinson, Basel, Switzerland) with sodium citrate anticoagulant before and after surgery.

Flow Cytometry

After the plasma was removed, red blood cells were lysed with ammonium chloride solution. For fluorescence-activated cell-sorting analysis, cells were re-suspended in 100.0 μL of phosphate-buffered saline (PBS). Nonspecific antibody binding was blocked using 20.0 μL FcR-blocking reagent (130-059-901, Miltenyi Bio-

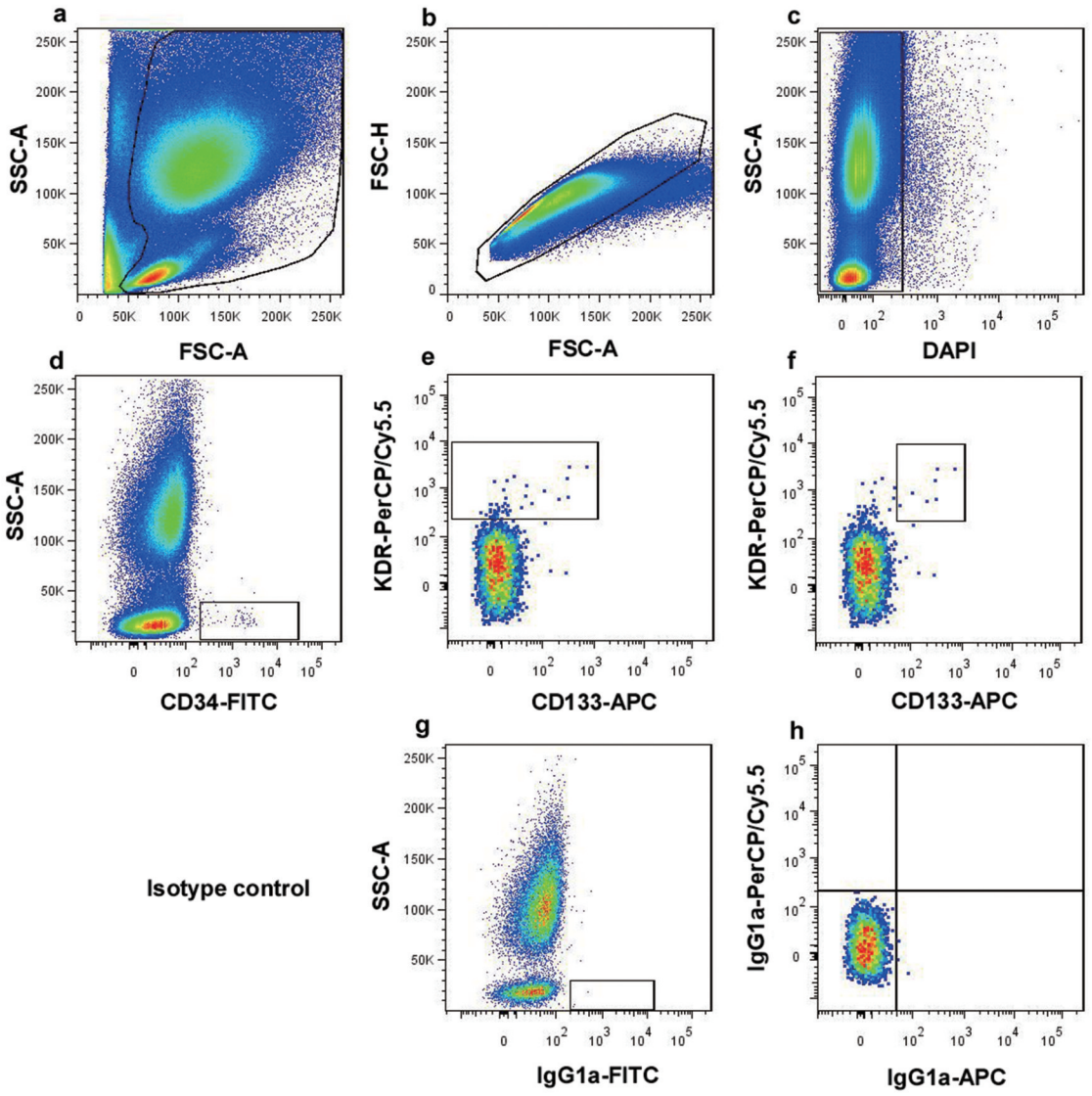

Figure 1. Strategy for Flow Cytometric Detection of Human Endothelial Progenitor Cells (EPC).
a. Platelets and cell debris were excluded in forward scatter (FSC)/side scatter (SSC) dot plots; b. Aggregated cells were excluded based on FSC height versus FSC area dot plots; c. Dead cells were excluded by DAPI; d and e. Show the CD34+KDR+EPC; f. Show the CD34+KDR+ CD133+EPC; g and h. Show the isotype control.

tec) for 20 minutes at room temperature before staining with conjugated antibodies. Immunofluorescent cell staining was performed with 6.0 μL of CD34-FITC (Becton Dickinson), 8.0 μL of CD309-PerCP/Cy5.5 (KDR, BioLegend) and 6.0 μL of CD133/1(AC133)-APC (Miltenyi Biotec). Each antibody was titrated by serial dilutions. IgG1- FITC/PE/APC PerCP/Cy5.5 antibody (Becton Dickinson) served as a negative control. These surface antibodies were incubated for 30 min at room temperature in the dark, followed by staining by DAPI (Sigma Aldrich) for dead cells (15-17). Data acquisition was performed on a FACS Aria cytometer equipped with FACS Diva 5.0 software (BD) and analyzed by Flowjo software (Tree Star). The instrument setup was standardized to reduce batch-to-batch differences by daily monitoring with Rainbow Beads (Becton Dickinson). A minimum of 5,000,000 events was acquired. The boundary between positive and negative cells was placed using fluorescence-minus-one controls and an internal control (16, 17). Figure 1 illustrates the sequential gating strategy to mark EPCs for in-

