Effect of adopting a new histological grading system of acute rejection after heart transplantation

Aggie H Balk, Pieter E Zondervan, Peter van der Meer, Teun van Gelder, Bas Mochtar, Maarten L Simoons, Willem Weimar

Abstract

Background—Treatment policy of acute rejection after heart transplantation has been changed after adopting the ISHLT endomyocardial biopsy grading system in 1991.

Objective—To determine the effect of this policy change on clinical outcome after transplantation.

Methods—The outcome of 147 patients who had a transplant before (early group, median follow up 96 months) and 114 patients who had a transplant after (late group, median follow up 41 months) the introduction of the ISHLT biopsy grading system was studied retrospectively. Initially “moderate rejection” according toBillingham’s conventional criteria was treated. From January 1991 grade 3A and higher was considered to require intensification of immunosuppression.

Results—There were some differences between the two groups: recipients (50 v 44 years) as well as donors (28 v 24 years) were older in the “late group” and more patients of this group received early anti-T cell prophylaxis (92% v 56%). Despite more extensive use of early prophylaxis more rejection episodes were diagnosed (2.4 v 1.4) and considerably more courses of rejection treatment were instituted in the late compared with the early group (3.2 v 1.5). There were no deaths because of rejection in the late group, however, more infections occurred within the first year (mean 1.8 v 1.4) and more non-skin malignancies within the first 41 months were diagnosed (8 of 57 v 6 of 147, 95% CIs of difference includes 0). The incidence of graft vascular disease in the late group has been comparable to the early group until now.

Conclusion—The interpretation of the ISHLT grading system resulted in lowering of the threshold for the diagnosis of rejection thereby increasing the number of rejections and subsequently the immunosuppressive load and its complications.

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Keywords: transplantation; biopsy grading system; rejection

The main challenge to transplant cardiologists is to keep the delicate balance between inhibition of rejection, the destructive immune response directed to the “non-self” organ, and prevention of immunosuppression related side effects.

Histological examination of endomyocardial biopsy (EMB) still is the most reliable method to detect and monitor rejection after heart transplantation. In an attempt to provide conformity in multicentre trials and publications a new, international, grading system for the histological diagnosis of acute rejection on EMB was formulated and published by a working group of the International Society for Heart and Lung Transplantation (ISHLT) in 1990.1 We adopted the ISHLT grading system and, like many others, subsequently elected to treat rejection grades 3A and higher. Our interpretation of the ISHLT system produced a shift of the threshold to intensification of immunosuppression. Before 1991 rejection treatment was instituted in cases of “moderate rejection” or more. For this we used Billingham’s conventional criteria (class 2 or higher) for which, besides interstitial mononuclear infiltrates, at least presence of hypereosinophilic myocytes with a pycnotic nucleus (myocytolysis), was considered a condition sine qua non.2 The shift of the threshold for treating rejection was caused by accepting at least the coexistence of mononuclear infiltrates with more than one focus of myocyte damage (ISHLT 3A and higher) rather than with myocytolysis/necrosis as the indication for rejection treatment.

The purpose of this study was to determine the effect of this change in policy on clinical outcome after cardiac transplantation.

Methods

We conducted a historical comparison of two groups of heart transplant patients in whom the surgical procedure and maintenance immunosuppression were identical.

STUDY POPULATION

The study population consists of all consecutive patients who received a cardiac allograft in our institution between June 1984 and September 1995. In the early phase of the programme the upper age limit for cardiac transplant candidates was 56 years. This upper limit was abandoned early in 1990. Patients with a creatinine clearance less than 30 ml/min, patients with diabetes mellitus requiring insulin, patients with clinical signs of vascular
disease outside the heart, and patients with malignancies within the past 10 years have been denied transplantation during the study period.

REJECTION SURVEILLANCE, PREVENTION, AND TREATMENT

Diagnosis of rejection was based on histological assessment of serial EMB. Biopsies were performed according to the following schedule: weekly for the first six weeks after transplantation, biweekly until three months, monthly until six months, and every eight weeks for the rest of the first year. After the first year, two to three EMB specimens per year were taken. The routine EMB schedule had been approved by the medical ethical committee of the University Hospital Rotterdam and allograft recipients had given informed consent. Each EMB specimen consisted of a minimum of four individual samples. Processing of the biopsy samples consisted of fixation in 10% neutral buffered formalin followed by embedding in paraffin wax and staining with haematoxylin and eosin.

Initially, moderate rejection or more (class 2 or higher) according to Bingham’s early criteria was considered to require treatment: treatment was instituted when interstitial infiltrates at least were accompanied by myocyteolysis (hyperesinophilic myocytes with a pyknotic nucleus), diagnosed on light microscopy (fig 1). Increased immunosuppression was given independent of the haemodynamic consequences of rejection. From January 1991 the presence of myocyte damage instead of myocyteolysis/necrosis was considered necessary for the diagnosis of acute rejection and the line between treatment/no treatment was drawn between ISHLT grades 2 and 3A: myocyte damage was left untreated when seen within only one focus of interstitial infiltrate (grade 2) as has been shown warranted later on by others. Immunosuppression however was intensified from grade 3A, when at least encroachment or damage of the myocytes was visible within more foci of interstitial infiltrates (fig 2). During the whole study period EMB specimens were graded by the same pathologists in close cooperation with the same transplant cardiologists.

Maintenance immunosuppression consisted of cyclosporine and low dose corticosteroids. Azathioprine was added only in cases of recurrent rejection (more than three episodes in the first year) and in patients who developed cyclosporine related severe renal failure. Different regimens of early postoperative immunosuppression have been used and evaluated in randomised trials (OKT3, 5 mg for seven days; horse-ATG for seven days; rabbit-ATG one dose of 8 mg/kg; BT563, an anti-interleukin 2 monoclonal antibody, 10 mg for seven days). Rejection was treated by methylprednisolone 1 g intravenously on three consecutive days and polyclonal (rabbit-ATG) or monoclonal (OKT3) anti-T cell antibodies in cases of persisting or frequently recurring rejection.

