Donor Serum SMARCAL1 Concentrations Predict Primary Graft Dysfunction in Cardiac Transplantation

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Background—Primary graft dysfunction (PGD) is a life-threatening complication in cardiac transplantation. A sensitive, specific, and easily measurable predictor in donors could facilitate PGD prevention.

Methods and Results—SMARCAL1 is a matrix-associated regulator of chromatin with helicase and ATPase activities, and its serum concentrations were significantly increased in a targeted protein array in donors whose grafts developed PGD. Therefore, this study analyzed SMARCAL1 serum concentrations by ELISA in 336 heart donors before and after aortic cross-clamping (ACC) and in recipients at 10, 30, and 60 minutes reperfusion. Demographic and hemodynamic parameters of donors and recipients as well as transplant procedure characteristics were documented. PGD (n=68) was defined as ventricular dilation and hypococontractility associated with systolic blood pressure <90 mm Hg, pulmonary capillary wedge pressure >20 mm Hg, and decreased mixed venous oxygen saturation necessitating mechanical circulatory support. SMARCAL1 serum protein concentration was significantly increased only before and after ACC in donors (P<0.0001) whose grafts developed PGD compared to those who did not. In receiver operating characteristic curve analysis, SMARCAL1 serum concentration at a cut-off level of ≥1.25 ng/mL before ACC in donors predicted PGD (P<0.0001, AUC=0.988, OR=17.050, 95% CI=5.200 to 55.901) with 96% sensitivity and 88% specificity. SMARCAL1 serum concentrations <1.25 ng/mL in donors before ACC resulted in 97% PGD-free outcome and SMARCAL1 concentrations ≥1.25 resulted in 83% PGD occurrence.

Conclusions—Donor serum SMARCAL1 may serve as a specific, sensitive, and noninvasive predictive marker in the assessment of cardiac graft quality. (Circulation. 2009;120[suppl 1]:S198–S205.)

Key Words: cardiac transplantation ▪ primary graft dysfunction ▪ marker ▪ SMARCAL1

Primary graft dysfunction (PGD) is the most common cause of early mortality accounting for 40% of deaths within the first month after cardiac transplantation. The incidence of PGD has been reported as high as 10% to 25% in heart transplant recipients. Increased pulmonary vascular resistance (PVR) associated with right heart failure, intrinsic donor organ dysfunction, preservation injury, and inflammation have been considered to contribute to PGD, although the predictive value of these parameters has not been fully characterized in randomized studies. Because of organ shortage, marginal donors are being increasingly accepted for heart transplantation, and a recent report indicates that marginal cardiac allografts are not associated with increased PGD risk.

Although we have used standardized recipient selection criteria and have rejected donors with decreased left ventricular (LV) systolic function, increased inotropic requirement, and LV hypertrophy, PGD continued to occur in about 20% of our patients during the last decade. A sensitive marker that could identify high-risk donors and complement the clinical risk factors of PGD could therefore improve donor selection and PGD prevention. Tissue stress attributable to ischemia-induced damage and endothelial dysfunction are believed to mediate cardiac allograft injury. Based on this reasoning, we have recently reported that hypoxia inducible factor (HIF)-1 mRNA expression in LV biopsies of donor cardiac allografts obtained after aortic cross clamping (ACC) can predict PGD.

The purpose of the present study was to find a noninvasive and rapidly responding PGD marker that would allow donor selection before organ harvest. To address this issue, we initiated a serum protein array in selected donors whose donated grafts developed PGD in comparison to those who did not. These analyses suggested SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin subfamily a-like 1 (SMARCAL1) to be associated with PGD. SMARCAL1 is an intracellular protein that acts as a DNA-dependent ATPase involved in transcription, DNA repair, and chromatin dynamics. Based on the unexpected increased
smear protein levels of the intracellular SMARCAL1 in array analyses of selected donors, we sought to determine whether SMARCAL1 could predict PGD in a subsequent patient cohort.

Methods

Patients

This study was approved by the Ethics Committee of the Medical University of Vienna. Serum samples of 40 donors were used for the initial array analyses. Then, a total of 336 consecutive cardiac allograft donors and recipients were included between January 1998 and September 2008. A total of 39 recipients had to be excluded due to informed consent not being given or because of the presence of hepatitis B or C antibodies. Tables 1 and 2 summarize the demographic and the most relevant characteristics of the recipients and donors. All recipients matched the American Heart Association guidelines for transplantation5 and had optimized pretransplant heart failure treatment. The patients received standard immunosuppression according to current standards.

