CD34\(^+\)CD140b\(^+\) cells and circulating CXCL12 correlate with the angiographically assessed severity of cardiac allograft vasculopathy

Andreas Schober\(^1,2\)*†, Mihail Hristov\(^2\)*†, Sieglinde Kofler\(^3\)*†, Robert Forbrig\(^1\), Barbara Löhre\(^1\), Nicole Heussen\(^4\), Zhou Zhe\(^2\), Shamima Akhtar\(^2\), Uwe Schumann\(^2\), Florian Krötz\(^1\), Marcus Leibig\(^1\), Andreas König\(^1\), Ingo Kaczmarek\(^5\), Bruno Reichart\(^5\), Volker Klauss\(^1\), Christian Weber\(^2,6\), and Hae-Young Sohn\(^1\)

\(^1\)Medizinische Poliklinik I, Kardiologie, University of Munich, Munich, Germany; \(^2\)Institute for Molecular Cardiovascular Research (IMCAR), RWTH Aachen University, Aachen, Germany; \(^3\)Medizinische Klinik I, Klinikum Großhadern University of Munich, Munich, Germany; \(^4\)Department of Medical Statistics, Medical Faculty, RWTH Aachen University, Aachen, Germany; \(^5\)Department of Cardiac Surgery, Klinikum Großhadern, University of Munich, Munich, Germany; and \(^6\)Institute for Preventive Cardiology, University of Munich, Munich, Germany

Received 2 June 2010; revised 6 September 2010; accepted 16 September 2010; online publish-ahead-of-print 28 October 2010

Aims
We sought to determine whether circulating vascular progenitor cells, such as endothelial progenitor cells (EPCs) or smooth muscle progenitor cells (SPCs), were associated with the severity of cardiac allograft vasculopathy (CAV).

Methods and results
CD34\(^+\)CD140b\(^+\) SPCs and CD34\(^+\)KDR\(^+\) EPCs were measured in the peripheral circulation of 187 adult heart transplant recipients by flow cytometry. Cardiac allograft vasculopathy was quantified by angiography using a CAV-specific scoring system. Cardiac allograft vasculopathy was present in 84 patients (44.7%) and was classified as mild in 59 and severe in 25 cases. Circulating SPCs were more frequently detectable in CAV patients than in patients without CAV. The number of CD34\(^+\)CD140b\(^+\) cells showed a stepwise increase in patients with moderate and severe CAV. Smooth muscle progenitor cell counts were higher in patients with coronary stent implant compared with unstented patients with CAV. In contrast, peripheral CD34\(^+\)KDR\(^+\) EPC counts were not changed in CAV patients. Plasma CXCL12 levels correlated with the degree of CAV and SPC counts. None of the different immunosuppressive drug regimes was related to the SPC count or the CXCL12 levels. A multivariate regression analysis revealed that the SPC count was independently associated with the presence of CAV.

Conclusion
Circulating SPCs, but not EPCs, and plasma CXCL12 concentrations are elevated in CAV patients, indicating that they play prominent roles in transplant arteriosclerosis.

Keywords
Transplantation • Cardiac allograft vasculopathy • Progenitor cells • Chemokines

Introduction
More than 5000 cardiac transplantations are performed each year for end-stage heart failure with a median survival of 13 years.\(^1\) The main cause of death in patients 5 years after transplantation is graft failure due to cardiac allograft vasculopathy (CAV).\(^1\) As a particular and accelerated type of coronary atherosclerosis, CAV develops early after transplantation in most patients and progresses steadily thereafter. A characteristic feature of CAV is the coexistence of focal, eccentric atherosclerotic plaques that affect epicardial coronary arteries and diffuse, concentric intimal thickening in intramyocardial arteries.\(^2,3\) An alloreactive immune response guided by T-cells induces the intimal hyperplasia by the accumulation of smooth muscle cells (SMCs).\(^4\)