INFECTION

Infection was defined as a symptomatic infectious episode with concurrent demonstration of the causative agent by culture or changes in serological status. Superficial, spontaneously healing, herpes simplex I lesions of the oral mucosa, and pityriasis versicolor have been excluded from the analysis. Prevention of oral candidiasis was by local application of amphotericin-B solution during the first three weeks after transplantation. Prevention of bacterial infection was by eradication of infection preoperatively and the use of intranasal mupirocin ointment and intravenous cephalosporins perioperatively. Cytomegalovirus (CMV) seronegative recipients received blood from CMV seronegative donors and were treated prophylactically with anti-CMV hyperimmunoglobulin (Cytotect, Biotest Pharma GmbH, Dreieich, Germany) in case of a CMV seropositive donor. CMV infection was defined as any appearance of immunoglobulin M, isolation of CMV from urine, throat or blood or demonstration of the immediate early antigen or pp65 antigen. CMV disease was diagnosed when infection coexisted with two of
**Table 1** Characteristics of the early (before 1991) and late (after 1991) transplant patients

<table>
<thead>
<tr>
<th></th>
<th>Early (n=147)</th>
<th>Late (n=114)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong> (male/female)</td>
<td>129/18</td>
<td>90/24</td>
</tr>
<tr>
<td><strong>Underlying heart disease</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>70</td>
<td>42</td>
</tr>
<tr>
<td>Ischaemic heart disease</td>
<td>71</td>
<td>66</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td><strong>Recipient age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>44 (11)</td>
<td>50 (10)*</td>
</tr>
<tr>
<td>Range</td>
<td>12–55</td>
<td>14–65</td>
</tr>
<tr>
<td><strong>Donor age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>24 (72)</td>
<td>28 (10)†</td>
</tr>
<tr>
<td>Range</td>
<td>11–35</td>
<td>12–45</td>
</tr>
<tr>
<td><strong>Donor/recipient sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Same sex</td>
<td>92</td>
<td>70</td>
</tr>
<tr>
<td>Female/male</td>
<td>42</td>
<td>28</td>
</tr>
<tr>
<td>Male/female</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td><strong>Blood group O</strong></td>
<td>52</td>
<td>51</td>
</tr>
<tr>
<td><strong>CMV mismatch (donor +ve/recipient –ve)</strong></td>
<td>29</td>
<td>18</td>
</tr>
<tr>
<td><strong>Mismatch</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-A+B (mean (SD))</td>
<td>2.9 (0.9)</td>
<td>2.9 (0.9)</td>
</tr>
<tr>
<td>HLA-DR (mean (SD))</td>
<td>1.4 (0.6)</td>
<td>1.4 (0.7)</td>
</tr>
<tr>
<td><strong>Anti-T cell prophylaxis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OKT3</td>
<td>83</td>
<td>105§</td>
</tr>
<tr>
<td>Horse-ATG</td>
<td>53</td>
<td>44</td>
</tr>
<tr>
<td>Rabbit-ATG</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Anti-interleukin 2 receptor</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

95% confidence intervals: * −9 to −4; † −6 to −2; ‡ −0.5 to −0.3.

**Table 2** Clinical outcome of the early (before 1991) and late (after 1991) transplant patients

<table>
<thead>
<tr>
<th></th>
<th>Early (n=147)</th>
<th>Late (n=114)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow up (months)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>96</td>
<td>41</td>
</tr>
<tr>
<td>Range</td>
<td>69–146</td>
<td>12–69</td>
</tr>
<tr>
<td><strong>Acute rejection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean number of episodes (SD)</td>
<td>1.4 (1.3)</td>
<td>2.4 (1.7)*</td>
</tr>
<tr>
<td>Patients with 0 episodes</td>
<td>38</td>
<td>14</td>
</tr>
<tr>
<td>Patients with &gt; 2 episodes</td>
<td>25</td>
<td>67</td>
</tr>
<tr>
<td>Mean courses of treatment (SD)</td>
<td>1.5 (1.5)</td>
<td>3.2 (2.3)*</td>
</tr>
<tr>
<td><strong>Mean number of infections within first year</strong></td>
<td>1.4 (1.3)</td>
<td>1.8 (1.6)‡</td>
</tr>
<tr>
<td>Overall mean number of infections (SD)</td>
<td>3.5 (2.8)</td>
<td>2.9 (2.4)‡</td>
</tr>
<tr>
<td>Number with CMV disease</td>
<td>24</td>
<td>18</td>
</tr>
<tr>
<td>Number with non-skin malignancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–41 months</td>
<td>6/147</td>
<td>8/57§</td>
</tr>
<tr>
<td>&gt; 41 months</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Number with skin malignancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–41 months</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>&gt; 41 months</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td><strong>Graft vascular disease</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 12 months</td>
<td>5/147</td>
<td>2/106</td>
</tr>
<tr>
<td>≥ 12 months</td>
<td>21/147</td>
<td>6/57</td>
</tr>
<tr>
<td>Mean LVEF at 1 year (SD)</td>
<td>65 (11)%</td>
<td>65 (11)%</td>
</tr>
</tbody>
</table>

Fifty seven patients had follow up of at least 41 months.

95% confidence intervals: * −1.4 to −0.6; † –2.2 to −1.3; ‡ −0.8 to −0.1; § −0.2 to −0.004.