Donor and procedure characteristics

<table>
<thead>
<tr>
<th></th>
<th>ICM</th>
<th>DCM</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>116</td>
<td>187</td>
<td>33</td>
</tr>
<tr>
<td>Recipient age, y</td>
<td>58±7</td>
<td>51±14*</td>
<td>40±17†</td>
</tr>
<tr>
<td>Male/Female</td>
<td>91/25</td>
<td>149/38</td>
<td>20/13†</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>20.4±8.3</td>
<td>18.1±8.1*</td>
<td>27.9±15.3†</td>
</tr>
<tr>
<td>Mean PAP, mm Hg</td>
<td>31.3±10.5</td>
<td>32.5±10.0</td>
<td>26.4±9.9†</td>
</tr>
<tr>
<td>PCWP, mm Hg</td>
<td>21.0±8.8</td>
<td>22.3±8.4</td>
<td>20.6±6.2</td>
</tr>
<tr>
<td>PVR, Wood units</td>
<td>2.6±1.2</td>
<td>2.6±1.5</td>
<td>1.9±0.9*</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>1.4±0.8</td>
<td>1.3±0.6</td>
<td>1.3±0.4</td>
</tr>
</tbody>
</table>

PGD was defined as ventricular dilation and hypocontractility assessed by transesophageal echocardiography associated with systemic hypotension (systolic blood pressure <90 mm Hg), increased pulmonary capillary wedge pressure (PCWP; >20 mm Hg), and decreased mixed venous oxygen saturation despite inotropic support.6,11,12 Affected patients were treated with prolonged reperfusion on cardiopulmonary bypass (CPB) with maximized drug therapy (including isoproterenol, norepinephrine, milrinone, epoprostenol, and inhaled nitric oxide). If weaning from CPB was still not possible, patients received postoperative mechanical circulatory support (intra-aortic balloon pump [IABP] or extracorporeal membrane oxygenation [ECMO]).

Diagnosis and Treatment of PGD

PGD was defined as ventricular dilation and hypocontractility assessed by transesophageal echocardiography associated with systemic hypotension (systolic blood pressure <90 mm Hg), increased pulmonary capillary wedge pressure (PCWP; >20 mm Hg), and decreased mixed venous oxygen saturation despite inotropic support.6,11,12 Affected patients were treated with prolonged reperfusion on cardiopulmonary bypass (CPB) with maximized drug therapy (including isoproterenol, norepinephrine, milrinone, epoprostenol, and inhaled nitric oxide). If weaning from CPB was still not possible, patients received postoperative mechanical circulatory support (intra-aortic balloon pump [IABP] or extracorporeal membrane oxygenation [ECMO]).

Serum Protein Array

For protein array a set of 40 randomly selected serum samples obtained before ACC in donors whose donated grafts developed PGD (n=20) and from those who did not (n=20) was obtained and pooled for each group. The protein array was performed on antibody Microarray 500 (Clontech; 507 proteins), according to the manufacturer’s protocol. The proteins in each sample were labeled with 2 different fluorescent dyes and incubated on microarray antibody-coated slides. The protein fluorescent signals of both slides were detected by the GenePix 4000B scanner (Molecular Dynamics). The
quantification of the signal was performed by calculating an internally normalized ratio (INR), yielding the abundance of an antigen in PGD sample relative to that in no PGD sample by using the automated Microarray Analysis Workbook (http://bioinfo.clontech.com). Proteins with INR values outside the threshold interval were considered differentially expressed.

