Transforming growth factor beta and myocardial dysfunction following heart transplantation

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Abstract

Objective: We analyzed the role of transforming growth factor-beta (TGF-β), a fibrogenic cytokine, in the development of left ventricular diastolic dysfunction following heart transplantation. Methods: We studied 152 heart transplant recipients who had survived for at least 24 months. We compared histopathological findings (staining of endomyocardial biopsy specimens using HemaToxlin Eosin and polyclonal antibodies), left ventricular function (Doppler echocardiography) and clinical course (NYHA status). Patients are classified into group A (n = 56 recipients) with immunohistochemical TGF-β staining score >7 and group B (n = 96 recipients) with a staining score <7. Results: Doppler echocardiographic evaluation demonstrated greater impairment of left ventricular diastolic function in recipients with higher TGF-β staining score. The average mitral deceleration time was 129 ± 6 ms for recipients group A compared to 167 ± 15 ms in group B. While the mean isovolumic relaxation time was 65 ± 8 ms for patients in group A compared with 82 ± 6 ms for recipients in group B (P = 0.0004 and 0.005, respectively). Immunohistochemical scoring correlated inversely with both mitral deceleration and isovolumic relaxation times (r = −0.74, P = 0.0004 and r = −0.66, P = 0.004, respectively). Mean NYHA status was 2.7 ± 1.3 for group A compared to 1.17 ± 0.4 in group B was (P = 0.002). Five years follow-up revealed persistent left ventricular diastolic impairment for recipients with higher immunohistochemical staining score. Mitral deceleration time and isovolumic relaxation time were 118 ± 11 and 62 ± 7 ms for group A compared to 156 ± 12 and 80 ± 5 ms for group B, P = 0.006 and P = 0.01, respectively. The actuarial development of subsequent coronary artery disease (> 50% stenosis) was 17 and 29% for recipients in group A compared to 4 and 6% for recipients in group B at 3 and 5 years follow-up, respectively (P = 0.01 and P = 0.005, respectively). Conclusions: TGF-β expression in cardiac allografts is associated with impaired graft function and limited survival. The pathogenesis of diastolic dysfunction may be an aberrant repair process following rejection due to increased TGF-β expression in transplant recipients. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Transforming growth factor-beta; Heart transplantation; Echocardiography; Left ventricular dysfunction

1. Introduction

Left ventricular diastolic dysfunction has been demonstrated in patients with coronary artery disease with or without systolic dysfunction, hypertension [1], valvular disease[2], cardiomyopathies [1] and a variety of systemic diseases [2]. However, the causes and implications of chronic diastolic left ventricular dysfunction of the human cardiac allograft are not clear. There is an evolving body of evidence that abnormalities of diastolic filling play an important role in the clinical status and prognosis of patients with heart disease [1–3]. Ross et al. [4] reported that impaired left ventricular diastolic function within 6 months of transplant was associated with an increased late mortality.

Cytokines and growth factors have many complex effects on all processes involved in fibrosis and collagen metabolism. Transforming growth factor-beta (TGF-β) is one of a number of cytokines and growth factors, which may influence matrix accumulation. The contribution of TGF-β to the development of impaired diastolic function in heart transplants is undefined. Increased expression of TGF-β in human fibrotic lesions has been previously reported [3]. In solid organ transplantation TGF-β has been implicated in the development of glomerulosclerosis mediated by its dual action of increasing deposition and decreasing degradation of extracellular matrix [5].

The involvement of TGF-β was examined in the present
study, in association with histological and clinical factors, in the generation of impaired left ventricular diastolic function following orthotopic heart transplantation.

2. Methods

2.1. Study population and study design

Between 1987 and 1997, 233 orthotopic heart transplantation were performed at Wythenshawe Hospital. The following patients have been excluded from this analysis:

- Recipients having heart re-transplantation (n = 1 recipient).
- Recipients who survived for less than 24 months after surgery (n = 32 recipients).
- Recipients who developed significant coronary artery disease (>50% stenosis of one of more major coronary artery) before 2 years post transplantation (n = 9 recipients).
- Recipients in who echocardiographic assessment of their left ventricular diastolic function could not be performed due to technical reasons (n = 22 recipients).
- Recipients who had persistent un-recovered impaired left ventricular diastolic function during the first 3 months after transplantation (n = 17 recipients).