tensive analysis (17). For each patient, a corresponding negative control with IgG1-FITC/PerCP/Cy5.5/APC was obtained. The numbers of CD34 + KDR + and CD34 + KDR + CD133 + EPCs were derived from the absolute number of white blood cells (WBCs) provided by a hematological analyzer (XE- 5000, Sysmex, Kobe, Japan) and the percentages of CD34 + KDR + and CD133 + CD34 + KDR + EPCs were determined by flow cytometry, using the following formula: percentage of EPCs × WBC count/100 (18).

Variables Measured

The primary outcomes were ARDS, renal dysfunction and heart failure. The arterial partial pressure of oxygen (PaO_2) and fraction of inspired oxygen (FiO_2) were measured before 4 and 20 hours after surgery. ARDS was defined as respiratory failure without cardiac dysfunction or fluid overload at 20 hours after surgery, which was objectively assessed by echocardiography, according to the Berlin definition of ARDS (19). Mild ARDS was defined as PaO_2/FiO_2 between 200-300 mm Hg with PEEP or CPAP$\geqslant$5 cm H_2O; moderate as 100 mm Hg $\leqslant$ $PaO_2/FiO_2 \leqslant$ 200 mm Hg with PEEP $\geqslant$5 cm H_2O, and severe as $PaO_2/FiO_2 \leqslant$ 100 mm Hg with PEEP $\geqslant$ 5 cm H_2O. Renal dysfunction was defined as a postoperative serum creatinine level over 177 μM with an increase of more than 62 μM; renal failure was defined as requiring dialysis or in-hospital death associated with acute renal dysfunction (20). Heart failure was defined as sudden deaths, congestive heart failure, and acute myocardial infarction.

Statistical Analysis

Data were analyzed by SAS 9.1 statistical software (SAS Institute Inc., Cary, NC). All quantitative data were examined for their distribution. Correlation analysis was used to examine the relationship between variables. Pearson and Spearman correlation analyses were used for normal and non-normal distribution data respectively.