**SMARCAL1 Assays**

Enzyme linked immunosorbent assay (ELISA) for SMARCAL1 (GEM ELISA, QED Bioscience) was performed according to the manufacturer’s protocol. A SMARCAL1 monoclonal antibody (20 μg/well, Abnova) was precoated onto a microplate and 100 μL of assay diluent (buffered protein base) was added to each well, followed by 100 μL of standard or serum sample. The wells were incubated overnight at 4°C, then blocked at room temperature for 30 minutes with 1% BSA-PBS-0.05% Tween 20 and washed. The secondary SMARCAL1 polyclonal antibody (10 μg/well; Abnova) conjugated to horseradish peroxidase was added and incubated for 2 hours at room temperature followed by 3 washes. Thereafter, 200 μL substrate solution (stabilized hydrogen peroxide; stabilized chromogen) was added and incubated for 25 minutes at room temperature to develop color. The reaction was stopped by adding 50 μL of 2 N sulfuric acid. The optical density was detected at dual wavelengths of 405 nm and 490 nm using an ELISA plate reader (Anthos).

**Quantitative Real-Time RT-PCR**

Total RNA was isolated from myocardial tissue samples using TRIzol (Invitrogen). The tissue was homogenized in a MagNA Lyser system (Roche) using MagNA Lyser Green Beads for 20 to 30 seconds at 6000 rpm. Homogenized samples were then mixed with 200 μL chloroform, incubated 2 to 3 minutes at room temperature and centrifuged at 12 000 g for 15 minutes at 4°C. RNA was collected and cDNA was synthesized using 2 μg total RNA and the M-MulV-RT kit (Fermentas). Real-time RT-PCR was performed on a LightCycler instrument (Roche) as described.10 The primer sequences were sense/antisense: SMARCAL1, 5'-GTTCACAAATCTCTGCC-3'/5'-CGTGTACATCCTGTC-3'; and β2-microglobulin, 5'-GATGATATGGGCCTGGT-3'/5'-CAATCCAAATGGCGG CATC-3'. mRNA expression units were determined after normalization to expression of the housekeeping gene β2-microglobulin as described previously10,11,12 and plotted for PGD patients as percentage relative to those in patients without PGD. Measurements were performed 3 times. The average value of the 3 PCR measurements in each sample was used for data analysis.

**Statistical Analysis**

Clinical parameters as well as donor and procedure characteristics were compared between patient groups by χ² test and analysis of variance (1-way ANOVA; Tukey test) according to the scale of the variable (continuous or categorical). Serum concentrations and mRNA expression levels in donor biopsies measured repeatedly were subjected to repeated-measures ANOVA by using the REPEATED statement in the MIXED procedure. The model included effects of the 2 groups (ie, PGD and no PGD) time point of sampling and the interaction term for time point of sampling and group. A covariance structure that resulted in the Akaike information criterion closest to zero was used. Post hoc comparisons were performed using the Tukey–Kramer method of adjustment. The impact of serum SMARCAL1 concentration to expression of the housekeeping gene β2-microglobulin was considered differentially expressed.

**Results**

**SMARCAL1 Is Associated With PGD**

In the targeted protein array screen of pooled serum samples in selected donors, we identified SMARCAL1 as a novel protein to be associated with PGD. The array also identified interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (data not shown) that have been previously described to be related to cardiac graft dysfunction.14–16 Figure 1A shows the array images of SMARCAL1 and indicates that its serum protein levels are increased in donors whose grafts developed PGD as compared to those who did not. The mRNA measurements in LV tissue indicated that SMARCAL1 expression increases significantly in donors whose grafts developed PGD as compared to mRNA expression levels of the non-PGD group (Figure 1B). This increase in SMARCAL1 LV mRNA expression was statistically significant before (P<0.0001) and after (P=0.0012) ACC in donors; although at all other time points SMARCAL1 mRNA expression was not signif-
Table 3. Outcome in Patients With and Without PGD

<table>
<thead>
<tr>
<th></th>
<th>No PGD</th>
<th>PGD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>268</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Length of ICU stay, days</td>
<td>8:9</td>
<td>16:15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Length of hospital stay, days</td>
<td>28:15</td>
<td>33:23</td>
<td>0.0523</td>
</tr>
<tr>
<td>3-month mortality, %*</td>
<td>10.7</td>
<td>36.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>1-year mortality, %†</td>
<td>13.6</td>
<td>44.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>5-year mortality, %‡</td>
<td>32.8</td>
<td>75.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Acute rejection incidence, %§</td>
<td>45.1</td>
<td>54.4</td>
<td>0.1717</td>
</tr>
<tr>
<td>3-month survivors only, %</td>
<td>55.8</td>
<td>50.0</td>
<td>0.2605</td>
</tr>
<tr>
<td>Allograft vasculopathy incidence, %</td>
<td>14.9</td>
<td>7.4</td>
<td>0.8897</td>
</tr>
<tr>
<td>Retransplantation incidence, %</td>
<td>2.2</td>
<td>4.4</td>
<td>0.3216</td>
</tr>
</tbody>
</table>

ICU indicates intensive care unit; PGD, primary graft dysfunction.