LVEF, left ventricular ejection fraction.

the following: fever of more than 38°C for more than two days, organ involvement or leucocytopenia or thrombocytopenia. Recipients who were seronegative for *Toxoplasma gondii* and received the heart of a seropositive donor were treated prophyactically with spiramycin in the early phase of the programme and with pyrimethamine later on.\(^9\)

**GRAFT VASCULAR DISEASE**

The occurrence of graft vascular disease was monitored by coronary arteriography.\(^10\) Initially, arteriography was performed annually. After analysis of sequential arteriograms of the first 119 one year survivors however, second and third year coronary arteriography was omitted in patients who had shown smooth epicardial branches on their first year arteriogram.\(^11\) Subsequently arteriography was performed each year or every two or three years depending on earlier findings. Graft vascular disease was considered present in cases of localised lesions (anything more than wall irregularities), tapering of branches or abrupt ending or disappearance of secondary or tertiary branches, or all three.

All heart transplant recipients have been followed up by transplant cardiologists of the Thoraxcenter, Rotterdam.

**STATISTICAL ANALYSIS**

Data are presented as mean (SD) values, medians or absolute numbers when appropriate. Group comparisons were performed using 95% confidence intervals analysis.\(^12\) The Kaplan-Meier method was used for analysis of survival rates.

**Results**

One hundred and forty seven patients received 148 cardiac allografts before the introduction of the ISHLT biopsy grading system and the change of our criteria for treating acute rejection. One patient underwent retransplantation after eight months and has been assessed as two separate cases. One patient died during the operation, the 147 patients who survived the operation constitute the early group. From January 1991 to October 1995, 119 consecutive patients were operated upon, 114 of whom survived the operation (late group). Table 1 shows the demographic and clinical characteristics. Because the incidence of acute rejection after the first year is very low and may be influenced by the low frequency of EMB the presented data concern the first year after transplantation only.

The late group differed slightly from the early group: recipients as well as donors were older and more patients received anti-T cell induction treatment. Inherent to a historical comparison, the follow up of the patients of the early group was shorter than of the patients of the early group (table 2). Despite the fact that 92% of the patients received early anti-T cell prophylaxis more rejection episodes were diagnosed in the late group, which resulted in considerably more courses of rejection treatment. During seven rejection episodes (in seven patients) of the early group the patient was haemodynamically compromised versus four episodes (in four patients) of the late group.

The increase in rejections diagnosed and treated in the late group was accompanied by more infections within the first year. CMV disease however did not occur more frequently.

The incidence of infection within the total follow up period was comparable in both groups despite a much longer follow up period in the early group.

The incidence of non-skin malignancy was higher in the group with the longer follow up compared with the late group: 12% versus 7%. When considering a 41 month follow up period (the median follow up duration of the late group) there were more non-skin malignancies in the late group. However, the difference in survival free from malignancy between the early and late groups was not statistically significant. The rates at one, three, and five

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*Histological grading system of acute rejection after heart transplantation*

605

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years were: 90%, 89%, and 82% versus 85%, 82%, and 76%, respectively. Skin malignancies occurred only in the group with the longest follow up.

The incidence of graft vascular disease, diagnosed by coronary arteriography (or by necropsy if no recent arteriogram was available) was not lower in the patients of the late group despite a higher overall immunosuppressive load.

In contrast with the early group, there were no deaths caused by acute rejection in the late group. Instead, however, three deaths were caused by infection (table 3).

Survival rates of the total study population were 90%, 85%, 81%, and 66% after one, three, five, and eight years, respectively. To date there are no differences in the survival rates between the patients who had a transplant before and after January 1991.

Discussion
To some extent the value of this study is limited by its retrospective nature and by the fact that two non-contemporaneous groups differing in early prophylaxis have been compared. Notwithstanding that we chose to report our findings because we consider them of interest for transplant cardiologists and pathologists. In our hands the ISHLT grading system lowered the threshold for the diagnosis of acute rejection, which cannot have been the intention of the working group. A lower threshold for the diagnosis of acute rejection resulted in a higher number of treatments for acute rejection and a decreased mortality by acute rejection on the one hand but in increased immunosuppressive load and hence its complications on the other hand.

Aiming at a lower incidence of rejection we used anti-T cell prophylaxis in several subsequent randomised trials thereby increasing the immunosuppressive load especially in most of the patients having a transplant after 1990.4,4

The immunosuppressive load of the heart transplant recipients, however, was determined mainly by courses of rejection treatment. So, lowering of the threshold for the diagnosis of rejection had considerable effects on the total immunosuppressive burden.

Myocytolysis, diagnosed on light microscopy, as a criterion for acute rejection needing additional immunosuppression has been questioned.13 Using this criterion however and considering the presence of myocytolysis a conditio sine qua non for instituting rejection treatment, the outcome of patients operated on in the early phase of our programme was excellent with 90%, 82%, and 67%, one, five, and eight year survival rates, respectively. Although much more interstitial infiltrates were accepted without treatment (when no myocytolysis/necrosis could be identified) before 1991 left ventricular function was equally good at the end of the first year compared with the function in the patients operated after 1990. In addition, left ventricular function remained good during the first five years in the patients of the early group.11 In our hands, rejection requiring treatment was diagnosed considerably more often using the presence of myocyte damage instead of myocytolysis/necrosis as a criterion. Our interpretation of the ISHLT system must have been wrong because the ISHLT system was, as far as we know, not meant to lower the threshold for the diagnosis of acute rejection. We therefore believe that such ambiguity of guidelines aiming at conformity requires revision of the system. Furthermore, for comparison of results in multicentre trials the use of a core laboratory seems mandatory.

Other centres also questioned the usefulness of the first version of the ISHLT system.11 We now question whether we have been right to change our policy after the adoption of a new grading system. The data presented here at least do not show favourable effects: fewer deaths by rejection are compensated by a higher morbidity because of infection and a tendency to more cases of malignancy.

The absence of an increased incidence of CMV disease may be explained by the use of anti-CMV hyperimmunoglobulin in CMV seronegative patients who received the heart of a seropositive donor. Longer follow up of the patients who received a transplant after 1990 will show whether death caused by malignancy in this group ultimately will surpass death by malignancy in the early group. Only a reduction of death caused by graft vascular disease eventually could compensate for that. The data up to now have encouraged us to reconsider our criteria for rejection requiring treatment.