One hundred and fifty-two patients survived over 2 years and were eligible to be included in our analysis. The age at transplantation was 45 ± 11 years and 90% were males. The standard technique [6] was performed in 84 recipients and the bicaual [7] procedure in 68 recipients.

Follow-up was complete to October 1999 or to the time of death and ranged from 24 to 60 months.

2.2. Endomyocardial biopsy

Endomyocardial biopsy (EMB) was performed via a percutaneous modified Seldinger right internal jugular approach with a 9F sheath and a Stanford–Caves biop tome using strict aseptic technique. Usually four to five satisfactory specimens were retrieved. All biopsy specimens were evaluated for rejection using International Society of Heart and Lung Transplantation (ISHLT) criteria [8].

2.3. Immunosuppression

Triple-drug immunosuppression with cyclosporin (3.5–5.0 mg/kg per day), azathioprine (1.5–2.5 mg/kg per day) and steroid (0.75–1.0 mg/kg per day) therapy was used in all patients. Cycloytic induction therapy (Antithymocyte globulin 2 mg/kg) was used in every patient during the initial 3 days. Cyclosporin therapy was adjusted to maintain serum trough level of 180–250 ng/ml during the first 2 years following transplantation. Azathioprine dose was adjusted to maintain white blood cell count of >4000/μL. Steroid dose was tapered gradually after the peri-operative induction to 0.125 mg/kg within 2–3 weeks after the operation. Acute rejection was treated with bolus methyl-prednisolone (500 mg daily for 3 days) and follow-up EMB was performed 1–2 weeks later to assess outcome of treatment.

2.4. Transthoracic echocardiography

Doppler echocardiographic studies were analyzed for every recipient. Echocardiographic studies are routinely performed at the same schedule of endomyocardial biopsy. An experienced echocardiographer performed conventional two-dimensional and Doppler echocardiography with patients in the left lateral decubitus position. All studies were performed with a Hewlett Packard Sonos 2000 or 2500 machine equipped with a 2.5 MHz transducer. M-mode and Doppler recordings were made at a sweep speed of 50 or 100 mm/s and studies were recorded on 0.75 ins SVHS videotape. The transmirtal flow velocity profile was recorded from the apical four-chamber view with the pulsed wave sample volume located at the tips of the mitral valve leaflets in diastole. The maximum E and A wave velocity and the time velocity integral of E and A wave were recorded. The time velocity integrals were measured by planimetry of the area under the mitral flow velocity curve. Mitral deceleration time (MDT) was measured as the time from peak E velocity to the point of intercept of the deceleration slope with the baseline. The isovolumic relaxation time (IVRT) represents the interval between aortic valve closure and mitral valve opening was recorded by pulsed wave Doppler evaluation with the sample volume midway between the mitral leaflets and the aortic annulus. Using this technique the timing of aortic closure and mitral opening could be identified on the same Doppler trace.

All studies were analyzed without prior knowledge of their immunohistochemical staining. Echocardiographic studies performed during on going cellular rejection (any grade) or studies performed in recipient who got significant angiographically detected coronary artery disease (>50% stenosis of left main stem, left anterior descending, circumflex coronary arteries) were excluded.

Left ventricular end-systolic and end-diastolic dimensions were recorded in the parasternal long axis view immediately distal to the mitral valve tips from the two-dimensional-guided M-mode image. Left ventricular ejection fraction was estimated qualitatively according to the usual practice in our laboratory. Colour-flow Doppler imaging was used to quantify mitral and tricuspid regurgitation. The left ventricular ejection fraction was calculated from 2-dimensional M-mode measurement, in accordance with the standard (sub method) clinical formula used by transplant Centre, where LVEF = EDV – ESV/E DV, and EDV = EDD3, ESV = ESD3. E/V: end systolic dimension, EDD: end diastolic dimension.

In all study subjects Doppler echocardiographic para-
2.4. Endomyocardial biopsy

A total of 3278 endomyocardial biopsies from the interventricular septum obtained during baseline follow-up and at the time of angiographic studies. For the purpose of this study, a rejection episode was defined as the presence in at least one biopsy of ISHLT grade >0 rejection. Subsequent positive biopsies were considered to be the same rejection episode if not separated by a rejection-free biopsy.