Circulating vascular progenitor cells have been implicated in different types of atherosclerotic vascular diseases.\(^5\) Whereas
reduced numbers of CD34+KDR+ endothelial progenitor cells (EPCs) appear to predict cardiovascular events, the role of EPCs in the progression of atherosclerosis is controversial due to stage-specific contributions.6–9 CD14/CD105-bearing mononuclear cells, a putative subset of smooth muscle progenitor cells (SPCs), can acquire α-smooth muscle actin (α-SMA) and are increased in patients with coronary artery disease.10 In acute coronary syndrome, however, SPCs are diminished, which has been implicated in plaque stability.11 In animal studies, vascular incorporation of recipient-derived vascular progenitor cells clearly constitutes a significant number of endothelial cells and SMCs in transplanted vessels and contributes to allograft vasculopathy.12,13 Similar results have been described in humans.14–17 Studies that correlate circulating EPC levels with the presence of CAV, however, show conflicting results.15,18

Chemokines play a pivotal role in the vascular remodelling of transplanted vessels by directing the recruitment of vascular progenitor cells.6,19 Notably, the CXC chemokine stromal cell-derived factor (SDF)-1α/CXCL12 mediates the mobilization and local recruitment of SPCs in a mouse model of CAV, thereby contributing to neointima formation.20 In addition, PDGF receptor-β (CD140b)-bearing SPCs are specifically mobilized into the circulation by CXCL12 after vascular injury in mice.21

We, therefore, hypothesized that increased circulating CD34+CD140b+ SPCs and plasma CXCL12 levels might be associated with progressive CAV, whereas EPCs might be elevated in CAV-free patients.

**Methods**

**Patient population**

Between October 2007 and September 2008, a total of 207 adult heart transplant recipients were screened during routine outpatient visits at the Medical Centre of the University of Munich. The exclusion criteria were cardiac transplantation within the last 3 months, cancer, acute inflammatory disease, acute rejection, myocardial infarction within the last 2 weeks, and major peripheral artery disease. Finally, 187 patients were enrolled in this study. The Ethics Committee of the University of Munich approved the study protocol, and written informed consent was obtained from all participating subjects.

**Cardiac allograft vasculopathy grading**

Coronary angiograms were performed and independently evaluated by two cardiologists. Cardiac allograft vasculopathy was quantified by a scoring system, which determines the location and severity of vessel stenoses (Table 1).22 On the basis of the sum of scores, each patient was categorized into one of the following groups: without CAV (score = 0), mild CAV (score 1–7), or severe CAV (score ≥8).

**Intravascular ultrasound**

Intravascular ultrasound (IVUS) data were obtained from the proximal part of the left anterior descending artery in 38 patients by continuous motorized pullback with an electronic sector scanner (Volcano, Eagle Eye catheter; 2.9F/20 MHz).23 Two blinded investigators performed the quantitative analysis using dedicated software (Volcano pCVH Review Software), and the parameters were calculated as reported.24

---

**Table 1**

**Cardiac allograft vasculopathy scoring system**

<table>
<thead>
<tr>
<th>Vessel stenosis</th>
<th>CAV score for each vessel stenosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LCA</td>
</tr>
<tr>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td>&lt;50%</td>
<td>3</td>
</tr>
<tr>
<td>50–70%</td>
<td>6</td>
</tr>
<tr>
<td>70–90%</td>
<td>9</td>
</tr>
<tr>
<td>&gt;90%</td>
<td>12</td>
</tr>
</tbody>
</table>

LCA, left coronary artery; LAD, left anterior descending artery; Cx, Circumflex coronary artery; RCA, right coronary artery.

---

**Flow cytometry**

Venous blood was drawn and anticoagulated with ethylenediamine-tetraacetic acid. Direct immunofluorescence staining was performed using a fluorescent isothiocyanate (FITC)-conjugated CD34 antibody (clone 581) with either a phycoerythrin (PE)-conjugated KDR antibody (clone 89106, R&D Systems) or a PE-conjugated CD140b antibody (clone 28D4). A mouse FITC-IgG1 antibody (clone MOPC-31C) in conjunction with PE-conjugated IgG2a (AbDSerotec) served as the isotype controls. After erythrocytes lysis, at least 50 000 events were acquired with an FACS Canto II flow cytometer (BD Biosciences). The cell populations were quantified as a percentage of the total cells in the lymphocyte side scatter gate (FACSDiva software, BD Biosciences). The absence of CD34+CD140b+ or CD34+KDR+ cells was assumed when no event was detectable. Reproducibility was determined on two subsequent days. The intra-class correlation coefficient was 0.50 for CD34+CD140b+, 0.67 for CD34+KDR+, 0.71 for CD34+, 0.67 for CD140b+, and −0.07 for KDR+ cells (n = 20). The expression of α-SMA in CD34+CD140b+ cells was determined in mononuclear cells after fixation (Cytofix, BD Biosciences) and permeabilization (0.1% saponin) by incubation with FITC-conjugated α-SMA (clone 1A4, Sigma), AlexaFluor 647-conjugated CD140b (clone J23-1044, BD Pharmingen), and PE-Cy7-conjugated CD34 antibodies (clone 8G12, BD Biosciences). Fluorescence-minus-one control experiments were performed by omitting the CD140b antibody. CD34+CD140b+ cells were sorted (FACS Aria, BD Biosciences) from human peripheral blood or bone marrow-derived CD34+ cells (Lonza) after labelling with antibodies against CD34 (clone 581) and CD140b (clone 28D4).