Table 3 Causes of death in the patients of the early (before 1991) and the late (after 1991) groups

<table>
<thead>
<tr>
<th></th>
<th>Early (n=147)</th>
<th>Late (n=114)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of deaths</td>
<td>48</td>
<td>16</td>
</tr>
<tr>
<td>Death in first 12 months</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Right ventricle failure</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Acute rejection</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Surgical complications</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Infection</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Malignancy</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Graft versus host disease</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Vascular disease</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Death between 12 and 41 months</td>
<td>5</td>
<td>6*</td>
</tr>
<tr>
<td>Infection</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Malignancy</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Vascular disease</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Unexplained graft failure</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Death after 41 months</td>
<td>29</td>
<td>1*</td>
</tr>
<tr>
<td>Infection</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Malignancy</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Graft versus host disease</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Multiorgan failure</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Vascular disease</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

* Of 57 patients with follow up of at least 41 months.

IMAGES IN CARDIOLOGY

The ductus venosus

Images of the ductus venosus obtained from a normal human fetus at 20 weeks of gestation. (A) and (B) Longitudinal fetal echocardiographic sections showing the ductus venosus connecting the umbilical vein to the inferior vena cava/right atrial junction. Note the decrease in diameter of the ductus venosus compared to that of the umbilical vein (A), and the acceleration of flow within its lumen demonstrated by aliasing on colour flow mapping (B). (C) Pulsed wave Doppler signal in the ductus venosus showing a characteristic continuous high velocity pattern (0.6 m/s) compared to 0.2 m/s normally detected in the umbilical vein. (Ant, anterior; Post, posterior; UV, umbilical vein; DV, ductus venosus; HV, hepatic vein; SVC, superior vena cava; Ao, aorta; RA, right atrium.)

J S CARVALHO
CASE STUDY

Dystrophin gene abnormalities in two patients with idiopathic dilated cardiomyopathy

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Abstract

Two new cases of dilated cardiomyopathy (DC) caused by dystrophinopathy are reported. One patient, a 24 year old man, had a family history of X linked DC, while the other, a 52 year old man, had sporadic disease. Each had abnormal dystrophin immunostaining in muscle or cardiac biopsy specimens, but neither had muscle weakness. Serum creatine kinase activity was raised only in the patient with familial disease. Analysis of dystrophin gene mutations showed a deletion of exons 48–49 in the patient with familial DC and of exons 49–51 in the other. Dystrophin transcription in cardiac tissue from the patient with sporadic disease showed abundant expression, predominantly of the muscle isoform. This study, together with previous reports, suggests that some patients with DC have a dystrophinopathy that can be diagnosed using a combination of biochemical and genetic analyses.

Keywords: dilated cardiomyopathy; dystrophin; Becker muscular dystrophy

Cardiac involvement is an integral part of DMD and BMD. In rare instances, however, patients can suffer from dilated cardiomyopathy (DC) as the only manifestation of a dystrophinopathy. This has been now recognised in families with typical X linked DC and in patients with sporadic disease. Unlike patients with DMD or BMD, these patients did not have symptoms of muscle weakness and the only sign of neuromuscular involvement was raised serum creatine kinase (CK) activity. Several patients had mutations clustered in the 5’ end of the gene, a region not affected by mutations usually found in DMD and BMD. DC has also been reported in asymptomatic female carriers of DMD and BMD.

Two cases of familial (X linked) and sporadic DC secondary to a dystrophinopathy are reported here.

Patients

The two patients described in this study originate from Italy. Ethical approval was obtained from the committees of the two hospitals involved in their assessment. Informed consent was obtained from each patient.

PATIENT 1

The first patient, a 24 year old man, was admitted in August 1991 after the recent onset of dyspnoea with mild physical activity. DC was diagnosed (left ventricular end diastolic diameter 73 mm; left ventricular end diastolic volume 107 ml/m²; left ventricular ejection fraction 27%) after exclusion of active myocarditis by endomyocardial biopsy and coronary artery disease by angiography. An electrocardiogram showed Q waves in the inferior and posterior leads and incomplete right bundle branch block. Sustained ventricular arrhythmias were detected after 24 hour Holter monitoring. Among the biochemical investigations, constantly raised serum CK activity (MM isoform, ranging between 540 and 867 U/l, normal < 200 U/l) was found. Neurological investigation failed to show any weakness or muscle wasting or hypertrophy. The patient denied symptoms of neuromuscular involvement such as cramps or myalgias associated

Duchenne’s (DMD) and Becker’s muscular dystrophies (BMD) are allelic X linked neuromuscular disorders. They result from mutations in the dystrophin gene that, when severe as in DMD (nonsense or out of frame mutations), lead to lack of expression of the sarcolemmal protein dystrophin. Up to one third of all cases of DMD and BMD arise from de novo mutations, so that a significant proportion of affected males have no family history of the condition. The absence of dystrophin in DMD causes severe and progressive muscle weakness, loss of ambulation before the age of 13, and death in the late teens or early twenties. BMD is a milder form of muscular dystrophy in which individuals are ambulant after the age of 16. These individuals usually have in frame deletions and residual expression of a partially functional dystrophin in the muscle.