2.5. Right heart catheterization

Right heart catheterization was performed annually with each biopsy using a multipurpose cordis 7F vascular catheter (Cordis, Miami, FL) connected to an AE 840 (Mikro Elektronik A/S) pressure transducer. Intracardiac pressures were recorded at the levels of the right atrium, right ventricular body and pulmonary artery. Pulmonary capillary wedge pressure was also recorded.

2.6. Coronary angiography and left heart catheterization

Each patient underwent annual surveillance coronary angiography during left heart catheterization performed from the femoral approach. The severity of coronary atherosclerosis was assessed in the left main stem coronary artery main coronary vessels (proximal 2/3 of the LAD, Circumflex, dominant right coronary arteries). Left intracardiac pressures were recorded with a fluid-filled pigtail catheter attached to micromanometer transducer.

2.7. Histological examination

A total of 3278 endomyocardial biopsies from the interventricular septum obtained during baseline follow-up and at the time of angiographic studies. For the purpose of this study, a rejection episode was defined as the presence in at least one biopsy of ISHLT grade >0 rejection. Subsequent positive biopsies were considered to be the same rejection episode if not separated by a rejection-free biopsy.

2.7.1. Immunohistochemistry

Only EMB specimens taken at least 2 years after transplantation were selected for immunohistochemical assessment. To avoid the immediate effects of current rejection, specimens taken from recipients in whom the previous biopsy result showed evidence of cellular rejection (any grade other than 0) were excluded from immunohistochemical staining (n = 633 biopsies). Specimens taken from patients requiring additional immunosuppression for any reason were also excluded (n = 163 biopsies).

Paraffin-embedded sections fixed in 4% formaldehyde were dewaxed and rehydrated with Citroclear, alcohol and water for 10 min then treated with 10% proteinase K (Dako) in Tris buffered saline (TBS). Non-specific binding was blocked with 10% normal swine serum (Chemicon International Ltd., Harrow, UK). Mouse anti-human TGF-β antibody was diluted 1/10 in TBS and applied to three of the four sections on each slide whilst the remaining section received only TBS without antibodies. The slides were incubated for 1 h, then washed and stained with a peroxidase-conjugated anti-mouse IgG at 1/1000 dilution (Sigma Immunochemical, USA) for 2 h, after which slides were transferred to fresh diaminobenzidine. The slides were counterstained with Meyer’s Haemalum, dehydrated and mounted with diethyl polystyrene xylene (DPX). To confirm the staining specificity, blocking studies were performed with recombinant TGF-β (R+D Systems, UK) to inhibit binding of anti-TGF-β antibodies. Macrophages were identified in sections using an indirect immunoperoxidase technique with a mouse monoclonal antibody, CD68 (reagent PGM-1, Dako, Bucks., UK).

2.7.2. Immunohistochemistry quantification

We employed the TGF-β scoring system, which was developed, by our laboratory and has been used to assess the immunohistochemical staining in heart and lung transplant recipients [9,10]. The system consists of evaluation of both cellular and fibrous scoring.

2.7.2.1. TGF-β staining assessment. The total scoring for TGF-β and CD + 68 scoring took into account two factors:

1. The absolute number of CD68-positive cells per each power field (0.202 mm²) was quantified with an eyepiece graticule. At least 40 fields were counted using a ×40 objective lens so the percentage standard deviation was less than 2% for at least five fields. The fields covered the entire field of each biopsy and any biopsy without 40 separate fields was excluded.

2. The presence and intensity of positive cellular staining with TGF (see Table 1).

2.7.2.2. Fibrosis assessment. Fibrosis was assessed in sections stained with haematoxylin and eosin. Slides were scanned using a ×10 objective lens. Each successive field was individually assessed for severity (as a percentage) of interstitial myocardial fibrosis (see Table 1). After examining the entire section the mean score of all the fields was taken as the fibrosis score.

By the end of the second post transplant year, each patient had of five to seven biopsies. Each single biopsy stained for:

- Immunohistochemical staining
  - TGF-β
  - CD + 68
- Haematoxylin and eosin.