Normally distributed variables including age, body mass index, and blood cells were expressed as mean ± standard deviation, and one-way ANOVA was used to compare the difference among groups. Otherwise, non-normal distribution data including CPB, cross-clamp, and surgery times were expressed as the median and 95% confidence interval, and differences between groups were assessed with the Kruskal-Wallis. Segmental data including smoking, hypertension, diabetes, NYHA, type of surgery, death, ARDS, acute renal dysfunction, heart failure and composite outcome event were expressed as a percentage, and differences between groups were compared using chi-square or Fisher exact tests. To analyze the impact of EPC homing on outcomes, we used pre-specified thresholds corresponding to patients' decreased EPC (low, medium, and high) at 4 hours after surgery. Multivariable Logistic Regression was used for correction of the outcome between groups by age, gender, body-mass index, smoking, hypertension, diabetes, and the NYHA Functional Classification. A nonparametric signed-rank test was used to compare differences of EPC counts and G-CSF, VEGF levels at different time points within the same group. $P<0.05$ was considered statistically significant.

RESULTS

A total of 138 patients were enrolled. Of these, 11 patients (5 incomplete data collection and 6 receiving PRBCs over 10 units) were excluded. Therefore, 127 patients were analyzed.

EPC Homing

EPCs (referring to CD34 + KDR + cells unless otherwisely defined) decreased from 42 (0-1236) cells/ml before surgery to 12 (0-190) cells/ml ($P<0.001$, Nonparametric Signed-rank Test) at 4 hours post-surgery, and were kept stable at 20 hours [14 (0-344) cells/ml] after surgery, suggesting that EPC homing may occur within 4 hours after surgery. Because EPCs may be lost in the heart lung machine, a decrease in EPC number does not necessarily equate to the number of homing EPCs, which includes both lost and homing EPCs. Therefore, the term "EPC deficit" was introduced, which is calculated as the difference between EPC counts pre- and 4 hours post-surgery. In a rat model with extracorporeal circulation, EPC deficit was positively correlated with the percentage of EPCs in both lung and kidney tissues ($P=0.019$ and $P=0.032$, respectively, $N=15$, Figure 2).