Up to one third of all cases of DMD and BMD arise from de novo mutations, so that a significant proportion of affected males have no family history of the condition. The absence of dystrophin in DMD causes severe and progressive muscle weakness, loss of ambulation before the age of 13, and death in the late teens or early twenties. BMD is a milder form of muscular dystrophy in which individuals are ambulant after the age of 16. These individuals usually have in frame deletions and residual expression of a partially functional dystrophin in the muscle. Duchenne’s (DMD) and Becker’s muscular dystrophies (BMD) are allelic X linked neuromuscular disorders. They result from mutations in the dystrophin gene that, when severe as in DMD (nonsense or out of frame mutations), lead to lack of expression of the sarcolemmal protein dystrophin. Up to one third of all cases of DMD and BMD arise from de novo mutations, so that a significant proportion of affected males have no family history of the condition. The absence of dystrophin in DMD causes severe and progressive muscle weakness, loss of ambulation before the age of 13, and death in the late teens or early twenties. BMD is a milder form of muscular dystrophy in which individuals are ambulant after the age of 16. These individuals usually have in frame deletions and residual expression of a partially functional dystrophin in the muscle.

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Dystrophin in dilated cardiomyopathy

PATIENT 1

The second patient, a 52 year old man, died shortly after cardiac transplantation in 1994. He was employed in the chemical industry and was physically active until the age of 50 when he presented with the first symptoms of cardiac failure. There was no family history of cardiovascular disease. The patient smoked 20 cigarettes daily and admitted moderate alcohol intake until the age of 50, when he developed a gastric peptic ulcer that eventually required surgical excision. On examination, aged 52, he was dyspnoeic at rest and a systolic murmur was present over the mitral valve. There was an additional third tone, but no raised jugular venous pressure or peripheral oedema. His liver was enlarged and neurological examination failed to show muscle atrophy or pseudohypertrophy. His muscle strength was normal, as was serum CK activity (84 IU). Negative T waves in leads V5–V6 were seen on the electrocardiogram. Echocardiography showed a dilated left ventricle (left ventricular end diastolic diameter 70 mm, left ventricular ejection fraction 20%). The right ventricle and both atria were also dilated. Moderate mitral and tricuspid valve regurgitation was also found. Right heart catheterisation disclosed pulmonary hypertension, (pulmonary artery pressure 68/30 mm Hg, mean 42 mm Hg), and a reduced cardiac index (2.0 l/min/m²). Despite medical treatment with angiotensin converting enzyme inhibitors, digitalis, and diuretics the patient deteriorated clinically and he underwent cardiac transplantation nine months later. In the early postoperative period he suffered from multiorgan failure and died shortly after.

Methods

IMMUNOHISTOCHEMICAL STUDY OF SKELETAL AND CARDIAC MUSCLE

A cardiac biopsy specimen obtained at cardiac transplantation, and a needle biopsy specimen of skeletal muscle taken from patient 1 were immediately frozen in liquid nitrogen cooled isopentane and stored at −70°C or in liquid nitrogen. The specimens were fully processed for histological, histochemical, and immunohistochemical examination. In particular, unfixed cryostat sections (6 µm) were immunostained using a panel of six ant dystrophin antibodies, including the monoclonal antibody DYS1808 (Ylem, Italy), as already described.

DNA STUDIES

DNA was isolated from leucocytes by standard methods. Multiplex DNA amplification of dystrophin exons was carried out according to previously described techniques.

REVVERSE TRANSCRIPTION AND POLYMERASE CHAIN REACTION

Total RNA was isolated from control frozen skeletal muscle and heart and from the left ventricle of patient 2. cDNA synthesis was performed using random hexanucleotide primers, following the procedure already described. Polymerase chain reaction was performed using forward primers designed to amplify the muscle, brain, and Purkinje cell isoforms and an exon 6 reverse primer as already described.

Results

IMMUNOHISTOCHEMICAL ANALYSIS

The skeletal muscle biopsy specimen from the propositus showed mild dystrophic changes, characterised by an increase in internal nuclei (12%), mild variability of fibre size with hypertrophic and hypertrophic fibres, and rare fibre splittings in an otherwise well preserved muscle. There were no signs of degeneration or regeneration and only a marginal increase in connective tissue. Immunohistochemistry
performed with the panel of antidystrophin antibodies showed variability in the immunoreaction between adjacent fibres (fig 2), the level of dystrophin expression being weaker than control muscle (fig 2).

Skeletal muscle samples were not available from patient 2. His cardiac biopsy specimen showed significant fibrosis, separating individual cardiomyocytes in some areas, and gross variability of fibre size mainly due to hypertrophic cardiomyocytes. A few cardiac cells undergoing degeneration were also seen. Immunostaining with antibodies directed towards the N terminal, mid-rod, and C terminal domain of dystrophin was strong and continuous, although there was some variability between adjacent fibres, mostly visible using N terminal antibodies (fig 3). There was no staining, however, using the terminal rod domain antibodies DYS-1808, suggesting the presence of a mutation in the genomic region encoding for this epitope (fig 3). Previous studies indicate that such region exists between exons 49 and 51.23

Western blot analysis22 showed a slight reduction in the amount of dystrophin expression with a small decrease in molecular weight (fig 4).

Figure 2 Immunohistochemical staining of skeletal muscle with Dys-III N terminus antidystrophin antibodies. (A) Normal muscle: all fibres equally and intensely stained (original magnification ×280). (B) Muscle from patient 1: staining is weaker than control muscle, suggesting a dystrophinopathy (original magnification ×280).

Figure 3 Top, immunohistochemical staining of cardiac muscle from patient 2 using antidystrophin antibodies. Bottom, protein dystrophin and epitopes of three of the antibodies used in the study. Top left, N terminus antidystrophin antibodies (Dys3) with a near normal reaction; top middle, no staining was visible with mid-rod domain 1808 antidystrophin antibodies; top right, reduced staining with Dys2 C terminus antidystrophin antibodies (original magnifications ×320).
Dystrophin in dilated cardiomyopathy

Dystrophin gene analysis

Multiplex polymerase chain reaction deletion study showed deletion of the central region of dystrophin (data not shown). In the patient with sporadic disease in frame deletion of exons 49–51 was found, while the propositus of the family with DC had a deletion of exons 48–49, which was subsequently confirmed in the obligate carriers (II:1 and II:7).