TGF-β staining assessment was calculated for each separate pieces and then added together to calculate the mean TGF-β score for each single biopsy. Slides were reviewed blindly twice. Similar TGF-β staining score was achieved in 87% of the biopsies. Slides with TGF-β staining or fibrosis scoring differences of more than 1 point were reviewed to reach an agreement regarding the final score of the slides.
2.7.3. Development of TGF-β scoring system and echocardiographic parameters for each patient

Mean TGF-β score and Doppler echocardiographic studies were calculated for each patient starting at the study beginning point at 24 months after the operation. In total 2685 endomyocardial biopsy specimens were stained for the entire study population. Patients were classified according to their degree TGF-β staining score. Echocardiographic assessment was repeated annually to assess the long-term progress of diastolic left ventricular function. In total 587 Doppler echocardiographic assessment during the time target (Patient death or 5 years after transplantation).

2.8. Statistical analysis

All statistical analysis were completed with SPSS software (window 7.5; SPSS, Inc, Chicago, IL). Results are expressed as mean ± standard of deviation. Data between the two groups were compared with unpaired t-test for parametric data and the Mann–Whitney test, or Fisher’s exact test where applicable. Parameters within the same group were compared using Student’s t-test. Some variables required log transformation to achieve approximate normality or constancy or additivity of scale. The regression and correlation analysis was used to compare the value of immunohistochemistry TGF score, and echocardiographic studies. Coronary artery disease was assessed using the Kaplan–Meier method and compared by log-rank test. A P value of less than 0.05 was defined as statistically significant.

3. Results (study beginning point: 2 years after transplantation)

3.1. Immunohistochemical staining score

TGF-β was immunolocalised in the myocardium, interstitium, and macrophages and areas of fibrosis (Fig. 2). Patients have been classified by the end point of this study according to their immunohistochemical staining into:

- Group A (n = 56 patients with immunohistochemical score ≥7).
- Group B (n = 96 with immunohistochemical score < 7).

There was no significant difference between the group A and group B in terms of age, sex, pre-operative trans-
pulmonary gradient, pulmonary vascular resistance, ischemic time, prevalence of the bicaval technique or cardiopulmonary bypass time (Table 2).

3.2. Doppler echocardiographic data

The Doppler echocardiographic data for the two groups is shown in Table 3. E/A ratio was significantly higher in group A compared to group B (Table 3). Mitral deceleration time (MDT) and IVRT were shorter in group A than group B.

Fractional shortening and ejection fraction were equally preserved in both groups (Table 3). No significant differences were demonstrated between the two groups in the prevalence or severity of mitral regurgitation.

3.3. Haemodynamic parameters

The mean arterial blood pressure was not significantly different between the two groups (Table 4). Twenty seven percent of recipients in group A (n = 12 patients) were on anti-hypertensive treatment medication (Calcium channel blocker) compared to 33% of recipients in group 2 (n = 32 patients). Three patients from group A and four patients in group B required more than single antihypertensive agent (P = 0.4). Right ventricular systolic and diastolic pressure, mean pulmonary arterial pressure and pulmonary capillary wedge pressure were statistically higher in group A (Table 4). Mean right atrial pressure was also significantly different between the two groups. Left ventricular end-diastolic pressure was higher for group A. Haemodynamic and echocardiographic variables were significantly different in recipients with the bicaval technique according to their TGF-β staining (Table 5).

3.4. Endomyocardial biopsy

The mean number of cellular rejection episodes was higher in the group A compared to those in group B (Table 6). The TGF-β score correlated with the number of rejection episodes of ISHLT during the first 2 years after transplantation.

TGF-β was strongly expressed in the EMB from patients with a restrictive filling pattern in group A in comparison to those in group B (Fig. 1, Table 4). Higher TGF-β expression