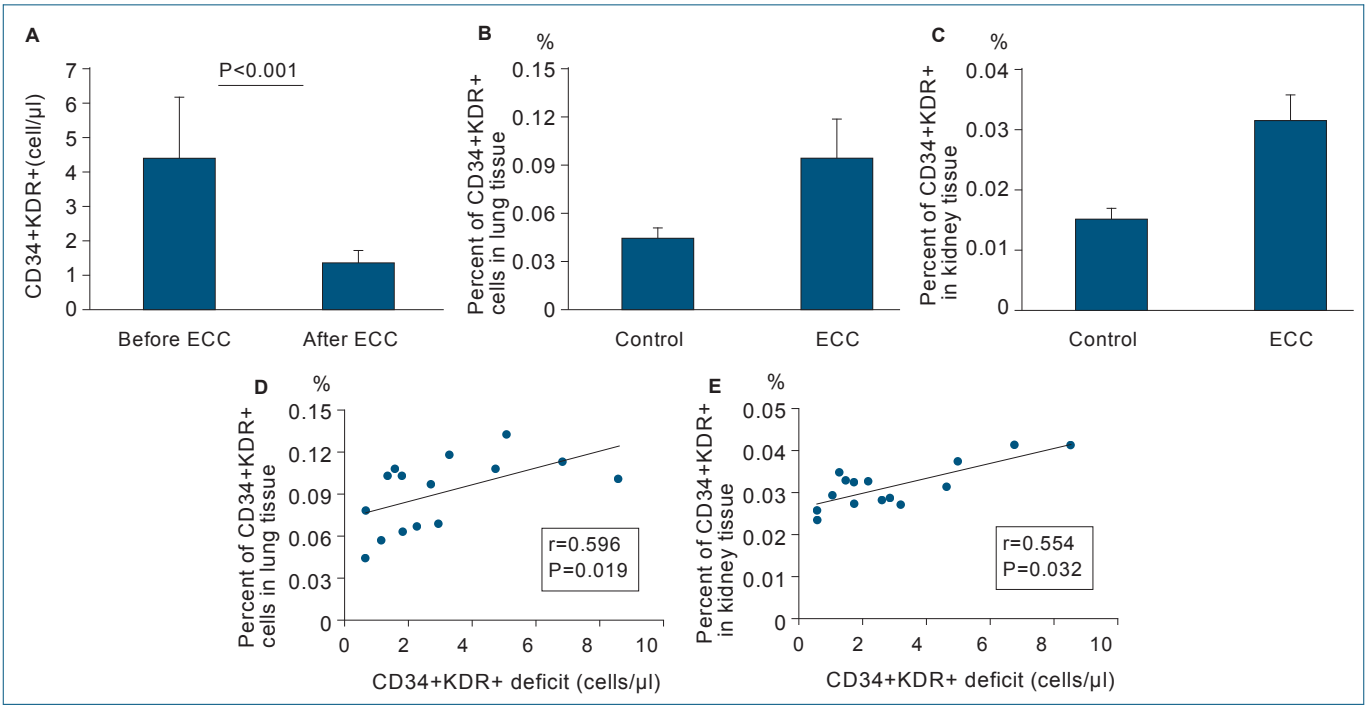

Figure 2. CD34+KDR+ Cell Deficit and Its Homing in Lung and Kidney Tissues.
A rat model of ECC was set up as we described before (Am J Respir Cell Mol Biol 2012; 47: 597-603.). Simply, rat was mechanically ventilated after anesthesia. After heparinization (375 unit/kg), the right common carotid artery and the left femoral vein were cannulated and connected to a roller pump (Stock II, Munich, Germany) primed with succinylated gelatine injection (B Braun, Germany) to establish extracorporeal circulation (ECC). ECC was going on for 2 hours. CD34+KDR+ cells were identified before and 4 hours after ECC (A, N=15), and CD34+KDR+ deficit was calculated as the difference between them. The lung and kidney tissues were also harvested 4 hours after ECC, and the single cell suspension was made to identify CD34+KDR+ cells in tissues (B, C). At least 1,000,000 events were investigated. Then the correlations between the percentage of CD34+KDR+ cells in tissues and its deficit in blood were investigated (D, E). Rat without ECC was served as control (N=5).

EPC Mobilization

VEGF level decreased from 45 (0-214) pg/ml before surgery to 4 (0-307) pg/ml at 4 hours (P< 0.001) and returned to 42 (0-667) pg/ml at 20 hours after surgery, suggesting that VEGF cannot rapidly mobilize EPCs after surgery. In the meantime, G-CSF levels increased from 5 (0-643) pg/ml before surgery to 34 (0-1382) pg/ml after 4 hours and continue to increase to 45 (0-694) pg/ml at 20 hours after surgery (P<0.001). To further explore the role of G-CSF, we divided patients into negative-deficit (EPCs increased at 4 hours, N=12) and a positive-deficit (EPCs decreased at 4 hours, N=115) groups. We found no significant differences in G-CSF levels between the 2 groups before (3.2 pg/ml vs 5.2 pg/ml, P=0.414) and 4 hours after (19.5 pg/mL vs. 36.7 pg/ml, P=0.313) surgery. Interestingly, VEGF was higher in the negative-deficit group than the positive-deficit group at 4 hours after surgery (19 pg/ml vs 5 pg/ml, P=0.032).

EPCs and Clinical Events, Lung Function

There were no differences in the demography and operative data among the 3 groups, based on the EPC deficit described above (Table 1). Two (one each in the low and medium groups) of 127 patients (1.6%) died from sequential multi-organ failure. The incidence of ARDS was 55.9% (37.8% mild, 18.1% moderate, and 0% severe). Incidence was higher in the low group than in medium and high groups, but not significantly (P=0.123). Similarly, acute renal dysfunction, and heart failure occurred more often in the low group (Figure 3). Hence, composite outcome events were significantly lower in the high group (P=0.000). After correction for age, gender, body-mass index, smoking, hypertension, diabetes and NYHA classification, multivariate analysis showed occurrences of composite out-

Table 1. Patient Characteristics.