**Proliferation assay**

CD34+CD140b+ cells were grown in Haematopoietic Progenitor Growth Medium (HPGM; Lonza) with or without CXCL12 (200 ng/mL, Peprotech) and incubated with 5-ethylthio-2-deoxyuridine (5 μM) for 48 h before labelling (Click-iT EDU Imaging kit, Invitrogen). The percentage of positive cells was determined by fluorescence microscopy.

**Transmigration assay**

CD34+CD140b+ cells (106) were loaded onto Transwell bare filters (5 μm pore size, Costar, Corning) in HPGM. CXCL12 (200 ng/mL) or vehicle was added to the bottom well. After 4 h, migrated cells in the bottom wells were counted by flow cytometry.
Flow chamber assay

The arrest of sorted CD34^+CD140b^+ cells on TNF-α-stimulated human umbilical vein endothelial cells (HUVECs) was determined in parallel-wall chambers under flow (1.5 dynes/cm^2, 5 min). HUVECs were pre-treated with CXCL12 (200 ng/mL) for 1 h before the perfusion with CD34^+CD140b^+ cells (10^5 cells/mL).

Immunofluorescence staining

CD34^+CD140b^+ cells or total peripheral leucocytes were fixed with methanol and incubated with a CXC4 (clone 12G5, R&D Systems) or CXCL12 antibody (clone 79018, R&D Systems), followed by a fluorescently labelled secondary antibody (anti mouse, Dylight 488-conjugated, KPL).

CXCL12 enzyme-linked immunosorbent assay

Plasma CXCL12 was quantified using an enzyme-linked immunosorbent assay according to the manufacturer’s protocol (RayBiotech, Inc.).

Statistical analysis

Continuous variables were summarized by means and standard deviations (± SD) or medians and interquartile ranges. These variables were compared by t-test, analysis of variance, or Mann–Whitney test. Categorical data were presented by frequencies and percentages, and compared with Fisher’s exact test. Logistic regression models were used to analyse the joint relationship between patient characteristics and compared with Fisher’s exact test. Categorical data were presented by frequencies and percentages, and pair-wise t-tests. A linear regression test was used to calculate the relationship between CXCL12 levels and circumferential cells. Repeatability was characterized by computing an intra-class correlation coefficient for each cell population. The overall significance level for all statistical tests was defined as 5%. As all statistical correlations were analysed by unpaired t-tests. A linear regression model was used to calculate the relationship between CXCL12 levels and circulating cells. Repeatability was characterized by computing an intra-class correlation coefficient for each cell population. The overall significance level for all statistical tests was defined as 5%. As all statistical tests were conducted entirely in an explorative manner, no α-adjustment for multiple testing was carried out. Thus, P values of ≤0.05 could be interpreted as statistically significant results with respect to the study population. All analyses were performed using SAS® statistical software (V9.1.3, SAS Institute).