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A: TGF-β ≥ 7 (n = 56)</th>
<th>Group B: TGF-β &lt; 7 (n = 96)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate /min</td>
<td>109 ± 13</td>
<td>91 ± 6</td>
<td>0.04</td>
</tr>
<tr>
<td>Right atrial pressure (mmHg)</td>
<td>16 ± 3</td>
<td>10 ± 2</td>
<td>0.03</td>
</tr>
<tr>
<td>Right ventricle end diastolic pressure (mmHg)</td>
<td>12 ± 6</td>
<td>8 ± 3</td>
<td>0.02</td>
</tr>
<tr>
<td>Pulmonary capillary wedge pressure (mmHg)</td>
<td>16 ± 3</td>
<td>11 ± 3</td>
<td>0.02</td>
</tr>
<tr>
<td>Left ventricular end diastolic pressure (mmHg)</td>
<td>16.5 ± 2.9</td>
<td>10.2 ± 2.6</td>
<td>0.03</td>
</tr>
<tr>
<td>Mean pulmonary artery pressure (mmHg)</td>
<td>25 ± 3.1</td>
<td>19 ± 4.0</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>99 ± 25</td>
<td>105 ± 19</td>
<td>0.5</td>
</tr>
<tr>
<td>Strokl Volume (ml/beat)</td>
<td>55 ± 18</td>
<td>66 ± 21</td>
<td>0.09</td>
</tr>
<tr>
<td>Cardiac index (l/min per m²)</td>
<td>3 ± 1.1</td>
<td>3.2 ± 1.1</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Table 4
Haemodynamic variables in patients grouped according to immunohistochemical TGF-β staining score (2 years after transplant)
was sustained in patients who developed a restrictive filling compared to patients who did not, and those who did not develop a restrictive filling pattern maintained a lower TGF-β expression over the same time span. (Table 4).

3.5. Correlation between restrictive filling pattern and TGF-β scoring

Increased TGF-β positive staining inversely correlates with MDT \( (r = -0.074, P = 0.0004) \) and IVRT \( (r = -0.66, P = 0.004) \) and positively correlates with E:A ratio \( (r = 0.66, P = 0.006) \). Increased TGF-β staining was also associated with both increased left ventricular end-diastolic pressure and pulmonary capillary wedge pressure \( (r = 0.47, P = 0.006, r = 0.41, P = 0.01) \).

3.6. Clinical status at 2 years after transplantation

Mean NYHA status for patients in group A was 2.7 ± 1.3 compared to 1.17 ± 0.4 for patients in group B \( (P = 0.002) \). Of the 56 patients in group A, 33/56 were in NYHA class III or IV or required anti-failure treatment compared with only 17/96 in group B (Table 6).

3.7. Follow-up (2–5 years after transplantation)

Restrictive filling pattern was persistent in recipients at group A and statistically significant compared to those at group B up to 5 years follow-up (Fig. 2). At the end of the study period, MDT was 118 ± 11 ms for recipients in group A compared to 156 ± 12 ms for those in group B \( (P = 0.006) \). Similar difference was noticed in measuring of the IVRT was 62 ± 7 ms for recipients in group A compared to 80 ± 5 ms for those in group B. The actuarial development of subsequent coronary artery disease (>50% stenosis) in one or more of the major coronary artery vessel was 17 and 29% for recipients in group A compared to 4 and 6% for recipients in group B at 3 and 5 years follow-up, respectively \( (P = 0.01 \) and \( P = 0.005 \), respectively).

4. Discussion

4.1. Relevance of left ventricular diastolic dysfunction after heart transplantation

The transplanted heart is affected by many factors that may alter left ventricular dynamics and adversely affect diastolic function. Acute diastolic dysfunction in the allograft is associated with rejection and is to be reversed by adequate anti-rejection therapy [11]. Interstitial myocardial fibrosis and stiffness of the left ventricle have been claimed to be the main pathophysiological features in chronic left ventricular diastolic dysfunction [12]. The mechanism of chronic left ventricular diastolic dysfunction in the non-rejecting allograft has not been fully studied.

Left ventricular diastolic dysfunction has been demonstrated in patients with coronary artery disease with or without systolic dysfunction [1], valvular disease [2], cardiomyopathies [2] and a variety of systemic diseases [3]. However, the causes and implications of chronic diastolic left ventricular dysfunction of the human cardiac allograft are not clear. There is an evolving body of evidence that abnormalities of diastolic filling play an important role in the clinical status and prognosis of patients with heart disease [10–12]. Ross et al. [4] reported that impaired left