		Grade of EPC deficit*			
Characteristics	**All Subjects (N=127)**	**Low (N=43)**	**Medium (N=42)**	**High (N=42)**	**P value**
Age-year†	47.0±9.3	49.5±9.0	45.7±9.1	45.7±9.6	0.089
Male sex- no. (%)‡	34 (26.8)	11 (25.6)	11 (26.2)	12 (28.6)	0.948
Body-mass index†	22.3±3.0	22.7±3.2	22.3±3.2	22.1±2.6	0.963
Smoker-no. (%)‡	24 (18.8)	9 (20.9)	6 (14.3)	9 (21.4)	0.717
Hypertension-no. (%)‡	8 (6.30)	4 (9.30)	3 (7.14)	1 (2.38)	0.531
Diabetes-no. (%)‡	2 (1.57)	1 (2.33)	0 (0)	1 (2.38)	1.000
NY HA ‡||					0.278
II-no. (%)	16 (12.6)	8 (18.6)	3 (7.1)	5 (11.9)	
III-no. (%)	111 (87.4)	35 (81.4)	39 (92.9)	37 (88.1)	
RBC ($\times10^{12}$/L) †	4.56±0.39	4.47±0.43	4.55±0.52	4.63±0.58	0.548
WBC ($\times10^{9}$/L) †	5.75±1.18	5.54±1.55	5.99±1.82	5.71±1.39	0.779
Neutrophil ($\times10^{9}$/L) †	3.48±1.00	3.35±1.33	3.67±1.55	3.42±1.23	0.769
Lymphocyte ($\times10^{9}$/L) †	1.68±0.40	1.64±0.41	1.68±0.60	1.71±0.53	0.732
Surgery information					
AVR/MVR/DVR ‡¶	58/15/54	19/6/18	18/4/20	21/5/16	0.898
Tricuspid Valvuloplasty ‡	56 (44.1)	22 (51.2)	16 (38.1)	18 (42.9)	0.470
Maze procedure ‡	27 (21.3)	11 (25.6)	10 (23.8)	6 (14.3)	0.376
CPB time (minute) §	120 (118-128)	120 (117-136)	130 (114-144)	117 (112-128)	0.954
Cross-Clamp Time (minute) §	81 (79-87)	82 (78-93)	84 (77-91)	80 (73-88)	0.812
Surgery time (hours) §	3.83 (3.79-4.02)	3.92 (3.75-4.17)	3.84 (3.71-4.11)	3.79 (3.63-4.04)	0.998

* Low EPC Group represents patients with EPC deficit of 9 or less, medium group EPCs deficit between 10 and 59, and high group, between 60 and 1103.
† Mean±SD; one-away ANOVA was used to compare the difference among three groups.
‡ Percentage; chi-square test or Fisher exact test were used for comparisons.
§ Median (95% confidence interval); Kruskal-Wallis Test was applied for comparison among three groups.
|| New York Heart Association (NYHA) Functional Classification.
¶ Aortic valve replacement, mitral valve replacement, and double valve replacement.