* included 327 (complete 3-month follow up); † included 304 (complete 1-year follow up); ‡ included 175 (complete 5-year follow up).

3-month (P=0.0038, AUC=0.777) and 1-year survival (P=0.0096, AUC=0.755), although neither SMARCAL1 nor PGD were independent predictors of 5-year survival.

Procedure and Patient Clinical Characteristics in Relation to PGD

In 68 (20.2%) patients enrolled in this study, PGD developed intraoperatively or within 24 hours after cardiac transplantation (Table 2). In analysis of donor, recipient, and procedure characteristics, recipient age, sex, underlying cardiac disease, diabetes status, LVEF, mean PAP, PCWP, PVR, time on waiting list until transplant, previous cardiac surgery, ventricular assist device, sex mismatch, CMV mismatch, and graft ischemic time were not significantly different between PGD and non-PGD group (Table 2). In donors, age, sex, and length of hospital stay did not significantly differ between the PGD and non-PGD group (Table 2). The analysis of recipient and procedure characteristics indicated pretransplant creatinine (P=0.0264), the extracorporeal circulation time (P=0.0091), reperfusion on CPB (P=0.0001), and use of posttransplant ECMO or IABP (P<0.0001) as being significantly different between the PGD and non-PGD groups (Table 2). In donors whose grafts developed PGD (n=296), at 10 (n=269), 30 minutes (n=286) aortic cross clamping (ACC) when PGD and non-PGD groups are compared, whereas no significant changes are seen in recipient serum before transplant (n=294) and after (P<0.0001) associated with PGD compared to non-PGD group (Figure 3). SMARCAL1 serum concentrations in pretransplant heart failure patients, in RS1-RS3 and at 1 to 4 weeks post-transplant, however, were not significantly different between PGD and non-PGD group (Figure 3). After the study was finished, we went on to learn about serum SMARCAL1 concentrations in donors before initiating organ harvest. Between April 2 and May 27, 2009, 10 heart transplants were performed in our center. The mean serum SMARCAL1 concentrations were 0.77±0.48 ng/mL in donors whose grafts did not develop PGD (n=8) and 3.51±2.05 ng/mL in donors whose grafts developed PGD (n=2).

Serum SMARCAL1 in Donors Independently Predicts PGD

Analysis of SMARCAL1 serum concentrations at the study sampling time points in all patients resulted in statistically significant higher concentrations of SMARCAL1 in DS1 (P<0.0001) and DS2 (P<0.0001) associated with PGD compared to non-PGD group (Figure 3). SMARCAL1 serum concentrations in pretransplant heart failure patients, in RS1-RS3 and at 1 to 4 weeks post-transplant, however, were not significantly different between PGD and non-PGD group (Figure 3). After the study was finished, we went on to learn about serum SMARCAL1 concentrations in donors before initiating organ harvest. Between April 2 and May 27, 2009, 10 heart transplants were performed in our center. The mean serum SMARCAL1 concentrations were 0.77±0.48 ng/mL in donors whose grafts did not develop PGD (n=8) and 3.51±2.05 ng/mL in donors whose grafts developed PGD (n=2).
When studying the SMARCAL1 and clinical data in univariate logistic regression analysis, only SMARCAL1 in DS1 ($P<0.0001$) and DS2 ($P=0.0019$) as well as recipient pretransplant creatinine levels ($P=0.0372$) were significant predictors of PGD (Table 4). To assess the mechanistic relevance of PGD-associated parameters, SMARCAL1 in DS1, DS2, and serum creatinine, as well as donor sex ($P=0.0639$) and ischemic time ($P=0.0583$), who met entrance level of $P=0.1$, were analyzed in multivariate logistic regression. When including DS1 SMARCAL1 and creatinine concentrations, donor sex, and ischemic time, SMARCAL1 ($P=0.0001$) and serum creatinine ($P=0.0314$) remained independent predictors of PGD (AUC=0.993), although when including DS2 SMARCAL1, creatinine, donor sex, and ischemic time, only SMARCAL1 ($P=0.0041$) predicted PGD (AUC=0.927; Table 5). In the ROC analysis, SMARCAL1 concentrations in DS1 at a cut-off level of $\geq 1.25$ ng/mL predicted PGD with 96% sensitivity and 88% specificity ($P<0.0001$, AUC=0.988). The corresponding PPV and NPV were 83% and 97%, respectively.