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Table 5

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A: TGF-β≥7 (n = 24)</th>
<th>Group B: TGF-β &lt; 7 (n = 46)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitral Deceleration Time (ms)</td>
<td>131 ± 4</td>
<td>162 ± 9</td>
<td>0.0008</td>
</tr>
<tr>
<td>Isovolumic relaxation time (ms)</td>
<td>68 ± 3</td>
<td>83 ± 4</td>
<td>0.01</td>
</tr>
<tr>
<td>E-wave velocity (ms)</td>
<td>84 ± 5</td>
<td>64 ± 5</td>
<td>0.007</td>
</tr>
<tr>
<td>A wave velocity (ms)</td>
<td>41 ± 2</td>
<td>53 ± 6</td>
<td>0.03</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>16 ± 2</td>
<td>10 ± 2</td>
<td>0.03</td>
</tr>
<tr>
<td>LV ED (mmHg)</td>
<td>15 ± 3</td>
<td>9 ± 2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

- PCWP, pulmonary capillary wedge pressure; LVED, left ventricular end diastolic pressure.

Table 6

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A: TGF-β (n = 56)</th>
<th>Group B: TGF-β (n = 96)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean TGF-β score</td>
<td>8.4 ± 1.2</td>
<td>4.1 ± 0.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Number of rejection</td>
<td>5.7 ± 1.9</td>
<td>2.6 ± 1.1</td>
<td>0.002</td>
</tr>
<tr>
<td>NYHA Class I</td>
<td>27% (16/58)</td>
<td>62% (60/96)</td>
<td>0.001</td>
</tr>
<tr>
<td>NYHA Class II</td>
<td>12% (7/56)</td>
<td>21% (19/96)</td>
<td>0.09</td>
</tr>
<tr>
<td>NYHA Class III</td>
<td>33% (18/56)</td>
<td>11% (11/96)</td>
<td>0.02</td>
</tr>
<tr>
<td>NYHA Class IV</td>
<td>28% (15/56)</td>
<td>6% (6/96)</td>
<td>0.006</td>
</tr>
</tbody>
</table>
ventricular diastolic function within 6 months of transplant was associated with an increased late mortality.

4.2. Pathophysiology of left ventricular diastolic dysfunction in heart transplant recipient

Stinson et al. [13] found a persistent elevation left ventricular end-diastolic pressure on exercise in patients studied 1 year after heart transplantation. Theoretically, particularly in view of the fact that myocardial dysfunction during rejection is at least in part due to leukocyte cytokines which are released acutely [14], recovery of function may be expected in these circumstances. Valentine et al. [15], however, have shown that this recovery may often be incomplete with the development of a chronic pattern of restrictive physiology characterized by an elevation of left ventricular end-diastolic pressure. This pattern is characterised by myocytes loss and fibrous replacement. The healing response may bring about an irreversible progressive decline in compliance and leading to chronically deranged diastolic function. Skowronski et al. [16] noticed that increased chamber stiffness following acute rejection remained abnormal despite resolution of histological abnormalities. Diastolic function abnormalities in the cardiac allograft need not relate solely to those caused

Fig. 1. (A) TGF-β staining of the myocardial biopsy. (B) CD + 68 macrophage staining of the myocardial biopsy.
directly by rejection. Very significant abnormalities may occur at various stages following transplantation and are attributable to factors as disparate as perioperative ischaemia, reperfusion injury and hypertension. Drug therapy with cyclosporin may contribute in some patients and, in-fact, it has been suggested that the mechanism of diastolic dysfunction caused by cyclosporin [17] may be mediated directly by myocardial fibrosis. Because some patients with chronic left ventricular dysfunction appear to develop clinically significant heart failure, it is important that the aetiology and pathology of diastolic abnormalities be explored. An early report [17] of myocardial fibrosis occurring in association with cyclosporin treatment prompted speculation that cyclosporin may contribute to diastolic abnormalities of the cardiac allograft. More recently, however, Greenberg [18] failed to confirm an association between myocardial fibrosis and the use of cyclosporin. In our study, we were unable to confirm a direct correlation between cyclosporin and impaired left ventricular function as the patients in both groups were on long term treatment of cyclosporin. However, as group A recipients have been suffering from more frequent rejection episodes, it is logical to conclude that the level of cyclosporin was needed to be higher than average during these rejection episodes.