Results

Patient characteristics

The angiographic CAV (score ≥1) was established in 44.7% of the patients. Moderate CAV was detected in 59 (31.4%; mean sum score 3.1 ± 1.9) and severe CAV in 25 patients (13.2%; mean sum score 14.3 ± 4). In 23 patients with CAV, the plaque index (39.7 ± 11.5 versus 29.7 ± 8.5%, P = 0.0031) and the mean plaque volume (8.2 ± 3.3 versus 5.9 ± 2.4 mm^3/mm, P = 0.016) were significantly increased compared with CAV-free patients as determined by IVUS. Cardiac allograft vasculopathy patients were slightly older and the time after transplantation was about 3 years longer (Table 2). Coronary stent implantation had been performed in 23 patients with CAV, who were therefore more often treated with anti-platelet drugs than unstented patients (Table 2). Of note, serum creatinine levels, BMI, and donor age were significantly higher in CAV patients compared with CAV-free patients (Table 2). Different immunosuppressant drug combinations were equally distributed in both groups. Sirolimus treatment was more frequent in patients without CAV, whereas cyclosporine (CsA) was more prevalent in CAV patients (Table 2).

Smooth muscle progenitor cell and endothelial progenitor cell counts in cardiac allograft vasculopathy

The flow cytometry analysis revealed that circulating CD34^+CD140b^+ cells were exclusively characterized by the expression of the SMC marker α-SMA, indicating an SMC-like phenotype (Figure 1). CD34^+CD140b^+ SPCs were detectable in a significantly higher proportion of patients with CAV (80.9%) than in CAV-free patients (59.5%) (P < 0.0001, Table 3). The percentage of patients with circulating CD34^+KDR^+ cells was similar irrespective of the presence of CAV (P = 0.5325, Table 3). Notably, the number of circulating SPCs was significantly increased in stented CAV patients compared with unstented CAV patients (0.15 ± 0.13 versus 0.10 ± 0.08; P = 0.0464; Figure 2A), implying that peripheral SPC counts are associated with clinically relevant CAV. In contrast, the number of EPCs did not differ between stented and unstented CAV patients (0.04 ± 0.06 versus 0.05 ± 0.08%; P = 0.6268; Figure 2B). Treatment with sirolimus was associated with a slight, but not statistically significant, reduction in the CD34^+CD140b^+ level (0.053 ± 0.074 versus 0.083 ± 0.096%; P = 0.0761). Accordingly, neither CsA treatment nor other immunosuppressant drugs, such as MMF or tacrolimus, resulted in a statistically significant change in the CD34^+CD140b^+ cell counts (data not shown). No association was observed between peripheral CD34^+CD140b^+ or CD34^+KDR^+ cell counts and recipient age, donor age, BMI, or serum creatinine (data not shown).

In patients with moderate CAV, the CD34^+CD140b^+ cell count was two-fold higher than in patients without CAV (0.095 ± 0.080 versus 0.048 ± 0.073%; P < 0.0001; Figure 3A). Compared with patients with moderate CAV, SPC numbers were further increased by 39% in patients with severe CAV (0.158 ± 0.134%; P = 0.0023; Figure 3A). The level of SPCs correlated with the CAV sum score (r = 0.3236; P < 0.0001) and the number of both distal (r = 0.3831; P < 0.0001) and proximal stenoses (r = 0.2111; P = 0.0013). In contrast, CD34^+KDR^+ cell counts did not statistically differ between patients without CAV (0.049 ± 0.067%) and with moderate (0.053 ± 0.084%) or severe CAV (0.045 ± 0.057%; Figure 3B). The counts for cells with only CD34, KDR, or CD140b expression did not correlate with the degree of CAV (Figure 3C), implying that increased CD34^+CD140b^+ cells are specifically associated with CAV severity.
Circulating CXCL12 levels

The CXCL12 levels in patients without CAV (103 ± 33 pg/mL, n = 79) were significantly lower than in patients with moderate (119 ± 48 pg/mL, n = 43, P < 0.05) or severe CAV (144 ± 58 pg/mL, n = 14, P < 0.01) (Figure 4). Compared with moderate CAV, CXCL12 levels were further elevated in patients with severe CAV (P < 0.05; Figure 4). Furthermore, CXCL12 concentrations correlated with the CD34+CD140b+ cell counts (r = 0.2967, P = 0.0005) (Figure 5A) but not with the CD34+KDR+ cell counts (r = −0.02804, P = 0.7459) (Figure 5B). In addition, no statistically significant difference in plasma CXCL12 concentration was observed in patients treated with sirolimus, MMF, tacrolimus, or CsA (data not shown).