To illustrate the relationship between circulating SMARCAL1 and PGD incidence, the evolution of SMARCAL1 serum concentrations in DS1, DS2, and RS1-RS3 was plotted for randomly selected patients with (Figure 4A) and without PGD (Figure 4B). The results indicated that SMARCAL1 concentration in DS1 remained below in non-PGD and increased above the cut-off level of 1.25 ng/mL in PGD patients.

### Table 4. Predictors of PGD in Univariate Logistic Regression Analysis

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>P</th>
<th>AUC</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS1 SMARCAL1</td>
<td>289</td>
<td>&lt;0.0001</td>
<td>0.988</td>
<td>17.050</td>
<td>5.200–55.901</td>
</tr>
<tr>
<td>DS2 SMARCAL1</td>
<td>286</td>
<td>0.0019</td>
<td>0.857</td>
<td>23.776</td>
<td>3.210–176.119</td>
</tr>
<tr>
<td>RS1 SMARCAL1</td>
<td>269</td>
<td>0.1603</td>
<td>0.621</td>
<td>3.233</td>
<td>0.628–16.633</td>
</tr>
<tr>
<td>RS2 SMARCAL1</td>
<td>294</td>
<td>0.1680</td>
<td>0.611</td>
<td>0.334</td>
<td>0.070–1.587</td>
</tr>
<tr>
<td>RS3 SMARCAL1</td>
<td>274</td>
<td>0.5357</td>
<td>0.500</td>
<td>1.595</td>
<td>0.364–6.987</td>
</tr>
</tbody>
</table>

#### Diagnosis
- ICM versus other: 336, 0.1131, 0.553, 1.569, 0.889–2.739
- DCM versus other: 336, 0.1110, 0.555, 0.641, 0.371–1.108

#### Donor age: 336, 0.1757, 0.552, 1.015, 0.993–1.036
- Recipient age: 336, 0.3597, 0.533, 1.010, 0.988–1.033
- Donor sex: 336, 0.0659, 0.560, 1.672, 0.967–2.892
- Recipient sex: 336, 0.3020, 0.529, 0.697, 0.352–1.383
- Sex mismatch: 336, 0.1456, 0.543, 1.550, 0.859–2.795
- CMV donor: 336, 0.9487, 0.502, 1.019, 0.580–1.788
- CMV recipient: 336, 0.1557, 0.549, 1.549, 0.847–2.836
- CMV mismatch: 336, 0.9085, 0.504, 0.966, 0.536–1.742

#### Donor cause of death
- Spontaneous cerebral bleeding versus other: 336, 0.3864, 0.529, 1.273, 0.737–2.196
- Isolated craniocerebral injury versus other: 336, 0.2589, 0.539, 0.730, 0.423–1.261

#### Ischemic time: 336, 0.0583, 0.578, 1.005, 1.000–1.011
- Hospital stay of donor: 336, 0.7873, 0.550, 1.011, 0.936–1.092
- PAP mean: 336, 0.7985, 0.519, 1.003, 0.977–1.030
- PCWP: 336, 0.8824, 0.497, 0.998, 0.966–1.030
- PVR: 336, 0.2936, 0.506, 1.104, 0.918–1.329
- LVEF: 336, 0.6500, 0.539, 1.008, 0.974–1.042
- NIDDM: 336, 0.5129, 0.509, 1.573, 0.405–6.117
- IDDM: 336, 0.2582, 0.532, 1.510, 0.739–3.084
- Pretransplant creatinine: 336, 0.0372, 0.608, 1.555, 1.027–2.355
- Time on waiting list: 336, 0.6779, 0.504, 1.000, 0.999–1.001
- Previous cardiac surgery: 336, 0.1385, 0.551, 1.502, 0.877–2.571
- Ventricular assist device: 336, 0.4020, 0.552, 1.333, 0.681–2.610

AUC indicates area under the curve; CI, confidence interval; CMV, cytomegalovirus; DCM, dilated cardiomyopathy; DS, donor serum; ICM, ischemic cardiomyopathy; IDDM, insulin-dependent diabetes mellitus; LVEF, left ventricular ejection fraction; NIDDM, non–insulin-dependent diabetes mellitus; OR, odds ratio; PAP, pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RS, recipients serum.