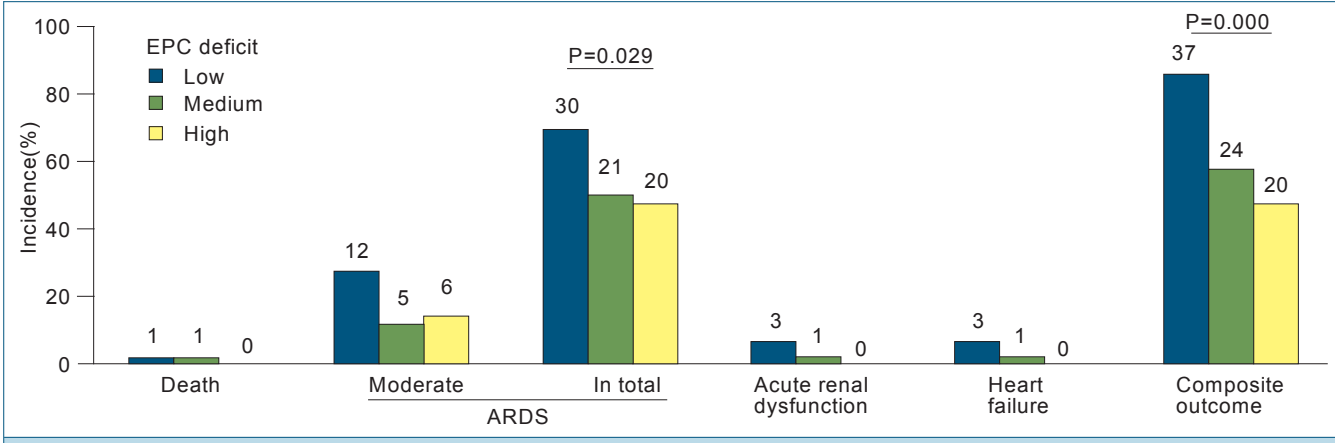

Figure 3. Primary Outcome Events among 127 Study Patients. Patients were Divided into 3 Groups Based on the Deficit of CD34+KDR+ EPC at 4 Hours after Surgery.
ARDS (Acute Respiratory Distress Syndrome) is defined based on JAMA 2012; 307: 2526-33. The higher EPC deficit, the lower incidences of renal dysfunction, ARDS and composite outcome events. The numbers above the bars are rounded incidence values, and P values shown are for the comparison among the 3 groups. The composite outcome events included all the other outcome events (death, renal dysfunction, heart failure, and ARDS).

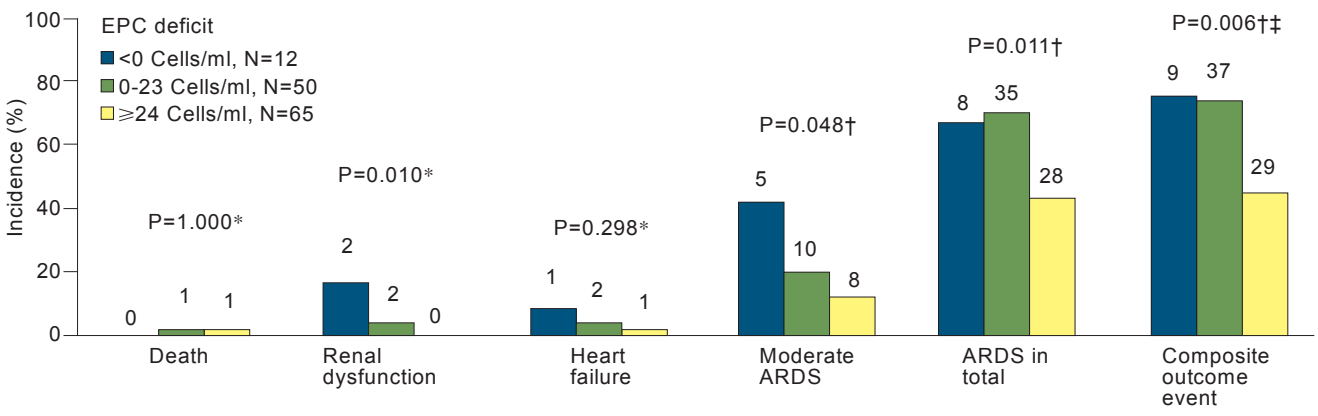

Figure 4. Primary Outcome Events among 127 Study Patients.
Patients were divided into 3 groups based on the deficit of CD34KDR EPC at 4 hours after surgery. ARDS (Acute Respiratory Distress Syndrome) is defined based on JAMA 2012; 307: 2526-33 and N Engl J Med 2006; 354: 353-65 respectively. The higher EPC deficit, the lower incidences of renal dysfunction, ARDS and composite outcome events. The numbers above the bars are rounded incidence values, and P values shown are for the comparison among the 3 groups. The composite outcome events included all the other outcome events (death, renal dysfunction, heart failure, and ARDS). * Fisher exact tests; † chi-square test; ‡ patient suffered with two organs dysfunction or over was calculated only once.

come events and ARDS were still significantly different among the 3 groups (P=0.007 and P=0.042, respectively).