Functional effects of CXCL12 on CD34+CD140b+ cells

Whereas CXCL12 expression was absent in CD34+CD140b+ cells (Figure 6A–C), CXCR4 immunostaining was clearly evident in both CD34+CD140b+ cells and leucocytes (Figure 6D–F). The surface expression of CXCR4 on CD34+CD140b+ cells...
was not different from CD34\(^+\)KDR\(^+\) cells, as determined by flow cytometry (data not shown). A functional analysis revealed that CXCL12 enhanced the migration but not the proliferation of CD34\(^+\)CD140b\(^+\) cells (Figure 6E and F). CXCL12 also supported the adhesion of CD34\(^+\)CD140b\(^+\) cells under flow conditions on TNA-\(\alpha\)-stimulated endothelial cells (Figure 6G).

**CD34\(^+\)CD140b\(^+\) cell count is independently associated with cardiac allograft vasculopathy**

In a multivariate logistic regression model, the factors associated with CAV were analysed (Table 4). Donor age, platelet count, and creatinine levels, but not the time after transplantation, recipient age, BMI, sirolimus, or CsA treatment, were identified to be associated independently with CAV in this model. Notably, peripheral CD34\(^+\)CD140b\(^+\) cell counts were also independently associated with the presence of CAV and appeared to add significant information beyond that provided by the other factors.

**Discussion**

We analysed putative CD34\(^+\)CD140b\(^+\) SPCs and CD34\(^+\)KDR\(^+\) EPCs in the circulation of heart transplant recipients and found a positive correlation of the SPC, but not the EPC counts, and of the plasma CXCL12 concentration with the severity of CAV. These findings are compatible with the hypothesis that CXCL12-mediated SPC mobilization is involved in the development of CAV.

CD34 is predominantly expressed in haematopoietic stem cells, and circulating CD34\(^+\) cells have previously been identified to comprise progenitors for endothelial cells and SMCs.\(^2\)\(^3\) Mobilization of CD34\(^+\) cells after coronary stent implantation is associated with

---

**Figure 1** Peripheral CD34\(^+\)CD140b\(^+\) cells express the SMC marker \(\alpha\)-SMA. CD34\(^+\) cells (A) were further analysed for CD140b co-expression (B, green). CD34\(^+\)CD140b\(^+\) cells expressed significant amounts of \(\alpha\)-SMA (C). Control experiments were performed by omitting the CD140b antibody (D).

**Figure 2** Peripheral CD34\(^+\)CD140b\(^+\) and CD34\(^+\)KDR\(^+\) cell counts in patients with cardiac allograft vasculopathy. Circulating CD34\(^+\)CD140b\(^+\) smooth muscle progenitor cells were increased by 50% in patients with cardiac allograft vasculopathy requiring coronary stent implantation compared with non-stented cardiac allograft vasculopathy patients (A) (\(\ast\) \(P = 0.0464\)). CD34\(^+\)KDR\(^+\) endothelial progenitor cell counts were similar in cardiac allograft vasculopathy patients irrespective of previous stent implantation (B) (\(P = 0.6268\)).

---

**Table 3** Presence of circulating CD34\(^+\)CD140b\(^+\) and CD34\(^+\)KDR\(^+\) cells

<table>
<thead>
<tr>
<th></th>
<th>w/o CAV</th>
<th>CAV</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients with CD34(^+)CD140b(^+) SPCs</td>
<td>50 (48.5%)</td>
<td>68 (80.9%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of patients with CD34(^+)KDR(^+) EPCs</td>
<td>55 (53%)</td>
<td>41 (48.8%)</td>
<td>0.5325</td>
</tr>
</tbody>
</table>
CD34⁺CD140b⁺ cells and circulating CXCL12 correlate with CAV

in-stent restenosis and increased outgrowth of SMCs. Recipient-derived SMCs accumulate in neointimal tissue of experimental transplant arteriosclerosis and CAV in humans. Recently, no difference in the CD34⁺ cell numbers was found between patients with and without CAV. The CD34⁺KDR⁺ subpopulation contains EPCs and has repeatedly been demonstrated to predict cardiovascular events. The absence of CD45 on CD34⁺ cells has been shown to be characteristic for EPCs. Moreover, CD34⁺CD45⁻ cells, but not CD34⁺CD45⁺ cells, exclusively express KDR, suggesting that CD34⁺KDR⁺ cells closely resemble CD34⁺CD45⁻ EPCs. In cardiac transplant patients, however, levels of EPC subpopulations, such as CD34⁺KDR⁺ or CD133⁺KDR⁺ cells, were not different in the presence of CAV, whereas increased EPC-colony-forming units were found in patients with an acute rejection episode. Our results confirm these findings in a larger cohort of patients demonstrating that neither the presence nor the number of CD34⁺KDR⁺ EPCs was related to CAV.