Transcription studies

Transcription of dystrophin muscle, brain, and Purkinje cell isoforms from the heart of patient 2 resulted in a pattern of isoform expression indistinguishable from that in control heart. Expression of the muscle isoform was high, while that of the brain was low. Purkinje cell isoforms were not expressed (fig 5).

Discussion

Cardiac involvement is very common in DMD and BMD. Almost all patients with DMD have signs of cardiac involvement in the late stages of their disease, while various authors have reported an incidence of cardiac involvement in patients with BMD of approximately 60–65%. Our group recently reported that the dystrophin gene is also involved in some patients with X linked DC. Dystrophin expression and transcription in these families showed that dystrophin was absent in the heart but not in skeletal muscle, thereby providing a biochemical explanation for severe cardiomyopathy. The fact that several patients with X linked DC carried unusual but similar mutations in the extreme 5' end of the gene suggests a link between these mutations and the prevalent cardiac involvement. In contrast, there is still no information on the precise prevalence of dystrophin abnormalities in a population of patients with DC. The only study that has addressed this issue was performed on 27 males with DC, but dystrophin deletion was not found, suggesting that the frequency of dystrophinopathy in the population of patients with DC is probably low.

Two further cases of DC without muscle weakness due to a dystrophinopathy are reported here. In each patient there was a deletion in the central rod domain of dystrophin, a region located in the dystrophin deletion “hot spot”. Identical mutations are typically associated with mild BMD. The reason for the lack of skeletal muscle involvement in our patients with a “typical BMD deletion” is unclear, but intriguing, especially considering that the patient with sporadic disease had normal serum CK activity. The mechanism of cardiac involvement in our patients is likely to be different from that in patients with mutations in the 5' of the gene, in whom cardiac expression of dystrophin could not be found. Indeed, high levels of cardiac dystrophin expression were seen in the patient with sporadic disease with a deletion of exons 49–51 using both immunocytochemistry and western blot analysis. Moreover, the transcription pattern in this patient was indistinguishable from that seen in normal heart—that is, prevalent expression of the muscle isoform with lower expression of the brain isoform. Unfortunately, cardiac muscle was not available from the other patient with a deletion of exons 48–49.

Various authors have reported a high incidence of cardiac involvement in patients with BMD with deletions involving exon 49, while deletion of only exon 48 are less frequently associated with a cardiomyopathy. It has been proposed that intron 48 might contain sequences relevant to the function of dystrophin in cardiac muscle. This intronic sequence would be removed or preserved by an intragenic deletion involving exon 48, depending on the break point within intron 48, while any deletion encompassing both exons 48 and 49 would remove these sequences. Another possibility is that exon 49 contains a domain with an essential function for the heart. Recent analysis of dystrophin has shown, for example, the presence of a calcium binding domain within the cysteine rich region, while a WW domain, involved in mediating protein protein interaction, is present in exons 62–64. Exon 49 is located in the central region of dystrophin, however, where the protein has an organisation similar to that of the coiled coil repeats of spectrin. Exon 49 is contained entirely in repeat 19 and therefore it seems unlikely to have a structure that might account for a specific and unique function in the heart.

We have no explanation as to why a cousin of the patient in the family with DC was affected by DMD, while the three remaining individuals had no signs of skeletal muscle involvement. One possibility is that a second mutation in the dystrophin gene. Such an eventuality has been reported, although it is rare. Unfortunately, DNA or skeletal muscle was not available from this patient.

Our results suggest that a deletion of the dystrophin region involved in the cases reported here can give rise not only to BMD, but also to DC. Intriguingly, serum CK activities can be normal, as highlighted by our patient with sporadic disease. High serum CK activi-
ties are therefore not essential for suspecting DC secondary to a dystrophinopathy.

In conclusion, two cases of DC secondary to a dystrophin mutation are reported. The findings suggest that involvement of dystrophin can be responsible for idiopathic DC, although its incidence is probably low. Combined genetic and biochemical studies on a larger patient population are needed to clarify the prevalence of dystrophin abnormalities in patients with DC.

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CASE STUDY

Non-atherosclerotic coronary artery aneurysms: two case reports

Hendrick M Y Chia, Kim H Tan, Graham Jackson

Abstract
Non-atherosclerotic isolated coronary artery aneurysm is not common. Two cases of non-atherosclerotic isolated coronary artery aneurysm, with similar presentations but different management strategies were presented. The patients were well four and six years later, respectively. The definition, incidence, causes, presentation, complications, investigations, management, and prognosis of coronary artery aneurysms are discussed. The difficulties of determining pathogenesis and different management strategies are highlighted. An isolated coronary artery aneurysm should be managed on its merits.

(Keywords: coronary artery aneurysms)

Aneurysmal coronary artery disease is characterised by abnormal dilatation of a localised or diffuse segment of the coronary artery tree. Before the advent of coronary angiography, all reported cases were based on postmortem findings. The largest angiographic series is the Coronary artery surgery study (CASS) registry where 4.9% of 20 087 patients were observed at angiography to have coronary artery aneurysms. In this series, a coronary artery aneurysm was defined as a dilatation with a diameter of 1.5 times the adjacent normal coronary artery. Ninety per cent of these were associated with atherosclerotic coronary artery disease, where vessel ectasia and poststenotic dilatations are not an infrequent finding. Tunick et al in their series, used the term "discrete aneurysm" to mean a localised spherical or saccular shaped dilatation of 1.5 times. They excluded fusiform aneurysms as they felt that these may be confused with vessel ectasia. In their series of 8422 patients an incidence of 0.2% was observed. Others have suggested a diameter of at least three times normal as the definition of coronary artery aneurysm so that the incidence will vary depending on definition.