In addition, we compared clinical events in negative- deficit (N=12) and positive- deficit (N=115) groups. Receiver-Operating Characteristic (ROC) analysis was performed on the positive-deficit group according to EPC deficits and composite outcome events. Based on the ROC curve, the Youden index was calculated to determine the optimal threshold value (23.5). Then, the positive-deficit group was divided further into low (below 23 cells/ml, N=50) and high (over 24 cells/ml, N=65) sub-groups. Compared with the high-positive group, both negative- and low- positive groups had an increased risk for acute renal dysfunction and heart failure (Figure 4), and also had 239% and 63% increased risk for moderate ARDS, and 68% and 66% increased risk for composite outcome events, respectively.

If EPCs cannot be mobilized rapidly, homing EPCs must be from pre-existing cells. Therefore, we compared clinic events among patients with low, medium, and high counts of pre- existing EPCs. One death occurred in the low and medium groups, respectively. The incidences of ARDS, acute renal dysfunction, heart failure and composite outcome events were lower in the high pre- existing EPC group than the other 2 groups (Table 2).

PaO_2/FiO_2 decreased significantly from a baseline of 431 ± 56 to 323 ± 66 mmHg at 4 hours, and continued to decline to 290 ± 70 mmHg at 20 hours after surgery. The EPC deficit positively correlated with PaO_2/FiO_2 at 20 hours (r=0.292, P=0.001). Furthermore, pre- existing EPC counts also positively correlated with PaO_2/FiO_2 at 20 hours (r=0.142, P=0.111). To minimize the impact of baseline PaO_2/FiO_2, we examined the correlation between pre- existing EPC counts and changes in PaO_2/FiO_2. This strengthened the relationship significantly (r=0.232, P=0.009). However, EPC counts at both 4 and 20 hours were poorly correlated with PaO_2/FiO_2 (r=- 0.014, P=0.876, and r=0.078, P=0.384, respectively).

Similarly, CD133 + CD34 + KDR + EPCs decreased from a baseline of 11 (0-170) to 0 (0-17) cells/ml 4 hours after surgery. A high count of pre- existing CD133 + CD34 + KDR + EPCs and the following deficit were also correlated with a low incidence of composite outcome events. These results further strengthen our conclusion.

DISCUSSION

In this cohort study, we demonstrated for the first time that EPC homing may occur early,

Table 2. Patient Outcomes According to Pre-existing EPC Before Surgery.

		Grade of pre-existing EPC count before surgery*			
Outcomes	**All subjects (N=127)**	**Low (N=43)**	**Medium (N=42)**	**High (N=42)**	**P value**
Die -no. (%) ‡	2 (1.57)	1 (2.33)	1 (2.38)	0 (0.00)	1.000
ARDS in total -no. (%) †	71 (55.9)	28 (65.1)	22 (52.4)	21 (50.0)	0.150
Mild -no. (%)	48 (37.8)	19 (44.2)	14 (33.3)	15 (35.7)	
Moderate -no. (%)	23 (18.1)	9 (20.9)	8 (19.1)	6 (14.3)	
Acute renal dysfunction -no. (%) ‡	4 (3.15)	2 (4.65)	2 (4.76)	0 (0.00)	0.187
Heart failure -no. (%) ‡	4 (3.15)	3 (6.98)	1 (2.38)	0 (0.00)	0.323
Composite outcome event -no. (%) †‖	81 (63.8)	34 (79.1)	26 (61.9)	21 (50.0)	0.004

* Low, Medium and high preexisting-EPC groups were lower than 23 cells/ml, 24-88 cells/ml, and over 89 cells/ml respectively.
† Percentage, differences were compared with chi-square test or ‡, Fisher exact test.
‖ Including death, pulmonary dysfunction, acute renal dysfunction and heart failure.

within 4 hours after cardiac surgery. A high number of homing EPCs may decrease risks of ARDS, acute kidney injury, heart failure and composite outcome. Since EPC homing depends on pre-existing EPCs, an increase in EPCs may improve cell homing, and thus contribute to a better surgical outcome.