**Discussion**

PGD continues to be the most important intra- and perioperative complication in heart transplantation.\(^1\)\(^2\)\(^17\) The etiology of PGD remains widely unclear and recognizing the param-
SMARCAL1 and Primary Graft Dysfunction

Table 5. Predictors of PGD in Multivariate Logistic Regression Analysis

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Statistical Significance</th>
</tr>
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<tbody>
<tr>
<td>Including DS1</td>
<td>AUC = 0.993, n = 289</td>
</tr>
<tr>
<td>SMARCAL1 concentration</td>
<td>P = 0.0012, OR = 39.452, 95% CI = 4.271–364.415</td>
</tr>
<tr>
<td>Pretransplant creatinine</td>
<td>P = 0.0388, OR = 2.772, 95% CI = 1.054–7.289</td>
</tr>
<tr>
<td>Donor sex</td>
<td>P = 0.6916, OR = 14.501, 95% CI = 0.018–14.501</td>
</tr>
<tr>
<td>Ischemic time</td>
<td>P = 0.0755, OR = 1.024, 95% CI = 0.998–1.052</td>
</tr>
<tr>
<td>Including DS2</td>
<td>AUC = 0.927, n = 286</td>
</tr>
<tr>
<td>SMARCAL1 concentration</td>
<td>P = 0.0041, OR = 117.807, 95% CI = 4.533–999.999</td>
</tr>
<tr>
<td>Pre-transplant creatinine</td>
<td>P = 0.0985, OR = 6.925, 95% CI = 0.697–68.797</td>
</tr>
<tr>
<td>Donor sex</td>
<td>P = 0.8708, OR = 1.208, 95% CI = 0.124–11.771</td>
</tr>
<tr>
<td>Ischemic time</td>
<td>P = 0.6261, OR = 0.994, 95% CI = 0.970–1.019</td>
</tr>
</tbody>
</table>

AUC indicates area under the curve; CI, confidence interval; DS, donor serum; OR, odds ratio.

Figure 4. Evolution of SMARCAL1 serum concentrations. SMARCAL1 serum concentrations increase above the cut-off level in 12 randomly selected donors whose grafts develop PGD (A) but remain below the cut-off level in 12 non-PGD patients (B). ACC indicates aortic cross clamping; rep., reperfusion.

The only clinical independent predictor of PGD identified in our patient cohort was pretransplant serum creatinine. The mechanisms by which recipient renal function could have influenced PGD development remain uncertain. One reason might be that intraoperative fluid balance management is more complex in patients with diabetes mellitus and associated macro- and microangiopathy involving the kidneys. Accordingly, patients with non–insulin-dependent diabetes mellitus in the PGD group had the highest mean pretransplant creatinine levels in this study. In line with our findings, increased pretransplant creatinine is reportedly a predictor of shorter survival and a risk factor for hospital mortality in heart transplant recipients. Of note, renal insufficiency increases the death risk in recipients who have assist devices as a bridge to transplant.

The ischemic time, which would expectedly and reportedly correlate with the incidence of PGD, was not a significant predictor of PGD in the present report. This phenomenon might be explained by the generally lower ischemic time of the allografts used in our recipients as compared to the graft ischemic times reported earlier. Furthermore, a direct comparison of our data set with earlier PGD data might be confounded by inclusion of a higher number of patients in and the exclusion of marginal donors from our study. The ISHLT registry and other reports have emphasized the overall impact of ischemic time on mortality at 1 year after cardiac transplantation. Our data suggest the occurrence of PGD as a significant cause of early (3-month) mortality. Thus, the occurrence of PGD per se, whether associated with independent of graft ischemic time as observed in our and a recent study, seems to be more important for posttransplant survival. Another factor that might confound the comparison of our data are the change in donor and recipient management in our center. For hemodynamic stabilization of donors, we have increasingly used vasopressors instead of inotropes. Toward recipients, the optimized heart failure treatment included pretransplant and if indicated intraoperative use of calcium channel sensitizer levosimendan during the last 4 years. Also, the number of recipients bridged to transplant by assist devices continuously increased in our center.