The pathogenesis of coronary artery aneurysm involves underlying destruction of the vessel media. This thinning of the media together with increased wall stress causes progressive dilatation of the segment of coronary artery resulting in the angiographic appearance of diffuse coronary ectasia as well as localised ectatic segments. These changes can also be described as fusiform or saccular aneurysms. It is therefore reasonable to surmise that coronary ectasia and coronary artery aneurysm may represent either end of the spectrum of a common pathological process.

The most common cause of coronary artery aneurysm is atherosclerosis as demonstrated in the CASS registry. Other causes of coronary artery aneurysms are not common and can be classified as: inflammatory—Kawasaki disease that occurs mainly in infants and children; Takayasu’s disease, polyarteritis nodosa, and systemic lupus; abnormal connective tissue synthesis—Marfan’s syndrome and Ehlers Danlos syndrome; infectious—septic emboli, syphilis, and Lyme borreliosis; tumour (rare)—primary cardiac lymphoma; congenital—coronary artery aneurysm and trauma (iatrogenic)—percutaneous transluminal coronary angioplasty, intracoronary Cook stents, and directional coronary atherectomy. Coronary artery aneurysms may be detected in the absence of symptoms; however, patients sometimes present with angina, myocardial infarction, or sudden death. If rupture into a heart chamber occurs, a continuous murmur may be apparent and decompensation may ensue. Fistula formation into the right atrium or right ventricle has been reported. Coronary artery aneurysms can be detected non-invasively using echocardiography, computed tomography, and magnetic resonance imaging. However, definitive diagnosis with coronary arteriography is usually needed accurately to delineate the anatomy and to ascertain the absence or presence of atherosclerosis elsewhere.

Because of the rarity of coronary artery aneurysm, the management of this condition is not clearly defined. The true incidence and natural history is uncertain as some patients may be entirely asymptomatic and the reported mortality may not reflect actual overall prognosis. Coronary artery aneurysms that present with life threatening complications such as heart chamber compression or fistula forma-
tion, require prompt surgical intervention. Some authors argue that coronary artery aneurysm of at least three to four times the size of the original vessel diameter is an absolute indication for surgical intervention because of the propensity for complications such as compression, rupture, or thrombosis. Others argue that discrete coronary artery aneurysms do not appear to rupture, and these patients appear to have a favourable long term prognosis; therefore, elective resection in asymptomatic patients is not warranted. This has been supported by a case report of a patient with asymptomatic coronary artery aneurysm who remained free from complications eight years after initial diagnosis. In asymptomatic cases, the risks of surgical intervention should be balanced by any potential risks if the aneurysm is left alone. In such cases, the benefits, if any, of antiplatelet or anticoagulation therapies are unknown.

The proximal and middle segments of the right coronary artery (RCA) are the most common sites for coronary artery aneurysm, followed by the proximal left anterior descending (LAD) and the left circumflex arteries. Coronary artery aneurysm of the left main stem is rare.

We report two unusual cases of coronary artery aneurysm arising from different arteries but with similar presentations.

Case 1
A 33 year old man with no previous history of ischaemic heart disease presented with acute central chest pain. He was a non-smoker, with no history of diabetes or hypertension. There was a positive family history of ischaemic heart disease. Total fasting cholesterol was 5.7 mmol/l. There was no history or clinical features suggestive of acute inflammatory or connective tissue disease at presentation.

ECG on presentation revealed ST segment elevation across the anterior chest leads, and he was thrombolysed with streptokinase. Sequential cardiac enzymes showed a rise of creatine kinase to a maximum of 785 U/l. Screening for autoimmune and inflammatory disorders were negative.

Coronary angiography revealed a large coronary artery aneurysm arising from the mid-LAD (fig 1), subtending an area of apical infarction, and flow distal to the aneurysm was noted to be slow. The rest of the coronary tree was normal.

The patient was initially managed conservatively with aspirin; however, he presented a month later with further chest pains and elevated ST segments across the anterior chest leads. He was thrombolysed with tissue plasminogen activator and this time the creatine kinase rose to a maximum of 761 U/l. A repeat study of coronary anatomy revealed similar findings to the first angiogram. Because of the slow flow of contrast to the distal LAD, it was postulated that the aneurysm was acting as a capacitance to blood flow, with stasis leading to thrombus formation and resulting distal emboli. He was therefore fully anticoagulated with warfarin, keeping the International Normalised Ratio (INR) at ~ 3.

In view of the two successive presentations of myocardial infarction and the site of the aneurysm, he underwent surgical excision of the coronary artery aneurysm and an internal mammary artery was anastomosed end to side to the proximal and distal origins of the aneurysm, thereby preserving the diagonal branch.

Sections of the coronary artery aneurysm examined histologically showed no evidence of atherosclerosis, active inflammation or necrosis. There was marked intimal thickening by fibromyxoid tissue with reduplicated elastic fibres and a hypocellular, hyalinised focus. The appearances were not specific of Kawasaki disease but could have represented the late stage of an inflammatory disorder or a focus of fibromuscular dysplasia.

The patient made a good recovery and has remained well four years after presentation.
Case 2
A 37 year old woman presented with severe retrosternal chest pains 12 days after giving birth to her sixth child. She was previously fit and well, and her recent labour was uncomplicated. She did not experience any chest pains during labour. She was an ex-smoker of seven years, with no history of diabetes or hypertension. Both her parents had a history of myocardial infarcts in their later years. Her fasting total cholesterol was 6.3 mmol/l. There was no history or clinical features suggestive of acute inflammatory or connective tissue disease.

Initial ECGs showed T wave inversion in leads II and aVL only but an ECG two days later showed deep T wave inversion with Q waves in the inferior leads. Serial cardiac enzymes showed a slight rise of creatine kinase to a maximum of 297 U/l on day 2. She was not thrombolysed. Screening for autoimmune and inflammatory disorders was negative.

Coronary angiography revealed a normal left coronary system, with no evidence of atheroma. The RCA, however, had a large aneurysm arising proximally (fig 2). Left ventriculography revealed mild inferior wall hypokinesia with otherwise normal left ventricular function.