EPC Homing Improves Surgical Outcome

Organ injury following cardiac surgery may result from systemic inflammation, which occurs in the early phases. For example, inflammatory mediators, such as IL-6 and IL-8, peaked at 4-24 hours after surgery (21). Similarly, leukocyte activation, characterized by release of neutrophil elastase, peaked at 4-6 hours (22). In the present study, PaO_2/FiO_2 decreased by 25% at 4 hours and circulating EPCs also homed to the injured organs during an early stage. It is very difficult to quantify EPC homing during CPB, because only circulating EPCs can be detected in a clinical study. In animal studies, we found that the decreased EPC (EPC deficit) during early stages is positively correlated with the number of EPCs in lung and kidney tissues, suggesting an EPC deficit can be used to assess EPC homing. Furthermore, we found that the number of homing EPCs also correlated with lung function, and was directly related to attenuation of ARDS, and acute kidney injury, and thus a positive composite outcome. Our results suggest that effective EPC homing may reduce CPB-associated organ injury.

EPC Homing Depends On Pre-Existing EPCs

Our data do not support that EPCs can be rapidly mobilized after surgery. However, there are reports indicating otherwise (23). We do not know the reasons for the discrepancy, however, differences in studied races and methods to identify EPCs were found between the reports. EPCs are quantified either by cell culture or by flow cytometry in most studies. Changes in circulating concentrations of EPCs are correlated to a variety of human pathologies seen in cell culture (24). However, this technique is not practical for diagnosis (17), because it relies on density gradient separation of mononuclear cells, followed by culture in fibronectin. EPCs are then enumerated manually based on staining the Di-LDL and Ulex europaeus I lectin. The inter-laboratory reproducibility of this method is poor due to multiple steps and involved subjective evaluation of staining. Therefore, cell culture is not recommended (25). However, EPCs can only be identified via multiple markers and since EPCs are extremely scarce in the peripheral circulation (between 0.01% and 0.0001% of the nucleated cells), flow cytometry becomes the preferred method (25-27). Using flow cytometry, we found EPCs cannot be rapidly mobilized after surgery, because there were no corresponding changes in G-CSF and VEGF levels. If EPCs cannot be mobilized rapidly within a few hours after surgery, the homing EPCs will be from pre-existing EPCs. In the present study, the finding that patients with high pre-existing EPCs had

better outcomes supports this hypothesis. Our results are consistent with recent reports that acute injuries induced by S. typhus vaccination injection (28) or acute hypoxia (29) do not cause immediate mobilization of vascular progenitors.

Homing EPCs

Interestingly, 9.4% of patients showed an increase in EPCs (negative- deficit) after surgery. These patients were older and had high incidences of moderate ARDS and composite outcome events. Since aging may modulate migration and homing capacity via structural changes in heparan sulfate, which is essential in the signaling pathways of VEGF for EPC homing (30), the negative- deficit may suggest a decline in EPC homing capacity and may limit the availability of EPCs at the site of injury. Furthermore, high plasma VEGF levels were found in these patients after surgery. High VEGF may facilitate EPC mobilization, but decrease the EPC gradient between blood and tissue for homing. These facts may explain, in part, the negative deficit of EPCs in these patients.

Limitations

Although a change in the number of EPCs may alter pathophysiological processes of disease, it may simply reflect an association, rather than a causal relationship. Therefore, further experiments are warranted to confirm the role of EPCs in reducing surgical complications.

Furthermore, our study was limited by the grade of EPC deficit. Because little is known with regarding to the threshold of the EPC-deficit, trisections of EPC were divided to observe the effect of EPC on outcome, which may be arbitrary.

At last, patients with pulmonary disease, or renal insufficiency, or hepatic insufficiency before surgery were excluded. Does EPC deficit improve the outcome of these patients? This need further study.

Although these limitations, we can concluded that EPC homing significantly increases within 4 hours after cardiac surgery, which closely correlates with a reduction in surgical complications.

Declaration of Interests

All authors have no financial support and potential conflicts of interest for this work.

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