Lima et al have reported PGD rates as high as 23% and 1-year survival rates of 79% in those patients (excluding marginal donors). We report here a PGD incidence of 20.2% and a 1-year survival of 56.5% in patients with PGD. Although the PGD rate was nearly identical at both centers, our 1-year survival rate in PGD patients is 20% below that reported by Lima et al, despite aggressive use of mechanical support in our center. As opposed to these survival rates, Segovia et al reported a survival rate of only 12% in their patient cohort with a PGD rate of 9.7%. The large divergence of PGD-associated survival rates might reflect the different stringency of criteria used to diagnose and treat PGD at different centers. The need for postoperative mechanical circulatory support has been considered conservative for PGD diagnosis, and high-dose inotropic support has therefore
been applied for PGD diagnosis. In our center, mechanical circulatory support was used as treatment in cases where PGD was diagnosed based on the hemodynamic parameters, as described earlier.

In this targeted screening, serum protein concentration of SMARCAL1 in donors was significantly increased associated with PGD. The protein encoded by this gene is a member of the SWI/SNF (SWItch/Sucrose NonFermentable) family of proteins. Members of this family have helicase and ATPase activities and are thought to regulate transcription of certain genes by altering their chromatin structure. Why serum SMARCAL1 in donors independently and specifically predicts PGD is unknown. However, evidence is accumulating that the affinity of histones for DNA and DNA-associated proteins is modulated by acetylation, phosphorylation, methylation, or ubiquitination of histone amino termini. The modification of histones, in turn, organizes the genome into open and condensed chromatin and thereby governs transcriptional activity and the availability of DNA for repair.

Nucleosome remodeling complexes are ATPase-dependent, involving SWI/SNF-related ATPases such as SMARCAL1, which was recently recognized as a helicase that acts throughout the genome to oppose the action of DNA-unwinding. Evidence was provided that a defect of SMARCAL1 function resulted in pleiotropic disorders.

Therefore, in the present study we hypothesized that SMARCAL1 might contribute specifically to a donor-related process that predisposes to PGD. This hypothesis is supported by the fact that differences in donor SMARCAL1 mRNA and serum concentrations are not translated to the recipients. Experimental studies are necessary to elucidate the underlying mechanisms by which SMARCAL1, a molecule on which our knowledge is evolving, affects graft function. A recent report strongly suggests that PGD is a donor-related process, as heart, lung, liver, and kidney grafts obtained from the same multi-organ donor developed PGD. SMARCAL1 might be one of the donor factors involved in PGD and its serum concentrations might therefore facilitate donor selection, in particular among marginal donors, where clinical parameters relevant to PGD yield a complex donor profile.

Although based on our original study design, the number of available donor serum samples obtained before initiating organ harvest is limited, the existing data suggest that serum SMARCAL1 might be beneficial in donor selection even before organ harvest.

This study included a relatively large number of cardiac transplant patients, yet it has limitations. Because the donor selection criteria weighed clinical risk factors to exclude marginal donors in our center for years, and although optimized pretransplant heart failure treatment was applied in all recipients, the factor bias in donor-recipient match, which might have varied during the study time, and the bias caused by rejecting marginal donors in our center cannot be accounted for in this report. A further limitation of the present report might be its single center nature, although a multicenter study would diversely assign priority to the multiple competing events resulting in PGD and PGD-associated mortality. Furthermore, because of the exploratory nature of the present study, randomized prospective studies are required that would implement the existing clinical criteria and supplement SMARCAL1 serum concentrations in donors to conclusively approve its utility as a PGD marker. As opposed to these limitations, the noninvasive nature and ease of the suggested SMARCAL1 serum quantification protocol along with the availability of ELISA as a routine clinical laboratory equipment, and finally the high sensitivity and specificity of SMARCAL1, make it an attractive donor-based PGD marker.

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Disclosures
None.

References


Donor Serum SMARCAL1 Concentrations Predict Primary Graft Dysfunction in Cardiac Transplantation

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