Uraemia has been shown to decrease peripheral EPC levels, which could at least partially explain the pathogenic role of impaired renal function in CAV development.

Compared with EPCs and EPC-like cells, the characterization of circulating SPCs is less well established. It has been consistently shown that peripheral blood CD34⁺ cells can differentiate into SMCs in vitro following the addition of PDGF-BB, which binds to the β-chain of the PDGF receptor (CD140b). CD140b is preferentially expressed in SMCs but not in endothelial cells, and it is essential for the recruitment of SMCs during embryonic blood vessel formation. Moreover, CD140b-expressing mouse SPCs are selectively mobilized and recruited into the neointima by CXCL12 after vascular injury. We, therefore, studied CD34⁺CD140b⁺ cells as putative SPCs in CAV. In contrast to CD34⁺KDR⁺ EPCs, the presence of CD34⁺CD140b⁺ cells was significantly associated with the presence of CAV, and the CD34⁺CD140b⁺ cell count increased in patients with moderate and severe CAV. The relatively modest correlation of the individual CAV sum score with the SPC count might be related to the limitations of the angiographic method to detect subtle variations in neointima formation. Although the SPC count was higher than

Figure 3 Circulating smooth muscle progenitor cell and endothelial progenitor cell counts in relation to the severity of cardiac allograft vasculopathy. The level of CD34⁺CD140b⁺ cells increased with the cardiac allograft vasculopathy severity (A) (*P < 0.01). No correlation was found between the level of CD34⁺KDR⁺ cells and the cardiac allograft vasculopathy degree (B). The expression of only a single marker, such as CD34, CD140b, or KDR, was not correlated with the severity of cardiac allograft vasculopathy (C).

Figure 4 Plasma CXCL12 levels correlate with the severity of cardiac allograft vasculopathy. The CXCL12 concentrations were significantly elevated in patients with severe and moderate cardiac allograft vasculopathy compared with cardiac allograft vasculopathy-free patients (*P < 0.05).
Figure 5  Circulating progenitor cells in relation to the plasma CXCL12 level. The linear regression analysis revealed that CXCL12 levels correlated with the number of circulating CD34+CD140b+ cells (P = 0.0005; A). There was no significant relationship between plasma CXCL12 and CD34+KDR+ cell counts (B).

Figure 6  Roles of CXCL12 and CXCR4 in CD34+CD140b+ cells. CXCL12 expression was not detectable in CD34+CD140b+ cells (A) but was in some leucocytes (C). CD34+CD140b+ cells (D) and leucocytes (F) were positive for CXCR4. Isotype control staining of CD34+CD140b+ cells revealed negligible background staining (B and E). CXCL12 treatment enhanced the migration (G; n = 3; P < 0.05) and adhesion (I; n = 2) but not the proliferation of CD34+CD140b+ cells (H; n = 3).
Table 4  Odds ratio and P value for the presence of CAV in the multivariate model

<table>
<thead>
<tr>
<th>Multivariate predictors</th>
<th>Odds ratio (95% Wald confidence limits)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count</td>
<td>0.989 (0.981–0.997)</td>
<td>0.0085</td>
</tr>
<tr>
<td>Donor age</td>
<td>1.056 (1.017–1.096)</td>
<td>0.0045</td>
</tr>
<tr>
<td>Creatinine</td>
<td>2.616 (1.259–5.436)</td>
<td>0.0100</td>
</tr>
<tr>
<td>CD34+CD140b+ SPC counts</td>
<td>18.034 (5.907–55.059)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

The covariates included in the initial model were age, BMI, time after HTx, donor age, creatinine levels, platelet count, presence or absence of sirolimus treatment, presence or absence of CVA treatment, and peripheral CD34+CD140b+ cell level (categorized into <0.05 and >0.05).

Conflict of interest: none declared.

References