4.3. The potential role of TGF-β

TGF-β modulates a number of crucial events potentially central to the genesis and maintenance of chronic graft injury. These include macrophage chemotaxis, suppression of lymphocyte function, fibroblast chemotaxis and proliferation in addition to the modulation of collagen synthesis [19]. TGF-β is also a strong stimulator of extracellular matrix synthesis [20]. Many different cells can synthesise and release TGF-β, including activated macrophages, lymphocytes and platelets [19,20]. Previous reports have confirmed increased expression of TGF-β in fibrotic lesions of human allografts [5].

4.4. Current study

We have demonstrated that TGF-β expression in the cardiac allograft is associated with impaired graft function implicating this cytokine as an important substrate for the development of diastolic dysfunction. In accordance with previous studies [21,22], we have documented an association between impaired left ventricular diastolic dysfunction of the human cardiac allograft and rejection incidence. These data suggest that cumulative immune mediated injury could be responsible for triggering and pathogenesis of left ventricular dysfunction.

The haemodynamic data in the present study are consistent with the Doppler echocardiographic findings. The relatively short isovolumic relaxation time in group A was paralleled by higher pulmonary capillary wedge pressures than in group B indicating a reduction in myocardial compliance characteristic of fibrosis. This fibrosis is a recognized consequence of several possible insults to the heart, of which recurrent inflammation is one. Thus increased fibrosis scores in patients with impaired left ventricular diastolic function may be reasonably attributed to higher rejection incidence in this group. Acute cardiac rejection is characterized by myocardial mononuclear cell infiltration and oedema in the perivascular and interstitial tissues [7,16]. These lead to temporary increased stiffness of the myocardium and changes in left ventricular filling properties. With more frequent rejection episodes, the amount and activity of mononuclear cell in the myocardium increases and is associated with an increasing cytokine production by these cells. Both cytokines and markers of activated cytotoxic T cells have been associated with diastolic dysfunction assessed by Doppler echocardiography.
The level of TGF-β expression in the EMB was the most potent predictor of diastolic dysfunction. The number of ISHLT grade rejection episodes correlated significantly with higher myocardial TGF-β deposition and the development of diastolic dysfunction. These findings suggest that frequent cellular rejection episodes during the first 2 post-transplant years predisposed to higher TGF-β production initiate a series of inflammatory and immunological responses characterized by over-expression of TGF-β. This culminates in myocardial fibrosis and subsequent impairment of left ventricular diastolic function.

4.5. Limitations

Our study was limited by the fact that the study population was analyzed retrospectively and restricted to recipients surviving for at least 2 years after transplantation who had undergone coronary angiography. We were unable, therefore, to provide information about the very early development of diastolic dysfunction. The rationale for this approach was based on the requirement to avoid the temporary influence of acute allograft rejection on the echocardiographic assessment, particularly given the poor reliability of immunohistochemical staining during rejection. By including no recipient in whom echocardiographic evaluation was performed during or within 6 months of an acute rejection episode and excluding all recipients with significant coronary artery disease our study has not suffered from their potential confounding effects on ventricular filling. The study is also limited by the fact that two different techniques have been used for heart transplantation, which may influence the accuracy of the haemodynamic and echocardiographic variables. However, the difference in the percentage of the bicaval technique recipients in each group was statistically insignificant. The haemodynamic variables of the left ventricular filling pressures and echocardiographic assessment was different between the bicaval technique recipients according to their TGF staining score. In addition, previous studies from our institution [23] and others [24] have not suggested any differences in the diastolic left ventricular function between the bicaval and standard technique heart transplant recipients.

4.6. Conclusion

This study is the first to highlight the potential role of TGF-β in the development of left ventricular diastolic dysfunction for long term survivors after heart transplantation. Increased understanding of the pathophysiology of diastolic dysfunction and the role of cytokines in its development will allow a clearer definition of recipients at high risk. We suggest that dealing with impaired left ventricular function in transplanted heart can be considered in stepwise fashion. First, individual identified by their echocardiographic pattern. Second, this high-risk group to be targeted for intra-coronary vascular ultra-sound even in the absence of angiographic evidence of coronary artery disease. Third, modification of the biopsy regime and immunosuppression may be important for these patients. Finally, we suggest that immunological strategies to manipulate cytokine expression in cardiac allografts may improve their function and survival.

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References