It was felt that the coronary artery aneurysm accounted for her symptoms; therefore, the patient was anticoagulated with warfarin. At follow up two months later a stress thallium study did not show any evidence of reversible ischaemia and the patient was well. Because the coronary artery aneurysm arose from the RCA, which was supplying only a small part of the left ventricle, it was felt that in the absence of symptoms or evidence of ischaemia, conservative management would be appropriate. The patient remained well six years after presentation.

Discussion
The diagnosis of congenital coronary artery aneurysm implies the exclusion of other acquired causes. In both the present cases, there was no evidence of atherosclerosis in the coronary arteries. Neither case had childhood histories suggestive of Kawasaki disease, nor was there evidence of acute inflammatory disease or other acquired causes. The histological specimen of case 1 was not conclusive. Although no histological specimens were available from case 2, it is possible that this case, as well as case 1, was a congenital abnormality. It is however impossible to rule out Kawasaki disease, as it can present undetected as a mild febrile episode in childhood, although this is uncommon. Coronary complications in Kawasaki disease usually occur six to 12 months following the acute illness.

Both our cases presented with evidence of infarction in the area of myocardium supplied by the culprit vessel. In the absence of atherosclerosis, it is likely the infarction was caused by thrombus formation within the coronary artery aneurysm resulting in embolisation to the distal artery.

Case 1 had surgical intervention because it was felt that the risk of further embolisation down the LAD was significant despite anticoagulation, and that there was potential for further damage to the left ventricle. In case 2, in view of the location of the coronary artery aneurysm in the RCA, it was felt that the risk of significant damage to the left ventricle was less than the risk of surgery, therefore, the patient was treated conservatively. Although little is known about the prevalence of distal embolisation or the benefits, if any, of antiplatelet or anticoagulation therapy, we felt that case 2 would benefit from long term anticoagulation because of her presentation with a myocardial infarction.

The prognosis of coronary artery aneurysm appears to be dependent on the presence or absence of associated stenotic coronary artery disease. The greater incidence of infarction in patients with coronary artery aneurysm may only be a reflection of an overall increased prevalence of atherosclerosis. This is underscored by the fact that myocardial infarction is reported to be no more prevalent in patients with non-atherosclerotic coronary artery aneurysm. Coronary artery aneurysms in Kawasaki disease are known to progress to obstructive lesions in later life. The prognosis of congenital coronary artery aneurysm how-
ever is more uncertain because of its rarity. Those reported in the literature tend to be cases that present with complications although there has been one case report of asymptomatic congenital coronary artery aneurysm that remained free from complications for eight years. Both our patients remain well four (case 1) and six (case 2) years after initial presentation.

In summary, we present two cases of non-atherosclerotic coronary artery aneurysm with similar presentations but different management strategies, both with favourable long term outcomes. These cases demonstrate the difficulties in determining the pathogenesis, and highlight the different management strategies, of isolated non-atherosclerotic coronary artery aneurysms. We believe the management of isolated coronary artery aneurysms should be individualised depending on their location and the clinical context.

Three year continuous abstinence in a smoking cessation study using the nicotine transdermal patch

Robyn L Richmond, Linda Kehoe, Abilio Cesar de Almeida Neto

Abstract
A total of 305 subjects from Sydney were randomly allocated to receive either an active (24 hour transdermal nicotine patch over a 10 week course) or placebo nicotine patch. All subjects participated in a multicomponent cognitive–behavioural smoking cessation programme over five weeks in two-hour group sessions. The continuous abstinence rates at three years (validated by expired carbon monoxide) were 13.8% for the active group and 5.2% for placebo group (p = 0.011). The active nicotine patch with behavioural therapy achieved more than double the abstinence rates early in treatment compared with placebo and this difference was maintained throughout the three year follow up. (Heart 1997;78:617–618)

Keywords: smoking cessation; nicotine patches

The nicotine transdermal patch has been shown to more than double 12 month continuous abstinence rates compared with placebo.1-3 Only one study has reported two year abstinence from smoking when the nicotine patch was used as an adjunct.4 The aim of this paper is to present continuous abstinence rates from the end of treatment to three years.

Methods
A total of 305 subjects from Sydney, Australia were randomly assigned to receive either an active nicotine patch or a placebo patch. Subjects in the active treatment group were given a 24 hour transdermal nicotine delivery system over a 10 week course: 21 mg of nicotine/day in patches for six weeks, and 14 and 7 mg for two weeks each thereafter. All subjects attended a multicomponent cognitive–behavioural smoking cessation programme consisting of five consecutive weeks of two-hour group sessions conducted at a hospital outpatient clinic. Components of the programme and subject characteristics have been described previously.5

The outcome measure for this paper was continuous abstinence determined by calculating the percentage of patients who had validated abstinence at every measurement point from the end of treatment through three years. Validation of self-reports of abstinence was based on expired carbon monoxide levels ≤ 10 ppm, or collateral confirmation obtained for three participants at the two and three year assessment points (for example, moved interstate). Other participants were classified as continuing smokers.

Distributions of the active and placebo groups were compared using the logrank test. For each case the time until restarting smoking was taken to be the last measurement point where abstinence was maintained. For those cases who did not restart smoking during the study, times to event were censored at the end of the three year study period.

Results
There was a significantly different continuous abstinence rate between the active and placebo patch groups (p = 0.0001) (fig 1). For both groups, the greatest relapse to smoking occurred in the first three months after the intervention, particularly in the first few weeks. However, the placebo group showed the greatest decline of abstainers in the early weeks and continued to show a lower level of abstainers.
Effect of adopting a new histological grading system of acute rejection after heart transplantation

Aggie H Balk, Pieter E Zondervan, Peter van der Meer, Teun van Gelder, Bas Mochtar, Maarten L Simoons and Willem Weimar

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